



Bioaccumulation and trophic transfer of perfluorinated alkyl substances (PFAS) in marine biota from the Belgian North Sea: Distribution and human health risk implications[☆]

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ABSTRACT

Per- and polyfluorinated alkyl substances (PFAS) are highly persistent chemicals, which pose a potential risk for aquatic wildlife due to their bioaccumulative behaviour and toxicological effects. Although the distribution of PFAS in marine environments has been studied worldwide, little is known on the contamination of PFAS in the southern North Sea. In the present study, the bioaccumulation and trophic transfer of Perfluoroalkyl acids (PFAAs) was studied in liver and muscle tissue of seven fish species and in whole-body tissue of two crustacean species, collected at 10 sites in the Belgian North Sea. Furthermore, the human and ecological health risks were examined.

Overall, perfluorooctane sulfonate (PFOS) was predominant in all matrices and other long-chain PFAS were frequently detected. Mean PFOS concentrations ranged from <LOQ to 107 ng/g (ww) in fish liver, from <LOQ to 24 ng/g ww in fish muscle and from 0.29 to 5.6 ng/g ww in crustaceans. Elevated perfluorotridecanoic acid (PFTrDA) concentrations were detected in fish liver from the estuarine and coastal region (<LOQ-116 ng/g ww), indicating a specific point source of this compound. Based on stable isotope analysis, no distinctive trophic transfer patterns of PFAS could be identified which implies that the bioconcentration of PFAS from the surrounding abiotic environment is most likely dominating over the biomagnification in the studied biota. The consumption of commercially important species such as the brown shrimp (*Crangon crangon*), plaice (*Pleuronectes platessa*), sole (*Solea solea*) and whiting (*Merlangus merlangus*) might pose potential health risks if it exceeds 17 g/day, 18 g/day, 26 g/day and 43 g/day respectively. Most PFOS measurements did not exceed the $QS_{\text{biota,h}}$ of 9.1 ng/g ww, however, the benchmark of 33 ng/g ww targeting the protection of wildlife from secondary poisoning was exceeded for 43% and 28% of the samples in plaice and sole.

1. Introduction

Per- and polyfluorinated alkyl substances (PFAS) are a diverse class of man-made compounds with the chemical structure generally formulated as “ $C_nF_{2n+1}R$ ” (Buck et al., 2011). They have distinctive physicochemical properties such as a strong covalent C–F bond and an amphiphilic nature (Buck et al., 2011). Therefore, PFAS have been widely used for more than 60 years in diverse commercial and industrial products, such as fire-fighting foams, food packaging, stain and water repellents, cosmetics, pharmaceuticals, electronics, insecticides and paints (Houde et al., 2006; Xia et al., 2013).

However, the wide application of PFAS goes along with their direct and indirect release into the environment via e.g. industrial emissions, wastewater effluent and the usage of consumer products (Buck et al., 2011). Due to the strong covalent C–F bond, PFAS are chemically and physically stable and resistant to environmental degradation (Buck et al., 2011; Blasco et al., 2016). Therefore, most PFAS are extremely persistent and considered to be accumulative in the environment (Houde et al., 2006). Bioaccumulation of PFAS in aquatic biota, such as invertebrates, fish, marine mammals and birds, has been reported worldwide (Houde et al., 2011). The detection of higher PFAS concentrations in higher trophic levels suggests the trophic transfer of PFAS in

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marine food webs (Haukås et al., 2007; Kelly et al., 2009). Biomagnification of PFAS provokes an increasing concern regarding their risks for secondary poisoning of top predators, including humans. Fish consumption is considered as a main PFAS-exposure route for humans, alongside with drinking water and dust inhalation (D'Hollander et al., 2010; Shoeib et al., 2005; Ericson et al., 2008; Augustsson et al., 2021). Several studies reported potential adverse effects of PFAS on the reproductive-, developmental-, endocrine- and immune system of both aquatic and terrestrial organisms (Ankley et al., 2005; Jensen and Leffers, 2008; Grandjean and Clapp, 2015). Epidemiological research has shown that long-term exposure to PFAS can be linked to an increased risk of cancer, impaired immune functioning and reduced fertility (Grandjean and Clapp, 2015; Vieira et al., 2013; Mastrantonio et al., 2018; Schiffer et al., 2014).

Due to an increasing concern on the global distribution and potential adverse health effects of PFAS, the 3M company (a main PFAS manufacturer) decided in 2000 to phase-out the production of perfluorooctane sulfonate (PFOS) and related products (Carlioni, 2009). Furthermore, PFOS and perfluorooctanoic acid (PFOA) were included in the Stockholm convention in 2009 and 2019 respectively (UNEP, 2019). Despite the phase-out and other regulatory measures, PFOS remains predominant in the environment and concentrations are often still very high, indicating the need for continuous monitoring of both legacy compounds and PFAS of emerging concern (Augustsson et al., 2021; Custer et al., 2010).

In general, relatively higher PFAS concentrations are detected in the northern hemisphere (Arctic, Europe and Asia) compared to the southern hemisphere (Houde et al., 2011; Dreyer et al., 2009). This trend is likely because most fluorochemical plants are located in industrialized and urbanized areas of the northern hemisphere (Houde et al., 2011). In several countries across Europe, the areas around PFAS factories and application sites have been identified as PFAS hotspots and point sources (Brandsma et al., 2019; Munoz et al., 2015; Pitter et al., 2020). Among the highest PFAS levels ever reported were those detected in the eggs of great tits (*Parus major*) near a fluorochemical plant (3M) in Antwerp, Belgium (Groffen et al., 2017). This fluorochemical plant is recognised as a PFAS hotspot, as high PFAS concentrations were detected in multiple terrestrial matrices such as soil, invertebrates, and mammals from the surrounding area (Lopez-Antia et al., 2017; Groffen et al., 2017; D'Hollander et al., 2014). Despite the high PFAS levels detected in the terrestrial environment, little is known on the contamination of the surrounding aquatic environment. Therefore, the Scheldt river, which flows adjacent to the 3 M production facility, is likely to be affected by industrial waste-water effluents, hence transporting PFAS towards the North Sea. In 2003, two studies on fish and invertebrate species reported a pollution gradient of PFOS along the Western Scheldt towards the North Sea (Hoff et al., 2003; Van De Vijver et al., 2003). However, these studies only targeted PFOS and since 2003 no additional research has been done on PFAS contamination in the North Sea and the Western Scheldt. Furthermore, inconsistent findings in literature indicate that biomagnification patterns of PFAS can differ among different geographical areas (Houde et al., 2011). Therefore, knowledge on the current distribution of PFAS and their potential biomagnification through the North Sea food web is lacking. However, this knowledge is crucial to evaluate the potential risks of PFAS in marine environments and to successfully derive and implement mitigation strategies.

In several studies, liver has been found to be a primary target tissue for PFAS accumulation in fish and marine mammals (Houde et al., 2011; Van De Vijver et al., 2007; Ahrens et al., 2009a). This can be explained by the high binding affinity of PFAS to proteins such as serum albumin and fatty acid binding proteins which are abundantly found in hepatocytes (Conder et al., 2008; Shi et al., 2012; Ng and Hungerbühler, 2013). However, monitoring the accumulation of PFAS in muscle tissue is still relevant concerning the potential risks for human consumption.

Therefore, the present study aims to investigate the spatial distribution and contamination profiles of Perfluoroalkyl acids (PFAAs) in

both liver and muscle tissue of marine fish and in the whole-body tissue of crustaceans from the Belgian North Sea. Furthermore, the trophic transfer of PFAS was assessed by means of stable isotope analysis. Lastly, the potential ecological and human health risks through the consumption of contaminated fish and crustaceans were investigated.

2. Material & methods

2.1. Sample collection

In total, 10 sites were sampled in the Belgian part of the North Sea and the Western Scheldt estuary (Fig. 1). In November and December of 2018, fish and crustaceans were sampled by a beam trawl during two sampling campaigns on the Research Vessel (RV) Simon Stevin and the RV Belgica respectively. At each sampling site, one haul with a duration of 30 min was performed and at least three individuals of each species were collected. The captured fishes were sacrificed with a blow on the head and stored on board in a -20°C freezer. In total, nine species were sampled, i.e., seven fish: *Gaidropsarus vulgaris* (three-bearded rockling), *Mullus surmuletus* (surmullet), *Solea solea* (common sole), *Pleuronectes platessa* (European plaice), *Arnoglossus laterna* (Mediterranean scaldfish), *Merlangius merlangus* (whiting), *Clupea harengus* (Atlantic herring) and two crustacean species: *Liocarcinus holsatus* (flying crab) and *Crangon crangon* (brown shrimp). *Limecola balthica* (Baltic clam) was only found at location 8 and 9 and was therefore only used for stable isotope analysis. All specimens were dissected under laboratory conditions and prior to dissection the total length and weight of each specimen was measured (Table S1-S2). The average length of each species per location was lower compared to the estimated first length at which they reach maturity (www.fishbase.se, Table S1). For the fish specimens, the liver and lateral muscle tissue were dissected and for the crustaceans all the internal soft tissue was dissected. The tissue samples were homogenised (stainless steel kitchen mixer: Bosch -MSM65PER) and stored in Polypropylene (PP) tubes in the freezer (-20°C) until further analysis. In between each specimen and tissue, the dissecting material was cleaned with ethanol (70%) and rinsed with acetonitrile (ACN, 100%, LiChrosolv, Merck Chemicals, Belgium) to avoid cross-contamination.

2.2. Sample extraction

For the extraction of biotic matrices, a method based on the principle of solid-phase extraction using graphitized carbon powder (ENVI-Carb), originally described by Powley et al. (2005), was used. Up to 0.5 g tissue was spiked with 10 ng of an isotopically mass-labelled internal standard mix solution (MPFAC-MXA, Wellington Laboratories, Guelph, Canada, containing $^{13}\text{C}_8$ -PFOS, $^{13}\text{O}_2$ -PFHxS, $^{13}\text{C}_4$ -PFBA, $^{13}\text{C}_2$ -PFHxA, $^{13}\text{C}_8$ -PFOA, $^{13}\text{C}_9$ -PFNA, $^{13}\text{C}_6$ -PFDA, $^{13}\text{C}_7$ -PFUnDA and $^{13}\text{C}_2$ -PFDoDA) was added. Thereafter, each sample was supplemented with 10 mL ACN, vortexed and placed in an ultrasonic bath (Branson 2510) for three times 10 min, with vortex-mixing in between periods. After vortexing, the samples were put on a shaking plate at 135 rpm for at least 16 h. Hereafter, samples were centrifuged (Eppendorf 5804 R) for 10 min at $1037\times g$ and 4°C . The supernatant of 10 mL was transferred to a 15 mL PP tube and concentrated by using an RVC 2-25 rotational-vacuum-concentrator (20°C) until approximately 0.5 mL. Once concentrated, the remaining 0.5 mL extract solutions were transferred to 1.5 mL Eppendorf tubes filled with 0.1 g graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Missouri, USA). Prior to the transfer, 50 μL glacial acetic acid (100%) was added to the carbon powder. The 15 mL tubes were rinsed twice by adding 250 μL ACN (vortexed in between) and then transferred to the same Eppendorf tubes. To mix the activated carbon with the sample, Eppendorf tubes were vortexed for at least 1 min. After centrifugation (10 min, $9279\times g$, 4°C) the supernatant was transferred to a new Eppendorf tube. Subsequently, the Eppendorf tubes were concentrated to dryness and resolved in 200 μL of a 2% ammonium hydroxide solution (Filter Service N.V., Belgium; diluted with ACN).

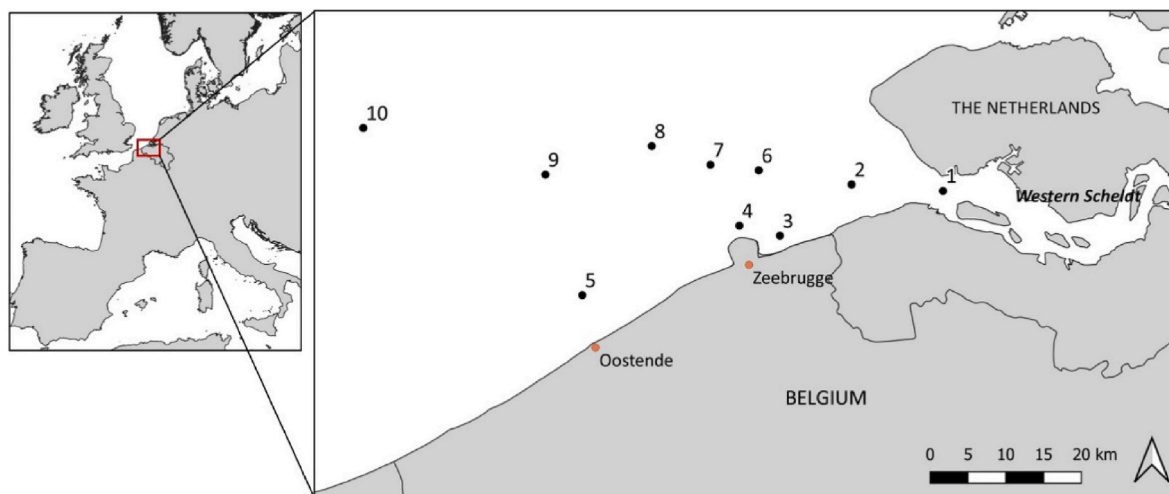


Fig. 1. Overview of the Belgian North Sea and the sampling sites, ranging from the mouth of the Scheldt estuary towards open sea.

Before PFAS analysis, a filtration step was added in order to remove remaining solids from the final extract. The samples were vortexed and filtered into injector vials by transferring them into a 10 mL syringe (Braun) to which an Ion Chromatography Acrodisc 13 mm Syringe Filter with 0.2 μm Supor polyethersulfone (PES) Membrane was attached.

2.3. UPLC-TQD analysis

Ultrahigh-performance liquid chromatography (UPLC) in tandem with electrospray (operating in negative modus) mass spectrometry (ES-MS/MS) linked to a triple quadrupole detector (ACQUITY, TQD, Waters, Milford, MA) was used to analyse PFAAs. Target analytes were perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), PFOA, PFOS, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), Perfluorodecane sulfonate (PFDS), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTTrDA) and perfluorotetradecanoic acid (PFTeDA).

Analytes were separated on an ACQUITY BEH C18 column (2.1 \times 50 mm; 1.7 μm , Waters, USA). An ACQUITY BEH C18-pre-column (2.1 \times 30 mm; 1.7 μm , Waters, USA), inserted between the injector and the solvent mixer, was used to retain any PFAS contamination from the system. The injection volume was set at 10 μL with a flow rate of 450 $\mu\text{L}/\text{min}$. As mobile phase solvents, 0.1% formic acid in water and 0.1% formic acid in ACN were used. The solvent gradient started at 65% of 0.1% formic acid in water, decreased to 0% in 3.4 min and returned to 65% at 4.7 min. PFAS were identified and quantified based on multiple reaction monitoring (MRM) of two diagnostic transitions for each compound (Table S3) (Groffen et al., 2019).

2.4. Quality control

Per 10 samples, one procedural blank and one reference sample were included during the extraction procedure and chemical analysis. For the procedural blank, 10 mL of ACN was used. As reference material, 0.5 g of sterilized fish muscle tissue from pike perch (*Stizostedion lucioperca*) (QUASIMEME Laboratory Performance Studies) was used (Van Leeuwen et al., 2011). The average concentrations measured in the reference samples were within the ranges of the interlaboratory study of the corresponding reference material (Table S4) (Van Leeuwen et al., 2011). The limit of quantification (LOQ) for each target analyte was calculated, in matrix, based on a signal-to-noise ratio of 10 (Table S5). During PFAS analysis, ACN was injected on a regular basis, as instrumental blank, to prevent the carry-over effect to the next sample.

2.5. Stable isotope analysis

Individual fish (muscle) and whole invertebrate specimens were pooled per species and per location (Table S6). The samples were freeze-dried at $-55\text{ }^\circ\text{C}$ for three days and homogenised into fine powder. Approximately 0.5 mg of the fish samples was weighed and encapsulated into a 5 \times 8 mm tin (Sn) capsule. The invertebrate samples can still contain some remnants from the exoskeleton. Therefore, approximately 0.5 mg of the invertebrate samples was encapsulated into a 5 \times 8 mm silver (Ag) capsule and 40 μL HCl was added to dissolve remaining calcium carbonate. Stable isotopes of N and C were measured using an EA1110 elemental analyser coupled to a Thermo DeltaV Advantage IRMS via a ConFlo IV interface at the Department of Earth and Environmental Sciences, KULeuven (Belgium). For the calibration of the stable isotope ratios, a combination of IAEA-600 (caffeine) and two in-house standards (leucine and freeze-dried tuna muscle tissue) were used. The two in-house standards were previously calibrated with certified reference standards. The estimated precisions for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were better than 0.05‰ and 0.13‰.

The stable isotope results are expressed in the standard notation as defined by:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{reference}}} \right) - 1 \right] \times 1000$$

with $R = {}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{13}\text{C}/{}^{12}\text{C}$, for nitrogen and carbon isotopes respectively.

Based on the $\delta^{15}\text{N}$ stable isotope results, the relative trophic level (TL) of each species per location was calculated using the formula of Post (2002):

$$TL_{\text{consumer}} = \left[\frac{(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{primaryconsumer}})}{\Delta\delta^{15}\text{N}} \right] + 2$$

where, TL is the trophic level of the organism of interest, $\delta^{15}\text{N}_{\text{consumer}}$ is $\delta^{15}\text{N}$ of the concerned organism, $\delta^{15}\text{N}_{\text{primary consumer}}$ is the $\delta^{15}\text{N}$ of a local primary consumer (*L. Balthica*), 2 is the trophic level of the primary consumer, and $\Delta\delta^{15}\text{N}$ is the trophic enrichment factor equal to 3.4 (Post, 2002).

2.6. Human and ecological health risk evaluation

The human consumption risk was assessed by calculating the maximum edible amount that can be daily consumed by a person of 70 kg without posing potential risks for human health. This risk was calculated for all fish and crustacean species on an individual basis using

the following formula described by Verhaert et al. (2017):

$$Q = \frac{W \times M}{C}$$

where Q is the maximum amount of contaminated fish or crustaceans that can be consumed by a person per day without posing health risks (g/day), W is human body weight (70 kg), M is the Minimum Risk Level (MRL) which is an estimate for the amount of a given compound that can be consumed by a person each day without posing detectable health risks (ng/kg body weight/day) and C is the concentration of the compound detected in the specimens (ng/g ww). The calculations are performed for three MRLs: the first two are defined by the Agency for Toxic Substances and Disease Registry (ATSDR) for PFOS (MRL_{PFOS} = 2 ng/kg/day) and PFOA (MRL_{PFOA} = 3 ng/kg/day) (ATSDR, 2021). ATSDR uses the point of departure approach, such as no-observed-adverse-effect levels, in which the most sensitive substance-induced endpoints are used as a basis for the derivation of MRLs. Based on laboratory animal data, hepatic, immune and developmental endpoints were found to be the most sensitive targets for the establishment of MRLs for PFOS and PFOA (ATSDR, 2021). The third MRL is defined by the European Food Safety Authority (EFSA) and assesses the combined exposure to PFOS, PFOA, PFNA and PFHxS (EFSA, 2020). To set this new threshold, the decreased immune response after vaccination due to PFAS consumption, was considered as the most sensitive endpoint. The EFSA risk level is set as a tolerable weekly intake (TWI) of 4.4 ng per kilogram body weight per week. For the determination of potential health risks in the present study, the TWI was converted to the tolerable daily intake of 0.36 ng/kg/day to compare with the ATSDR MRLs (ng/kg/day). As PFHxS was not detected in the present study the sum of PFOS, PFOA and PFNA was compared with the EFSA MRL.

To assess the potential ecological risks, accumulated PFOS concentrations were compared with the Quality Standards for biota (QS_{biota}) which were set by the European Commission under the Water Framework Directive (European Union, 2013). The human health-based threshold (QS_{biota, hh}), namely 9.1 µg PFOS/kg ww, was compared with the accumulated PFOS concentrations in muscle tissue. To test for compliance with the benchmark that was specifically derived for the protection of wildlife against secondary poisoning (QS_{biota, secpois} = 33 µg PFOS/kg ww), the sum of the accumulated PFOS concentrations in muscle and liver tissue was used (European Union, 2014).

2.7. Statistical analysis

Statistical analyses were conducted in R-Studio (R 3.0.1+) and GraphPad Prism 7 with $p \leq 0.05$ as the level of significance. Samples with concentrations below the LOQ were given a value corresponding to their LOQ/2 (Bervoets et al., 2004). This was only the case for 0.92% of all measurements in fish muscle tissue, 1.2% of all measurements in fish liver tissue and 2.5% of all measurements in crustaceans (Tables S5-7). Therefore, left-skewing of the data is considered to be minimal for this dataset.

To examine whether there were significant differences for each PFAS compound among locations and species, multiway ANOVA analyses were performed. All locations were directly compared for three species (*M. merlangus*, *P. platessa* and *S. solea*), which were captured at almost all locations. Post hoc comparisons were calculated using Tukey's honestly significant difference (HSD) adjusted post hoc test. Each fitted model was diagnosed for normality of residuals using the Shapiro Wilk test (function shapiro.test from package stats) and for homogeneity of variances using Levene's test (function leveneTest from package car) or graphically analysing the spread level plot (function spreadLevelPlot from package car). In case of a non-normal distribution, the non-parametric Kruskal Wallis Chi-squared test was performed followed by a non-parametric Dunn post hoc test. Correlation analyses were performed of PFAS concentrations between liver and muscle and between

PFAS concentrations and trophic levels per location. The Pearson correlation coefficient (r) was used if all model assumptions (i.e. linearity of residuals, homogeneity of variances and normal distribution of residuals) were met. In the case of infringement for one of the model assumptions, the non-parametric Spearman correlation coefficient (r) was used as alternative test. To compare measured concentrations between liver and muscle tissue, paired t-tests were performed. Based on the stable isotope data, SIBER – analyses (Stable Isotope Bayesian Ellipses in R) were done to study the extent of isotopic niche overlap among the species and to compare the isotopic niche widths (Jackson et al., 2011).

3. Results

3.1. Spatial PFAS distribution and profiles

A summary of the PFAS concentrations in fish and crustacean species at each site is given in Tables S7 – S9. Target analytes PFBA, PFBS, PFPeA, PFHxA, PFHxS, PFHpA, PFDS and PFTeDA are not shown as they were not detected in any matrix. In all matrices, only long-chain compounds were detected, with seven compounds in fish muscle and liver tissue and five compounds in crustaceans. Overall, irrespectively of fish species and location, significantly higher PFAS concentrations were found in liver tissue compared to muscle tissue for all compounds ($p < 0.001$). Significant, positive correlations between liver and muscle concentrations were observed for PFOS ($p < 0.001$, $r_{\text{Spearman}} = 0.67$) and PFOA ($p = 0.002$, $r_{\text{Spearman}} = 0.32$). For none of the other compounds significant correlations were found between liver and muscle tissue.

Comparison of PFAS profiles among locations was only done for those species collected at ≥ 7 sampling sites, i.e., *P. platessa*, *S. solea* and *M. merlangus*, *C. crangon* and *L. holsatus* (Fig. 2). In liver tissue, PFTeDA concentrations of all three fish species were significantly higher at location 4 compared to all other sampling sites (*P. platessa*: $p < 0.001$, *S. solea*: $p < 0.001$, *M. merlangus*: $p < 0.001$) (Fig. 2c–e, Table S10). For *M. merlangus*, PFTeDA was also significantly higher at location 2 compared to all other sites, except for location 4 ($p < 0.001$) (Table S5). At location 4, higher PFOS concentrations in liver of *S. solea* were detected compared to location 2 ($p = 0.002$), 6 ($p = 0.006$) and 8 ($p = 0.015$). In muscle tissue of *M. merlangus* and *P. platessa*, PFOS concentrations were significantly higher at location 4 compared to location 2 (*M. merlangus*: $p = 0.029$, *P. platessa* $p = 0.011$) (Fig. 2c–e). In *P. platessa*, also significant higher PFOA concentrations were found at location 4 compared to location 1 ($p = 0.016$) and 6 ($p = 0.002$). For crustaceans, significantly higher concentrations were only found for PFUnDA in *L. holsatus* at location 7 compared to location 1 ($p = 0.021$) and 3 ($p = 0.021$) (Fig. 2a and b).

3.2. Species – specific PFAS profiles

When comparing mean PFAS profiles among species, PFOS was found to be predominant in almost all species and all matrices except for *C. harengus* and *G. vulgaris*. For *C. harengus*, PFOA (47%) was found to be dominant in liver tissue and for *G. vulgaris* the highest relative contribution was found for PFTeDA (30%) in liver tissue and for PFUnDA (22%) in muscle tissue. In liver tissue (Fig. 3a), the relative contribution of PFOS was 58% in *P. platessa*, 52% in *S. solea*, 46% in *M. surmuletus*, 48% in *A. laterna*, 25% in *C. harengus*, 47% in *M. merlangus* and 28% in *G. vulgaris*. The highest mean PFOS concentration in liver tissue was found for *P. platessa* (30.5 ng/g ww) and further decreased in the following order: *S. solea* (25.1 ng/g ww) > *M. surmuletus* (14.5 ng/g ww) > *A. laterna* (13.8 ng/g ww) > *M. merlangus* (7.00 ng/g ww) > *C. harengus* (5.59 ng/g ww) > *G. vulgaris* (1.49 ng/g ww). The second dominant PFAS in liver tissue was PFTeDA in *P. platessa* (17%), *S. solea* (18%) and *M. merlangus* (19%). In *C. harengus*, *M. surmuletus* and *A. laterna* the second dominant compounds in liver were PFOS (25%), PFDA (16%) and PFUnDA (18%), respectively. By comparing the Σ PFAS concentrations in liver tissue among fish species, significant higher

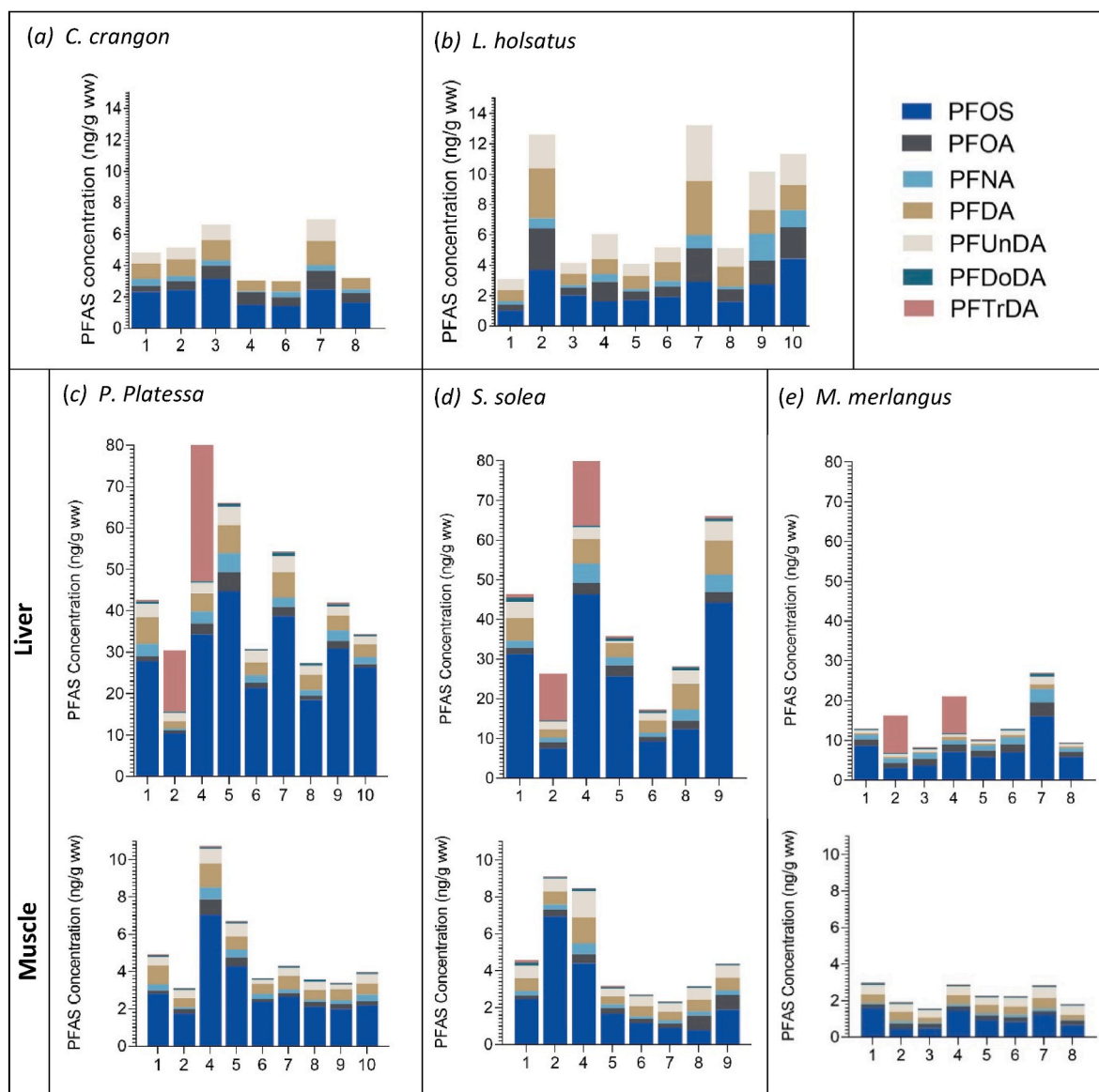


Fig. 2. Spatial distribution of PFAS profiles and concentrations of 2 invertebrate species (a) *C. crangon*, (b) *L. holsatus* and three fish species (c) *P. platessa*, (d) *S. solea* and (e) *M. merlangus* in liver and muscle tissue ($N = 3-4$ replicates per species per location).

concentrations were found for *P. platessa* and *S. solea* compared to *M. merlangus* ($p < 0.001$) and *G. vulgaris* ($p < 0.001$). Mean Σ PFAS concentrations in liver tissue were the highest in *P. platessa* (52.5 ng/g ww) and further decreased in the following order: *S. solea* (47.9 ng/g ww) > *M. surmuletus* (31.5 ng/g ww) > *A. laterna* (28.5 ng/g ww) > *C. harengus* (22.7 ng/g ww) > *M. merlangus* (14.9 ng/g ww) > *G. vulgaris* (5.38 ng/g ww).

In muscle tissue (Fig. 3b), the relative contribution of PFOS was 61% in *P. platessa*, 60% in *S. solea*, 47% in *M. surmuletus*, 63% in *A. laterna*, 62% in *C. harengus*, 41% in *M. merlangus* and 13% in *G. vulgaris*. The highest mean PFOS concentration was found for *P. platessa* (3.03 ng/g ww) and further decreased in the following order: *A. laterna* (2.83 ng/g ww) > *S. solea* (2.76 ng/g ww) > *C. harengus* (2.04 ng/g ww) > *M. surmuletus* (1.56 ng/g ww) > *M. merlangus* (0.96 ng/g ww) > *G. vulgaris* (0.29 ng/g ww). The second dominant compound was PFDA in *P. platessa* (14%), *S. solea* (15%), *A. laterna* (12%) and PFUnDA in *M. merlangus* (20%) and *C. harengus* (14%). For *M. surmuletus*, PFOA, PFDA PFUnDA had the same relative contribution of 13%. Significant lower Σ PFAS concentrations in muscle tissues were found in *G. vulgaris* compared to *A. laterna* ($p < 0.001$), *M. surmuletus* ($p = 0.014$), *P. platessa*

($p < 0.001$), *S. solea* ($p = 0.002$) and in *M. merlangus* compared to *A. laterna* ($p = 0.007$) and *P. platessa* ($p < 0.001$). The mean Σ PFAS concentration was 4.95 ng/g ww for *P. platessa* and further decreased in the following order: *S. solea* (4.59 ng/g ww) > *A. laterna* (4.48 ng/g ww) > *M. surmuletus* (3.35 ng/g ww) > *C. harengus* (3.31 ng/g ww) > *M. merlangus* (2.32 ng/g ww) > *G. vulgaris* (2.17 ng/g ww). Significant differences among fish species per individual PFAS compound in liver and muscle tissue are summarized in Tables S11-S12. No significant differences were found between *L. holsatus* and *C. crangon* with mean Σ PFAS concentrations of 7.60 ng/g ww and 4.73 ng/g ww, respectively.

3.3. Stable isotope analysis: trophic transfer of PFAS and isotopic niche overlap

An overview of the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and the corresponding relative trophic levels of all species per location is shown in Table S13-S15. Mean relative trophic levels range from 2.7 for *L. holsatus* up to 3.6 for *M. merlangus* (Fig. 4). *M. merlangus* has significantly higher TLs compared to all other species ($p < 0.001$) except for *G. vulgaris*. Significantly higher TLs were also found for *G. vulgaris* compared to

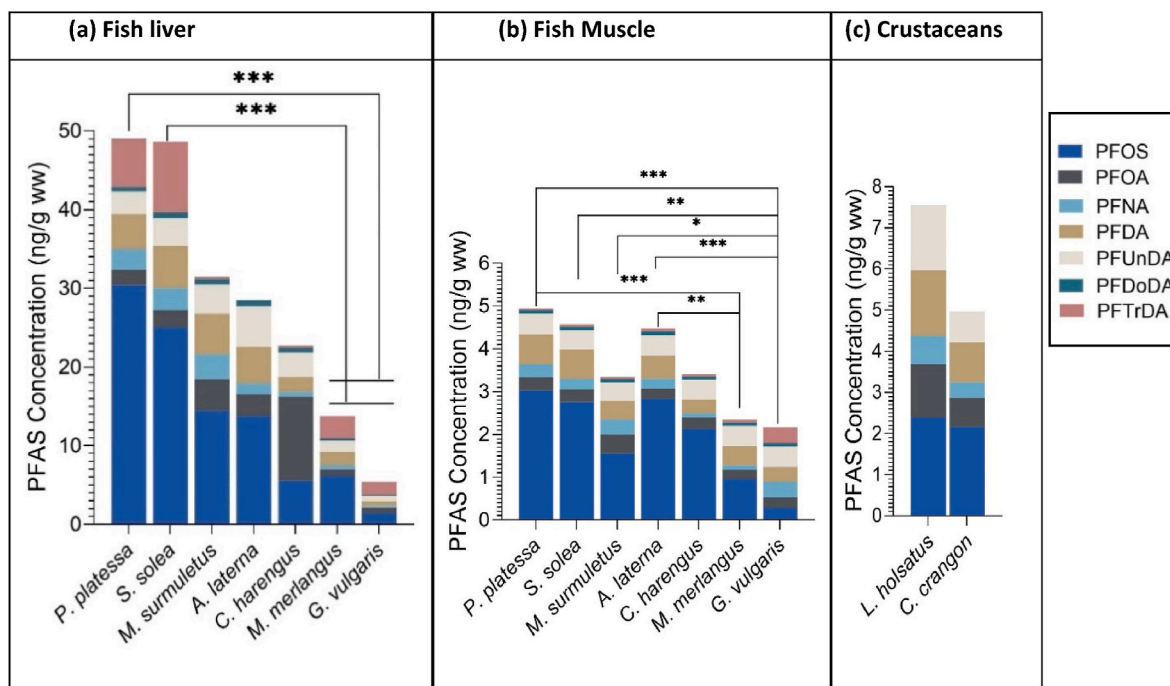


Fig. 3. PFAS profiles and concentrations (ng/g ww) in liver (a) and muscle (b) tissue of seven fish species, *P. platessa* ($N = 29$), *S. solea* ($N = 23$), *M. surmuletus* ($N = 9$), *M. merlangus* ($N = 26$), *A. laterna* ($N = 9$), *C. harengus* ($N = 10$), *G. vulgaris* ($N = 11$) and two invertebrate species, *L. holsatus* ($N = 29$) and *C. crangon* ($N = 20$). Significant differences (sign.) are indicated by asterisks, with **: $p < 0.05$, ***: $p < 0.01$, ****: $p < 0.001$.

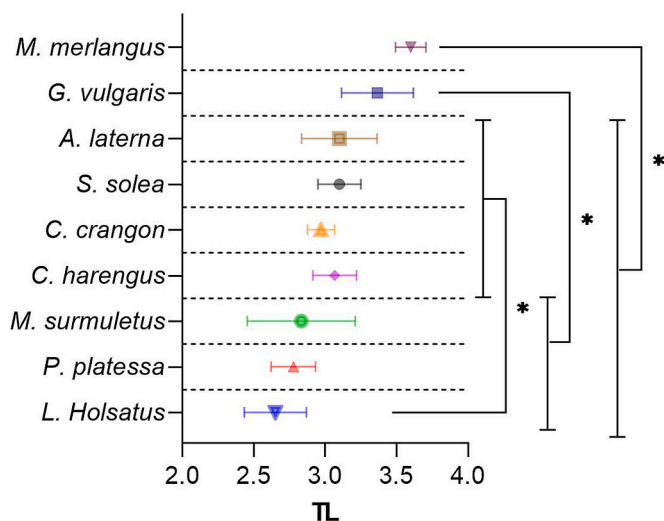


Fig. 4. Mean Relative Trophic levels with standard deviations (SD) of seven fish species, *P. platessa* ($N = 9$), *S. solea* ($N = 9$), *M. surmuletus* ($N = 3$), *M. merlangus* ($N = 8$), *A. laterna* ($N = 3$), *C. harengus* ($N = 3$), *G. vulgaris* ($N = 3$) and two crustacean species, *L. holsatus* ($N = 10$) and *C. crangon* ($N = 7$). Significant differences are indicated by **.

M. surmuletus ($p = 0.022$), *P. platessa* ($p < 0.001$) and *L. holsatus* ($p < 0.001$). For *L. holsatus*, TLs were significantly lower compared to *S. solea* ($p < 0.001$), *C. harengus* ($p = 0.029$), *A. laterna* ($p = 0.014$) and *C. crangon* ($p = 0.022$). In addition, SIBER analysis were done, and isotopic niche overlaps were found among *M. merlangus*, *P. platessa*, *S. solea*, *C. crangon* and *L. holsatus* (Fig. 5). The corrected standard ellipse area (SEA_c), which indicates the size of the isotopic niche, was the largest for *L. holsatus* (1.09‰^2), followed by *S. solea* (1.07‰^2) > *P. platessa* (0.58‰^2) > *C. crangon* (0.48‰^2) > *M. merlangus* (0.43‰^2).

The trophic transfer of PFAS through biota was studied by looking at the relationship between the TLs from all species and the accumulated

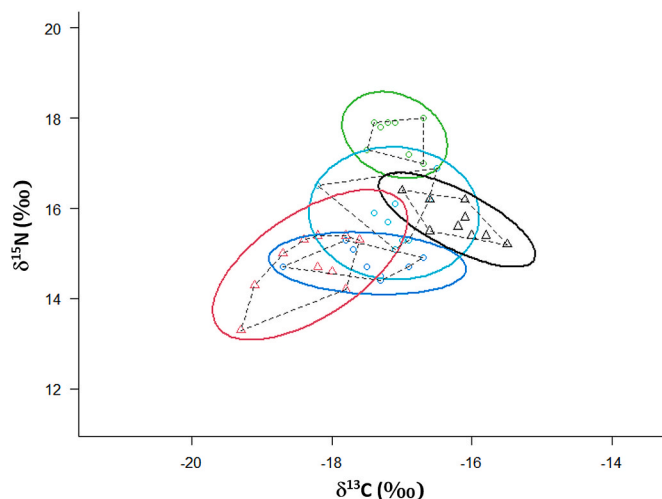


Fig. 5. Isotopic niche overlap among *M. merlangus* (green ellipse, $N = 8$), *P. platessa* (dark blue ellipse, $N = 8$), *S. solea* (light blue ellipse, $N = 9$), *C. crangon* (black ellipse, $N = 9$) and *L. holsatus* (red ellipse, $N = 10$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

PFAS concentrations. This was done for both muscle and liver tissue of the fish species and for each location separately to exclude the effect of differences in exposure concentrations among the locations. For liver tissue, no significant correlations were found. In muscle tissue, TLs were significantly negatively correlated to PFOA at location 4 ($r_{\text{Pearson}} = -0.85$, $p = 0.014$), location 5 ($r_{\text{Pearson}} = -0.92$, $p = 0.027$) and location 8 ($r_{\text{Pearson}} = -0.81$, $p = 0.025$) (Fig. 6a). Significantly negative correlations were also found with PFUnDA at location 5 ($r_{\text{Pearson}} = -0.90$, $p = 0.038$) (Fig. 6b). At location 8, significantly positive correlations were found between TL and PFTrDA ($r_{\text{Pearson}} = 0.90$, $p = 0.037$) (Fig. 6c).

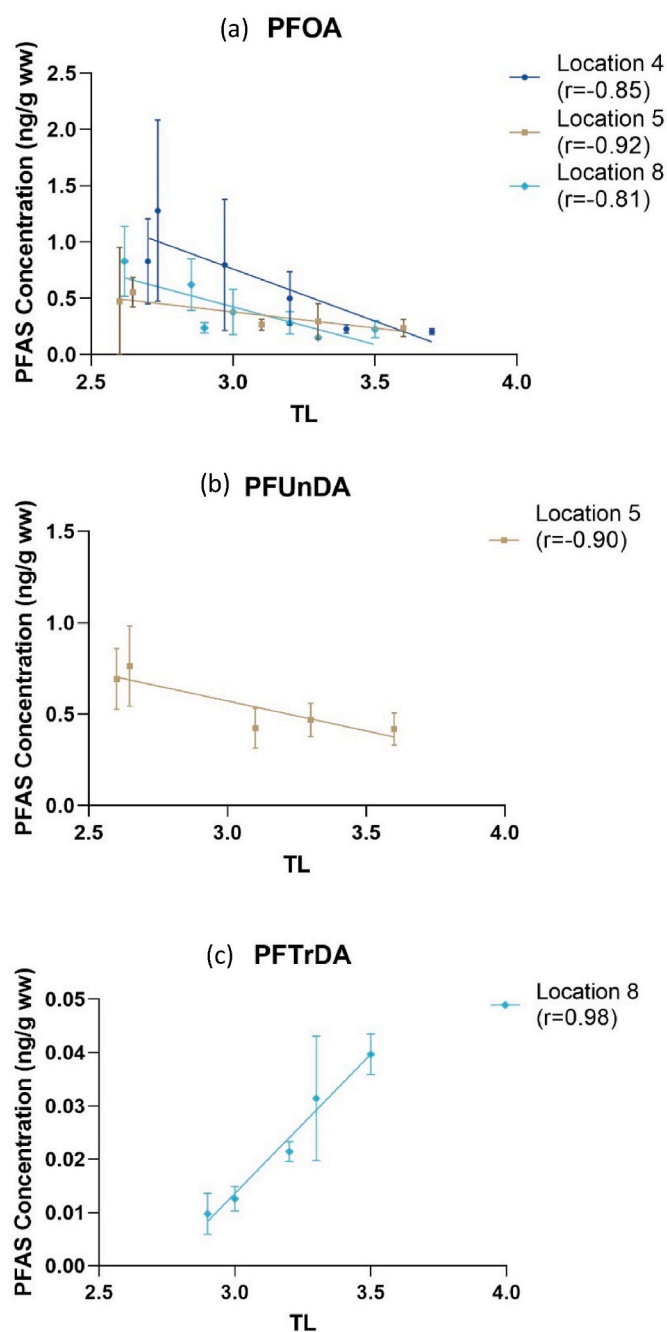


Fig. 6. Mean PFAS concentrations (ng/g ww) with SD in muscle tissue of fish species and whole body tissue of crustaceans correlated with the relative trophic levels for the corresponding species for (a) PFOA, (b) PFUnDA, (c) PFTTrDA.

3.4. Human health risks

The mean and min – max ranges of the maximum consumable amount of contaminated organisms by a person of 70 kg per day are reported per species and for three MRLs (ATSDR_{PFOS}, ATSDR_{PFOA}, EFSA) in Table 1. For all species, the lowest edible amount by a 70 kg person was calculated with the MRL from EFSA, which is the strictest threshold. Based on this threshold, the highest consumption risk was found for *A. laterna* followed by *C. crangon* > *P. platessa* and *L. holtsatus* > *M. surmuletus* > *S. solea* > *C. harengus* > *M. merlangus* > *G. vulgaris*. The MRL from ATSDR for PFOS (2 ng/kg/day) is stricter compared to the MRL for PFOA (3 ng/kg/day) and mean PFOS concentrations in all species are higher compared to the mean PFOA concentrations.

Therefore, the maximum edible amount by a 70 kg person is for all species the lowest based on the MRL for PFOS.

4. Discussion

4.1. Spatial PFAS distribution and profiles

The Scheldt river is likely to be affected by PFAS contaminated industrial wastewater effluents due to the proximity of the perfluorochemical plant 3M in Antwerp. The integrated model of Delhez & Carabin (2001) indicates that the Scheldt river plume extends along the Belgian coast to Zeebrugge and Oostende, with the highest pollution levels in the centre of the plume. Therefore, the Scheldt river is considered as a potential source of PFAS pollution towards the North Sea. In the study of Van De Vijver et al. (2003), a pollution gradient was found for PFOS in shrimp (*C. crangon*) and crab (*C. maenas*) sampled along the Western Scheldt towards the North Sea with slightly lower concentrations in open waters. A similar pattern was found in the study of Hoff et al. (2003), in which PFOS concentrations were assessed in two fish species, *P. platessa* and *Tricopterus luscus*, from the Scheldt river and Belgian North sea. Therefore, higher concentrations of PFAS were expected at sampling sites located in the mouth of the estuary (locations 1 and 2) and along the Belgian coastline (locations 3–7) compared to open water (locations 8–10). Overall, such pattern was not identified in the present study. However, the pollution gradient in the study of Hoff et al. (2003) was explained by the effect of tissue dilution, since the decreasing PFOS concentrations towards the North Sea were linked to the increasing fork length of the studied fish specimens. In the present study, no significant differences were found for the total fish lengths of *S. solea*, *P. platessa* and *M. merlangus* between sampling sites and the species were considered to have the same size order at each sampling site (Fig. S1). Therefore, the effect of fish size and thus tissue dilution cannot be used as a potential explanation for the spatial distribution pattern in the present study.

The Scheldt river plume might be diluted in the estuarine part due to ocean currents and tidal waves, which can explain the absence of a distinct pollution gradient. A geographical study on PFAS accumulation in eel (*Anguilla anguilla*) in the Netherlands showed remarkably lower ΣPFAS levels in eel collected in the mouth of highly polluted rivers (6.2–12.9 ng/g ww) compared to inland waters such as the Ghent-Terneuzen canal (133 ng/g ww) (Zafeiraki et al., 2019). In a recent study of Teunen et al. (2021), accumulated PFOS concentrations further upstream in the Scheldt river ranged from 19.8 to 28.8 ng/g ww in muscle tissue of the European eel (*A. anguilla*) and 25.9 ng/g ww in perch (*Perca fluviatilis*) which are remarkably higher compared to the PFOS levels in the current study. Differences in bioavailability of PFAS among sites might also explain the lack of gradient. Physicochemical characteristics of water and sediment, e.g. salinity and TOC, might result in a stronger sorption of PFAS to sediments, and thus a lower bioavailability for fish (Munoz et al., 2017). However, in the present study, such characteristics were not investigated.

PFOS concentrations in the present study are lower in both fish (*P. platessa*) and crustaceans (*C. crangon* and crab sp.) compared to the studies from 2003 (Table 2). The most probable explanation is the phase-out of PFOS and PFOA in 2000 by the perfluorochemical manufacturer 3M (Carlioni, 2009). Several temporal studies report a decrease in PFOS concentrations in biota over time (Houde et al., 2011; Ahrens et al., 2009a). For example, PFOS levels in European eel (*A. anguilla*) from the Netherlands have shown a decrease by a factor 2 to 4 from the 1990s to 2008 (Kwadijk et al., 2010). A significant decrease of PFOS was also found in shellfish from French coasts after the mid-90s (Munsky et al., 2019). However, despite the presumably decreasing PFOS concentrations in the Western Scheldt and Belgian North Sea, PFOS remains the predominant compound. The steady decline of PFOS might be explained by the additional exposure to precursor compounds such as perfluoroalkane sulfonamidoacetic acids (FASAAAs), sulfonamides

Table 1

Human health risks determined as the maximum allowable quantity of fish and crustaceans that can be consumed per day without posing health risks (Q in g/day). The risks are calculated based on three minimum risk levels (MRLs) for mean PFAS concentrations and concentration ranges (min – max) of all species.

MRL:	ATSDR		EFSA
	PFOS 2 ng/kg/day	PFOA 3 ng/kg/day	PFOA + PFOS + PFNA + PFHxS 0.63 ng/kg/day
Q for fish species (g/day)			
<i>A. laterna</i>	55 (30–90)	982 (583–1488)	15 (8–23)
<i>C. harengus</i>	163 (26–647)	882 (589–1730)	33 (8–94)
<i>G. vulgaris</i>	678 (180–1637)	952 (535–1345)	106 (31–243)
<i>M. merlangus</i>	207 (47–468)	1055 (590–2105)	43 (14–76)
<i>M. surmuletus</i>	113 (52–211)	559 (307–1039)	23 (11–41)
<i>P. platessa</i>	75 (12–227)	1028 (166–3522)	18 (3–49)
<i>S. solea</i>	122 (6–464)	857 (315–1562)	26 (2–54)
Q for crustaceans (g/day)			
<i>L. holsatus</i>	110 (25–464)	334 (48–1640)	18 (4–63)
<i>C. crangon</i>	102 (31–479)	434 (133–1591)	17 (7–44)

(FASAs), and sulfonamidoethanols (FASEs). The biotransformation of precursors to PFOS is considered to have an important contribution to the predominance of PFOS in marine wildlife (Ullah et al., 2014; Gebbink et al., 2016). Since no precursor compounds were targeted in the present study, further identification of the potential sources of the current PFOS contamination is limited.

Elevated PFTrDA concentrations were found in liver tissue of *P. platessa*, *S. solea* and *M. merlangus* at location 2 and 4. Overall, PFTrDA concentrations ranged from <LOQ – 116 ng/g (ww), with the highest concentration measured in *P. platessa* sampled at location 4 (near the harbor of Zeebrugge). In Sweden, PFTrDA was detected in liver tissue of several marine fish species such as Atlantic cod with concentrations ranging from 0.18 to 4.4 ng/g (ww) (Faxneld et al., 2014). In the Pearl River Delta region, South China, PFTrDA was detected in muscle and liver tissue of nine freshwater fish species with concentrations ranging from <0.03 to 1.1 ng/g and 0.27–22 ng/g, respectively. (Pan et al., 2014). In the study of Munschy et al. (2019), a profile shift from PFOS to long-chain perfluoroalkyl carboxylic acids (PFCA) was found in shellfish from French coasts, with PFTrDA (0.018–1.36 ng/g ww) showing one of the highest concentrations of all PFCA. However, in comparison to the mentioned studies, PFTrDA concentrations in the present study are higher and site specific. These observations might indicate a specific source of PFTrDA in the Belgian estuarine and coastal region. In general, a profile shift from Σ C7–C12 towards Σ C4–C6 PFCA was expected because of the replacement of long chain PFAS with shorter chain homologues which are assumed to have a lower bioaccumulation potential (Buck et al., 2011). However, in China the estimated discharges of long chain PFCA (Σ C7–C12) from rivers towards estuaries and seas did not

decrease over time (2000–2019), which indicates a continued release of these compounds (Muir and Miaz, 2021). In Europe, data on the estimated discharge of Σ C7–C12 PFCA via rivers is lacking from recent years but median Σ C7–C12 PFCA concentrations in coastal and oceanic waters showed an increase from 2010 to 2019 compared to 2000–2009 (Muir and Miaz, 2021). A potential source of long chain PFCA in the Belgian North Sea could be the shipping traffic around the port of Zeebrugge and Antwerp. However, little is known about the effect of coastal ship traffic on the release of PFAS, and more specifically PFTrDA, into marine environments. Another direct source can be the locality of multiple paper and textile industries along the Gent – Terneuzen canal that ends up in Western Scheldt. Bioaccumulated PFAS concentrations in perch from the Gent – Terneuzen canal ranged from 50 to 60 ng/g ww and the PFAS profiles were mainly dominated by long chain PFAS (Teunen et al., 2021). Additionally, another potential indirect source can be long-range atmospheric transport of PFAS compounds. PFAS fingerprints in fish from Norwegian freshwater environments that were known to have long-range atmospheric transport as major input source, were characterized with high percentages of long chained PFCA (Langberg et al., 2022). However, the number of samples analysed in the present study is limited, which hinders the identification of potential contamination sources. Further research should be done to identify the major production processes, uses and potential releases of PFTrDA and other long chain PFCA to understand the increased patterns along the Belgian coast.

4.2. Species – specific PFAS profiles

Overall, similar contamination profiles were found among fish and invertebrate species. PFOS was predominant in all matrices and species except for *C. harengus*, where PFOA was predominant in liver tissue. This observation is likely to be linked to differences in the feeding ecology of *C. harengus*, compared to the other species. *C. harengus* forages in the pelagic environment and typically feeds on planktonic crustaceans, molluscs and fish larvae (Whitehead, 1985). All other fish species are demersal that typically feed on zoobenthos, such as molluscs and polychaetes (Muus and Dahlstrom, 1978). Based on a global survey, PFOA appears to be the predominant compound in oceanic waters (Yamashita et al., 2005). PFOS shows an increased adsorption onto sediments with increasing salinity and is found to be predominant in marine sediments (You et al., 2010; Chen et al., 2012). Therefore, *C. harengus* is, due to its pelagic feeding behaviour, more likely to experience a greater exposure to PFOA compared to sediment-associated species. The higher tendency of PFOA to accumulate more in pelagic species has also been reported for the Baltic herring (*C. harengus*) and smelt (*Hyperoplus lanceolatus*) in the Finnish Baltic Sea with concentrations in muscle tissue ranging from <LOQ–1.20 ng/g and <LOQ–0.52 ng/g ww, respectively (Kumar et al., 2022).

Table 2

Comparison of mean PFOS concentrations and/or ranges (ng/g ww) in 2019 vs 2003 in fish muscle of *P. platessa* and in two crustaceans *C. crangon* and crab sp. (2019: *L. holsatus*, 2003: *C. maenas*).

	2019 (present study)	2003
Estuary:		
<i>P. platessa</i>	2.52 (0.62–4.47)	<10–87 (Hoff et al., 2003)
Crab sp.	2.38 (0.53–5.61)	–
<i>C. crangon</i>	2.38 (0.93–3.86)	301 ± 62 (Van De Vijver et al., 2003)
Coastal zone:		
<i>P. platessa</i>	5.47 (1.04–11.9)	<10–39 (Hoff et al., 2003)
Crab sp.	1.79 (0.35–3.82)	120 ± 15 (Van De Vijver et al., 2003)
<i>C. crangon</i>	2.49 (1.34–4.55)	120 ± 10 (Van De Vijver et al., 2003)
Open waters		
<i>p. platessa</i>	2.03 (1.10–3.67)	<10–17 (Hoff et al., 2003)
Crab sp.	2.93 (0.91–4.77)	93 ± 36 (Van De Vijver et al., 2003)
<i>C. crangon</i>	1.63 (0.29–2.83)	40 ± 13 (Van De Vijver et al., 2003)

After PFOS, PFTrDA is the second largest contributor in liver tissue except for *A. laterna*, *M. surmuletus* and *C. harengus*. A possible explanation is that these species could not be collected at location 4 and 2 where significant higher PFTrDA concentrations were found. The highest mean PFAS concentrations among all biota were found in *P. platessa* and the lowest concentrations were measured in *G. vulgaris*. Overall, differences in PFAS contamination levels among species could be caused by multiple factors that influence PFAS accumulation patterns such as diet, migration routes and metabolic differences. Species might also be affected by other exposure routes due to differences in spawning and nursery behaviour. Estuaries form important nursery grounds for demersal fish as they provide food and refuge for juveniles (van Beek et al., 1989; Mathieson et al., 2000). Therefore, juveniles from demersal fish species might be exposed to higher concentrations of toxicants in their early life-stages compared to species with nursery grounds in deeper waters. Furthermore, PFAS concentrations may vary among species due to differences in metabolic and elimination processes (Galatius et al., 2013). However, in-depth knowledge on species-specific characteristics of PFAS accumulation and the underlying metabolic processes of the species sampled in this study, is lacking.

4.3. Trophic transfer of PFAS

Although the trophic range studied in this study is too small to investigate biomagnification patterns, the positive correlation between TL and PFTrDA in fish muscle and crustaceans at location 8 implies the potential biomagnification of this compound throughout the North Sea food web. The negative correlations between TL and PFOA and PFUnDA at locations 4, 5 and 8, suggests the potential biodilution of these compounds. However, we must stress that at most locations and for most compounds, no significant correlations were found which suggests the absence of biomagnification and biodilution patterns. Even though only limited studies on PFAS bioaccumulation in the North Sea are available, biomagnification was expected since high mean Σ PFAS concentrations were reported for three North Sea top predators which were sampled between 1999 and 2002: 757.8 ng Σ PFAS/g ww in the harbor seal, 439.9 ng Σ PFAS/g ww in the white-beaked Dolphin and 355.5 ng Σ PFAS/g ww in the harbor porpoise (Galatius et al., 2013). However, in contrast to the study of Galatius et al. (2013) which considers air breathing aquatic mammals, the present study focusses on gill breathing organisms. The trophic transfer of PFOS and several other PFAS in gill breathing organisms appears to be less extensively because of the efficient respiratory elimination of PFAS via gills, facilitated by the high water throughput (De Silva et al., 2020; Vidal et al., 2019). The elimination of these compounds via lung to air is rather limited, resulting in a more extensive biomagnification of PFAS in studies including air-breathing mammals (Bossi et al., 2005; Houde et al., 2006). Therefore, the present study implies that PFAS bioconcentration from the surrounding abiotic environment is most likely dominating over biomagnification in the studied species. The absence of significant biomagnification patterns in aquatic food chains has been reported before (Mazzoni et al., 2020). On the other hand, the lack of significant correlations in the present study might also be explained by the results of the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope analysis. The $\delta^{15}\text{N}$ range and $\delta^{13}\text{C}$ range provide an estimate of the trophic length and the diversity of basal carbon resources, which are commonly used to define the isotopic niche of species and communities (Perkins et al., 2014). Although isotopic niche and ecological niche are not the same, they are considered to be closely correlated (Jackson et al., 2011). Consequently, based on the isotopic niche overlap among *M. merlangus*, *P. platessa*, *S. solea*, *L. holsatus* and *C. crangon*, we can assume that these species have a similar ecological niche. This confirms that the trophic range studied in this research is too restricted to draw solid conclusions on the trophic transfer of PFAS in the North Sea. For *M. merlangus* the highest TL was calculated and among the lowest PFAS concentrations were detected in both liver and muscle tissue. However, SIBER analysis show that *M. merlangus* has the smallest isotopic niche

area, mainly due to its narrow $\delta^{13}\text{C}$ range in comparison with the other species. This implies that the diversity in basal resources might have an influence on the bioaccumulation levels of PFAS.

Overall, inconsistent findings in literature indicate that biomagnification or biodilution patterns of PFAS are complex and likely to be affected by multiple factors such as biotransformation, ecological characteristics, and the physicochemical properties of PFAS, sediment and water. Considering the limitations of the present study, we highlight three major research recommendations for future studies on the trophic transfer of PFAS. First, we suggest to extend the trophic sampling range with an interval of at least two trophic levels (Borgå et al., 2012; Kidd et al., 2019). Hereby, it is recommended to consider both gill and air breathing organisms in the study design to gain complete insights in the foodweb. Secondly, we recommend identifying precursor compounds to assess the confounding effect of biotransformation. Therefore, the Total oxidizable Precursor assay (TOP) is suggested as useful tool to quantify the concentration of PFAS precursors (Zhang et al., 2019; Amin et al., 2021). Lastly, the impact of abiotic factors (i.e., pH, conductivity, dissolved organic carbon (DOC) and oxygen level) and the accumulated concentrations in environmental compartments (water and sediment) must be considered.

4.4. Human health and ecological risk assessment

The lowest maximum edible amount of fish and crustaceans that can be consumed without potential risks for human health was for all species based on the EFSA guidelines. The EFSA threshold has been newly set in 2020 and assesses the combined exposure to PFOS, PFOA, PFNA and PFHxS while the ATSDR thresholds assess the individual risk for PFOS and PFOA. The potential human health risk exposed through the consumption of contaminated fish, depends on the amount of fish that is daily consumed and on the human body weight. In the present study a human body weight of 70 kg was considered in all calculations and conclusions. Very limited information is available on the species-specific fish consumption in Belgium including details on the origin of the consumed fish and crustaceans. Therefore, the maximum tolerable amount of contaminated fish that can be consumed per day without posing health risks (Q-values) were compared to the mean per capita fish consumption in Belgium (65 g/day) (EUMOFA, 2017). However, not only the consumption of wild fish captured in the Belgian North Sea is included in this value, but also the consumption of imported and farmed fish. Additionally, regional differences in fish consumption can also lead to an over- or underestimation of the potential risks. Therefore, we would like to strengthen that the mean per capita fish consumption of 65 g/day is only used as a guideline and no strict conclusions can be drawn based on this value. Based on the EFSA guidelines the mean Q-values were, for all fish species, lower than the reported mean consumption, except for *G. vulgaris* (106 ng/kg/day). This means that the maximum tolerable fish consumption based on the EFSA MRL is exceeded for almost all species. Based on the ATSDR MRLs for PFOA, mean Q-values were up to 16 times higher compared to the mean per capita fish consumption in Belgium, indicating a limited risk for PFOA exposure. For the PFOS MRL, mean Q values were slightly higher compared to the mean consumption of 65 g/day for all species, except for *A. laterna* (55 g/day). When considering the minimum range of the Q-values for PFOS, the maximum tolerable daily consumption is exceeded for all fish species except for *G. vulgaris*. However, most individuals analysed in this study were juveniles since the average length per species per location was lower compared to the estimated first length at maturity (Table S1). As only large adult fish are commercially available, the effects of both biomagnification and tissue dilution on PFOS concentrations in adult fish should be studied. Therefore, in-depth analysis and monitoring of PFAS contamination in fish available at commercial markets is highly recommended and necessary to confirm our findings. The consumption of fish is considered as one of the most important exposure routes of PFAS to humans and potential risks to local

communities have been reported worldwide (Schuetze et al., 2010; Zhao et al., 2011; Pan et al., 2014; Christensen et al., 2017; Groffen et al., 2018). Once in an adult life stage, most species from this study tend to migrate further offshore and might end up in other international waters, especially commercially important species such as *P. platessa*, *S. solea*, *M. merlangus* and *C. harengus* (Blaxter and Parrish, 1965; De Veen, 1978; Henderson and Holmes., 1989; Koutsikopoulos et al., 1995). Hence, the accumulated PFAS concentrations and the subsequent potential risks reported in this study are crucial for international fisheries in all neighbouring countries of the North Sea.

Besides the potential risk for human health, the ecological risks of PFAS on marine ecosystems should also be considered. In 2013, a Quality Standard (QS) for PFOS in biota was set by the European Union under the Water Framework Directive (European Commission, 2013). To protect aquatic food webs and top predators, including humans, a human health-based threshold value of 9.1 µg PFOS/kg ww ($QS_{\text{biota, hh}}$) was derived. In the present study, the $QS_{\text{biota, hh}}$ was only exceeded in three single fish samples, i.e., for *P. platessa* at location 4 and 5 and for *S. solea* at location 2. In crustaceans, the $QS_{\text{biota, hh}}$ was not exceeded for any of the samples. These findings indicate no potential health risks for aquatic ecosystems, specifically for the secondary poisoning of humans, which is contradicting with the previously calculated human health risks. This can be explained by the current $QS_{\text{biota, hh}}$ that is still based on the EFSA MRL from 2008 (15 ng/kg/day) which is almost 240 times higher compared to the updated MRL from 2020 (0.063 ng/kg/day) (EFSA, 2008; EFSA, 2020).

Additionally, a specific benchmark of 33 µg PFOS/kg was derived for the protection of wildlife against secondary poisoning ($QS_{\text{biota, secpois}}$) (European Union, 2014). Since aquatic top predators are likely to consume whole specimens, it is more appropriate to also include the accumulation in other tissues and organs, instead of only muscle tissue. Therefore, the sum of the accumulated PFOS concentrations in muscle and liver tissue was used for comparison to the $QS_{\text{biota, secpois}}$. This resulted in an exceedance for 43% of the samples for *P. platessa* and 28% of the samples for *S. solea*. For all other species no exceedance of the $QS_{\text{biota, secpois}}$ was found. These findings indicate that the consumption of juvenile *P. platessa* and *S. solea* can pose potential health risks to predatory species such as harbor porpoises (*Phocoena phocoena*) and harbor seals (*Phoca vitulina*). *P. platessa* and *S. solea* are both ecological and economical important species, whose populations are affected by multiple threats such as overfishing, habitat degradation and climate change (Rochette et al., 2010; Teal et al., 2008). Therefore, not only the risks for secondary poisoning need further clarification but also the potential effects of PFAS on the population dynamics of these species must be studied.

In general, assessing the ecological risks of PFAS is a multifaceted challenge requiring data and expertise from both laboratory toxicity tests versus field and monitoring studies. The toxicity of PFAS on marine fishes and invertebrates have been much less well studied compared to freshwater species, making it even more difficult to assess the ecotoxicological effects of PFAS in marine ecosystems (Ankley et al., 2021). Although the widespread usage and concern about PFAS, risk assessment programs are still mainly focussed on a limited number of compounds. Therefore, the combination of field monitoring and toxicity testing of a broad range of PFAS is crucial to strengthen mitigation measures.

5. Conclusions

The present study shows that PFOS is still the predominant PFAS compound in marine biota from the Belgian North Sea, despite the phase-out in 2000. The extend of the Scheldt river as potential source of PFAS towards the North Sea remains unclear and must be further investigated. Remarkably high PFTrDA concentrations were found in liver tissue of *P. platessa*, *S. solea* and *M. merlangus* in the estuarine and coastal region, indicating a specific point-source of this compound.

Among all investigated species, the highest mean ΣPFAS concentrations were found for *P. platessa* in liver tissue (52.5 ng/g ww) and the lowest mean ΣPFAS concentrations were found for *G. vulgaris* in muscle tissue (2.17 ng/g ww). This study shows that SIBER analysis are a good method to visualize stable isotope data and to link this information on ecological niches with accumulated PFAS concentrations. Because of the isotopic niche overlap and the restricted TL range of the studied species, no solid conclusions on the trophic transfer of PFAS could be made. However, since almost no information is available on the PFAS accumulation in fish and invertebrate species in the North Sea, this study provides important insights on the current state of PFAS contamination and distribution. Regarding the human health risks discussed in this study, it can be concluded that the monitoring of PFAS in commercially available fish is strongly advised. Finally, potential ecological risks for secondary poisoning were found for *P. platessa* and *S. solea* based on the benchmark of 33 ng/g ww ($QS_{\text{biota, secpois}}$), while no risks were identified based on the human health based $QS_{\text{biota, hh}}$ (9.1 ng/g ww). This indicates the importance to interpret and discuss both standards in future studies.

Author statement

Cara Byens: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **Lies Teunen:** Conceptualization, Supervision, Writing – review & editing. **Thimo Groffen:** Formal analysis, Supervision, Writing – review & editing, Funding acquisition. **Robin Lasters:** Supervision, Writing – review & editing. **Lieven Bervoets:** Conceptualization, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2022.119907>.

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