



## Exposure and magnification of PFAS in a temperate estuarine food web, including top predators

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### ABSTRACT

The Western Scheldt is a polluted temperate estuary in the Netherlands and a hotspot for per- and polyfluoroalkyl substances (PFAS), leading to environmental concerns. To assess PFAS contamination, several biota types were sampled in 2023. Reference data was available from 2006 to 2008 and reference material was collected from the Wadden Sea. A spatial gradient in stable isotopes and PFAS concentrations was observed in the estuary. PFAS concentrations were positively correlated with trophic level. For most biota, concentrations were significantly lower than 2006–2008, but significantly higher than the Wadden Sea. Whole-body burdens for marine mammals were extrapolated from liver concentrations to allow further comparison between trophic levels. Biota Magnification Factors for perfluorooctane sulfonic acid (PFOS) ranged between 0.1 and 34, and the Trophic Magnification Factor was 5.7. PFOS concentrations exceeded the European threshold level in flounder and, partly, other fish. Results point at risk of sublethal effects in estuarine biota at several trophic levels.

### 1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have been produced since the 1950s and are found ubiquitous in the environment, stemming from point source emissions as well as from transport via air, run-off from land, rivers, and ocean currents (Kurwadkar et al., 2022; Panieri et al., 2022). PFAS are proteonophilic given their high affinity to bind to proteins in biota (Teunen et al., 2022), and several PFAS, especially perfluorooctane sulfonic acid (PFOS), have been shown to accumulate in biota across all environmental compartments (Panieri et al., 2022). This raises significant concerns, as PFAS exposure has been linked to various adverse health effects in both wildlife and humans, such as negative effects on the immune system, endocrine system, growth and reproduction (Molina et al., 2006; Wang et al., 2011; Liu and Gin, 2018; Panieri et al., 2022).

Extensive knowledge gaps still exist concerning the behavior, fate and effects of PFAS in the environment, especially for complex ecological systems, such as estuaries, that are highly influenced by human activities. Estuaries are both dynamic and productive, and form the interface between rivers and oceans. By definition, they are strongly connected to the terrestrial system. Estuaries are therefore particularly exposed to manmade contaminants as they integrate contaminants from multiple sources. Furthermore, the variability in physico-chemical characteristics, such as salinity, temperature and nutrient concentrations, strongly influences both the presence of species and trophic interactions. While some information on PFAS magnification in estuarine food webs is available (Munoz et al., 2017, 2022; Li et al., 2021), these studies typically concern lower food webs up to the trophic level of fish. By excluding top predators, such as marine birds and mammals, relevant information on the food webs characteristics of PFAS is lacking.

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The Dutch Western Scheldt estuary is highly affected by local shipping and the industrialized and urbanized hinterland, with direct connections to the urban and port areas of Antwerp and Ghent (Belgium), and Vlissingen (the Netherlands). It is considered one of the most contaminated estuaries in Europe (Gaulier et al., 2021). More specifically, the Western Scheldt estuary has been shown to contain elevated concentrations of PFAS in both surface water and biota compared to other areas in the Netherlands (Jonker, 2024). Several PFAS point sources, such as PFAS production and processing industry, and waste water treatment and waste incinerator plants, can be found along the estuary and directly upstream (Hoff et al., 2004; D'Hollander et al., 2014; Jonker, 2024), with diffuse sources from the hinterland of the Scheldt river also contributing to the PFAS load (Teunen et al., 2021). Based on new data showing high PFAS concentrations in shrimp and fish, the Dutch National Institute for Public Health and Environment issued a warning to civilians to minimize the consumption of fishery products from this estuary (van den Heuvel-Greve et al., 2022; Zwartsen and Boon, 2022). This led to further concerns about the environmental health of the estuary. Although some data on PFAS exposure in biota of the estuarine Western Scheldt food web exist, they are predominantly fragmented and/or over 15 years old (Hoff et al., 2003; Van de Vijver et al., 2003; de Vos et al., 2008; Byns et al., 2022; Jonker, 2024).

The objective of this study was to assess the current PFAS exposure in the estuarine food web of the contaminated Western Scheldt and to place these findings into perspective, both temporally (using historically available data from 2006 to 2008) and spatially (with the Wadden Sea as reference location, containing similar species but without significant point sources of pollution). In order to do this, the Western Scheldt food web was sampled across trophic levels, from invertebrates and fish to the level of local top predators, aiming to provide a comprehensive overview of PFAS distribution across the food web. The results were used to assess the exposure, biomagnification and trophic magnification of PFAS, as well as the potential implications of PFAS exposure for the environmental health of the estuary. To the best of our knowledge, this is the first study to assess the trophic transfer of PFAS in a temperate estuarine food web, up to the level of top predators.

## 2. Material & methods

### 2.1. Western Scheldt estuary

The Western Scheldt estuary receives freshwater from the river Scheldt, that runs from northern France and Belgium (upstream) westwards through the Netherlands, and ultimately drains into the North Sea. It has a salinity gradient ranging from 5 to 18 ‰ in the east (mesohaline) to 18–30 ‰ in the west (polyhaline), and a tidal amplitude of 4–5 m (Meire et al., 2005).

### 2.2. Sampling

To assess the exposure, bioaccumulation, and biomagnification of PFAS in the Western Scheldt, three top predators in this estuary were examined: the common tern (*Sterna hirundo*), the harbor porpoise (*Phocoena phocoena*), and the harbor seal (*Phoca vitulina*). The Wadden Sea served as a reference site based on the lack of point sources of PFAS of similar magnitude and its considerable distance from the Western Scheldt (200 km), thereby minimizing the likelihood of recent foraging overlap of the studied top predators between these two locations. Common tern samples were collected from a breeding colony in each region, while samples from harbor porpoise and harbor seal were collected from individuals that had washed ashore (Fig. 1). Invertebrate and fish samples were collected at four locations in the Western Scheldt (Ghent-Terneuzen Channel, Middelplaat, Ossensisse sandflats, Valkenisse sandflats) and two locations in the Dutch Wadden Sea (near the island of Griend, and the western Wadden Sea) (Fig. 1).

### 2.3. Common tern

Common tern eggs were collected from breeding colonies in the Western Scheldt (Terneuzen harbor) and in the Wadden Sea (isle of Griend) at the beginning of the breeding season in May 2023 (Fig. 1, Supporting information Table S1). Single eggs were randomly taken from ten different nests, wrapped in clean paper towel, placed in an egg carton for safe keeping and frozen at  $-20^{\circ}\text{C}$ .

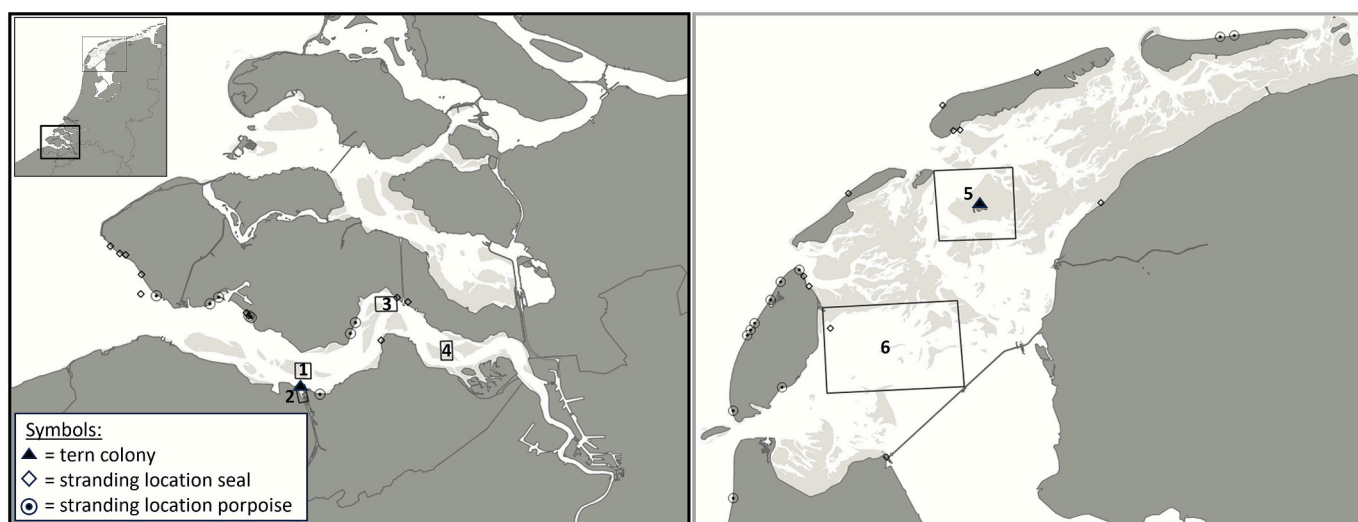


Fig. 1. Sampling locations of invertebrates and fish in the Western Scheldt (left) and Wadden Sea (right) in 2023. Sample collections at locations 1, 2 and 5 took place in the spring, whereas the collections at locations 3, 4 and 6 took place in the fall. Western Scheldt locations: 1 = Middelplaat, 2 = harbor Terneuzen - entrance to the Ghent-Terneuzen Channel, 3 = Ossensisse sandflats, 4 = Valkenisse sandflats. Wadden Sea: 5 = island of Griend and surroundings, 6 = Wadden Sea west. Bird eggs were collected from the colonies of Terneuzen (Western Scheldt) and Griend (Wadden Sea). Stranded porpoises and seals were collected from the beaches in both areas (see symbols).

## 2.4. Marine mammals

Liver (for PFAS, moisture and lipid analysis) and muscle (for isotope analysis) samples were collected during post-mortem investigations of juvenile harbor seals and harbor porpoises that were found stranded in the Western Scheldt ( $n = 7$  and  $n = 10$  respectively) and the Wadden Sea ( $n = 11$  and  $n = 10$  respectively) (see Supporting information Tables S2–S3 for details). In addition, samples were collected from an adult harbor seal that was found stranded in the Western Scheldt. The juveniles were of particular interest for this study as they are more prone to contain the highest PFAS concentrations (Foord et al., 2024). Furthermore, individuals of this age class are assumed to be less mobile and predominantly stay in and near the Western Scheldt for a longer period, making them good models for studying PFAS bioaccumulation locally.

Harbor porpoise samples were selected from the ongoing statutory post-mortem investigations on fresh carcasses by the Faculty of Veterinary Medicine, Utrecht University, following internationally standardized guidelines (Jsseldijk et al., 2019). For harbor seals, on the contrary, there is no statutory post-mortem monitoring. Therefore, stranded corpses were specifically collected between October 2021 and July 2023 and stored frozen ( $-20\text{ }^{\circ}\text{C}$ ) until later simultaneous necropsy.

For both marine mammal species, stranding date, location, sex, total length (cm), and weight (kg) were recorded (Tables S2–S3). Additionally, the nutritional condition code (NCC) was assessed visually, based on the dorsal musculature, presence/absence of visceral fat and quantitative assessment of blubber thickness. Scoring was done on a six-point scale with NCC1 representing very fat and muscular animals and NCC6 severely emaciated animals.

## 2.5. Lower trophic levels

Sampling of the lower trophic levels took place in May–June 2023 for the common tern prey base (Tables 1, S4) and in September–October 2023 for the harbor porpoise and harbor seal prey bases (Tables 2, S5). Most of the invertebrate and all fish samples were collected from a ship, using a 3-meter wide (shrimp) beam trawl with a mesh size of 2 cm. Catches were directly sorted on a stainless-steel table and target species were selected. Fish were anesthetized by transferring them to seawater containing an overdose of the aquatic anesthetic AQUI-S (active ingredient Isoeugenol). Clean lab gloves were used for sorting, and changed between samples and after contact outside the sorting tray. Between samplings, all used materials were extensively rinsed with tap water and dried using clean paper towels. Marine worms and shellfish were collected by hand from the sand flats during low tide.

In the onboard lab, wet weight, length and number of individuals were recorded per sample (Tables S4–S5), and samples were stored in

glass containers at  $-20\text{ }^{\circ}\text{C}$  until further processing. Fish being too large for the glass containers were wrapped in aluminum foil and placed in ziplock bags in the freezer. Lugworms were pooled into three subsamples of which the total wet weight was recorded, and stored in glass containers at  $-20\text{ }^{\circ}\text{C}$ . For fish, body sizes that were representative for the diets of terns, porpoises and seals were collected. Gut depuration processes did not take place in the benthic invertebrate species (clams and worms), as the research's purpose was to assess PFAS concentrations in the diet of (higher) trophic levels (terns, porpoises and seals).

Sampling locations for invertebrates and fish were based on foraging locations of the top predators. Both the Terneuzen harbor, in front of the Ghent-Terneuzen Channel, and Middelpaalt on the Western Scheldt are important feeding areas for common terns of the Terneuzen breeding colony, while the area northwest of the island of Griend serves as a key feeding area for the Wadden Sea breeding colony (Fig. 1). Therefore, these locations were selected to sample invertebrates and fish as a potential prey source for the terns in spring. The Ossensisse and Valkenisse sand flats in the Western Scheldt are regularly used by seals as haul out sites. It is likely that the young seals forage in the vicinity, particularly during or just after the pupping season in late summer, early fall. Therefore, sample collection of potential diet sources of seal diet, and their prey, was conducted at and around these sand flats in the Western Scheldt. Both sampling locations and a few seal prey species (herring and whiting) were considered representative for harbor porpoises. This means that the samples collected as prey for harbor seals were also used for harbor porpoises, with pouting and goby added as additional prey sources. As reference location for seals and porpoises, sampling of shrimp and fish was conducted in the western Wadden Sea in the fall.

## 2.6. Chemical analysis

All samples, except those from top predators, were processed as whole individuals and consisted of multiple individuals (Tables 1–2). Common tern and marine mammal samples were treated per individual. All samples were fully homogenized using a blender prior to further analysis.

To determine the trophic position in the Western Scheldt food web, the stable isotope ratios  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were determined in the invertebrates, fish, common tern (eggs) and marine mammal (muscle) samples. Samples were freeze-dried for 24 h, homogenized with pestle and mortar, and  $\pm 2\text{ g}$  freeze-dried material was used for analysis. For  $\delta^{13}\text{C}$  analysis, samples were acidified with HCl prior to analysis, to remove inorganic carbon. Samples were analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in an Element Analyzer (EA, Firma Thermo Electron, Flash EA 1112 analyzer) coupled to an isotope ratio mass spectrometer (IRMS, Delta V Firma Thermo Electron). The isotope values are expressed in parts per thousand (‰), relative to the Vienna Pee Dee Belemnite (VPDB)

**Table 1**

Number of invertebrate, fish and common tern samples collected per sampling location in the Western Scheldt ( $n = 25$ ) and the Wadden Sea ( $n = 21$ ) in May 2023. Whole organism samples consisted of multiple individuals per sample, whereas top predator samples (tern eggs) consisted of one individual per sample. The targeted lengths of the prey fish (4–8 cm) were based on the preferred diet size of common terns.

Species	Sample type	Number of individuals per sample	Western Scheldt (Terneuzen colony)	Western Scheldt (harbor/entrance Ghent-Terneuzen channel)	Western Scheldt (Middelpaalt)	Wadden Sea (Isle of Griend)	Wadden Sea (Mainly northwest of Griend)
Brown shrimp ( <i>Crangon crangon</i> )	Whole organism	50		1	3		3
Goby ( <i>Pomatoschistus/Gobius</i> sp.)	Whole organism	30 <sup>a</sup>		–	3		3
Pouting ( <i>Trisopterus luscus</i> )	Whole organism	30		1	3		3
Atlantic herring ( <i>Clupea harengus</i> )	Whole organism	30 <sup>a</sup>		1	3		2
Common tern ( <i>Sterna hirundo</i> )	Egg	1	10			10	

<sup>a</sup> Target number of individuals was not always reached for each sample (see Table S9).

**Table 2**

Number of invertebrate, fish, porpoise (liver, muscle) and seal (liver, muscle) samples collected per sampling location in the Western Scheldt ( $n = 66$ ) and the Wadden Sea ( $n = 30$ ), in September–October 2023. Whole organism samples consisted of multiple individuals per sample, whereas top predator samples (porpoise and seal liver/muscle) consisted of one individual per sample. The targeted lengths of the prey fish (8–25 cm) were based on the preferred diet size of harbor seals and harbor porpoises.

Species	Sample type	Number of individuals per sample	Western Scheldt	Western Scheldt (Ossensisse sandflats)	Western Scheldt (Valkenisse sandflats)	Wadden Sea	Wadden Sea West
Peppery furrow shell ( <i>Scrobicularia plana</i> )	Whole organism	65 <sup>a</sup>		3	3		
Lugworm ( <i>Arenicola marina</i> )	Whole organism	50		3	3		
Brown shrimp ( <i>Crangon crangon</i> )	Whole organism	100		3	3		3
Shore crab ( <i>Carcinus maenas</i> )	Whole organism	30		3	3		
Goby ( <i>Pomatoschistus/Gobius</i> sp.)	Whole organism	30 <sup>a</sup>		3	3		3
Pouting ( <i>Trisopterus luscus</i> )	Whole organism	30 <sup>a</sup>		3	2		
Atlantic herring ( <i>Clupea harengus</i> )	Whole organism	30		2	1		
Whiting ( <i>Merlangius merlangus</i> )	Whole organism	30 <sup>a</sup>		3	1		
European flounder ( <i>Platichthys flesus</i> )	Whole organism	30 <sup>a</sup>		3	3		3
Harbor porpoise ( <i>Phocoena phocoena</i> )	Liver + muscle	1	10			10	
Seal ( <i>Phoca vitulina</i> )	Liver + muscle	1	8			11	

<sup>a</sup> Target number of individuals was not always reached for each sample (see Table S9).

standard and atmospheric nitrogen, respectively.

Moisture content in the samples was determined using a gravimetric method. Each sample was mixed with shell sand, weighed, dried in an oven at 105 °C for 3 h, then cooled in a desiccator and weighed. Ash content determination involved gradually heating and drying weighted samples in a crucible on a hotplate, followed by ashing in a muffle furnace at 550 ± 15 °C for 22 h. The samples were then cooled in a desiccator and reweighed.

The lipid content of samples was determined using a modified version of the Bligh and Dyer method (de Boer, 1988), based on cold chloroform-methanol extraction.

Concentrations of 16 PFAS components were determined in a total of 142 samples (Tables S6 and S8), consisting of: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTeA, PFBS, PFHxS, PFHpS, PFOS, and PFDS. Mass labelled and unlabeled standards for all compounds were obtained from Wellington labs. PFAS analyses were performed following Kwadijk et al. (2010). In short, a subsample (1–5 g) of each homogenized sample underwent ultrasonic extraction using acetonitrile. The resulting extract was dried over a glass filter with sodium sulfate, followed by a cleanup step with activated carbon. The final extract was analyzed by Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS) using an Agilent 1200 LC pump coupled with a Waters Quattro micro MS. Separation was performed using a 150 × 2.1 mm (3.5 µm) Zorbax C18 column (Agilent) with 25 mM ammonium formate in demi water as solvent A and 25 mM formic acid in methanol as solvent B. Quantification was performed in ESI negative mode (Table S7). The calibration curve for each compound consisted of eight points between 300 and 0.5 ng/ml and had an  $R^2 > 0.998$ .

Quality assurance and quality control samples were added to each set of up to 20 samples. These consisted of procedural blanks, in-house reference materials (commercial mussel *Mytilus edulis* tissue samples for moisture content, eel *Anguilla anguilla* tissue samples from Dutch waters for lipid analysis, and *M. edulis* tissue samples spiked with PFOS-PFOA-PFNA-PFDA-PFUnA-PFDoA for PFAS analysis), and recovery tests containing <sup>13</sup>C-labelled PFAS components for all analyzed PFAS components. No PFAS components were detected in any of the blank samples. For the in-house reference material, all results fell within normal

variation (<20 %). Internal standard recovery of all <sup>13</sup>C-labelled PFAS components was between 70 and 130 %. Samples with concentrations above the calibration curve were reanalyzed with a lower sample intake, while concentrations below the lowest calibration curve were reported as below the Limit of Quantification (<LoQ). PFBA and PFHxA were not observed above the LoQ in any of the samples and were excluded from further analyses. Therefore, the Sum-PFAS was ultimately based on 14 PFAS components (Sum-14PFAS). The PFAS method is routinely tested in intercomparison exercises (QUASIMEME), scoring Z-scores of –1.4 to 1.4 for PFOS (2020–2025).

## 2.7. Data processing

Tissue PFAS concentrations were expressed on a 100 % dry weight basis to remove variance as a result of differences in moisture content. All values below the LoQ were treated as '0'. Furthermore, the fraction of PFOS of the Sum-14PFAS was determined.

Due to the limited amount of data per species and/or location in most datasets, it was not possible to verify the normal distribution of all data. Therefore, the non-parametric two-tailed Mann-Whitney test was used to assess the statistical significance of differences in concentrations between species, locations, and recent versus historic data. Differences were considered statistically significant at  $p < 0.05$ . Boxplots and Mann-Whitney tests were performed using Graphpad Prism, version 10.1.0.

The trophic level (TL) within the food web was determined for each sample based on the stable nitrogen isotope results, using the following equation (Post, 2002):

$$TL_{\text{consumer}} = \lambda + ((\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{base}}) / \Delta^{15}N),$$

where  $\lambda$  is the trophic position of the organism used to estimate  $\delta^{15}N_{\text{base}}$  (e.g.,  $\lambda = 2$  for primary consumers),  $\delta^{15}N_{\text{secondary consumer}}$  ( $\delta^{15}N_{\text{sc}}$ , or any higher consumer) is measured directly, and  $\Delta^{15}N$  is the trophic fraction of nitrogen in  $\delta^{15}N$  per trophic level. The latter was kept at 3.4, as a generic fractionation for secondary and tertiary consumers in aquatic environments (Van der Zanden and Rasmussen, 2001). Clams consistently showed the lowest  $\delta^{15}N$  values ( $14.52 \pm 0.78$  ‰) in our dataset and were therefore used as baseline organism ( $\lambda = 2$ ) in this study.

The food chain length (FCL) of the Western Scheldt estuary was calculated according to Van der Zanden and Fetzer (2007), as following:

$$FCL = (\delta^{15}\text{N}_{\text{toppredator}} - \delta^{15}\text{N}_{\text{base}}) / \Delta^{15}\text{N} + \lambda$$

with all symbols being the same as described above and  $\delta^{15}\text{N}_{\text{toppredator}}$  being the maximum  $\delta^{15}\text{N}$  in our dataset.

Two types of Biomagnification Factor (BMF) were calculated for the Western Scheldt food web. This was done for PFOS only, as this was the only PFAS component with 0 % non-detects in the Western Scheldt samples (Table S8). The BMFs were calculated for PFOS concentrations that were based on 100 % dry weight.

A lab-derived BMF was calculated using the equation (Conder et al., 2012):

$$BMF = \frac{C_{\text{predator}}}{C_{\text{prey}}}$$

with  $C_{\text{predator}}$  being the PFOS concentration of the predator species and  $[C_{\text{prey}}]$  the PFOS concentration of the prey species.

A field-derived BMF was calculated using the equation (Conder et al., 2012):

$$BMF_{TL} = 10^{\left[ \frac{\log_{10}(C_{\text{predator}}/C_{\text{prey}})}{TL_{\text{predator}} - TL_{\text{prey}}} \right]}$$

with  $TL_{\text{predator}}$  being the TL value of the predator species and  $TL_{\text{prey}}$  the TL value of the prey species.

Finally, the Trophic Magnification Factor (TMF) was calculated in the Western Scheldt food web, also only for PFOS. The following equations were used (Borgå et al., 2012):

$$[\text{Contaminant}] = 10^{b \cdot TL}$$

$$TMF = 10^b$$

where  $b$  is the regression slope of the log-normal relationship. Regression slope was retrieved using R (R core team, 2024), and plotting was performed with *ggplot2* and *ggprism* (Wickham, 2016; Dawson, 2021). For both the BMF and TMF, a value of  $>1$  points at biomagnification, whereas a value  $<1$  implies biological dilution of the specific contaminant in the food web compartment.

In marine mammals, PFAS concentrations are predominantly measured in specific organs, such as liver or blood, as whole-organism concentrations are difficult to assess in these large bodied animals. However, comparing contaminant levels determined in organs of individual top predators with levels in organisms from lower trophic levels that are based on multiple individuals using the whole-body, may lead to a mismatch, resulting in an overestimation of the PFAS concentration at the top predator level (Houde et al., 2006; Borgå et al., 2012). A well-supported biomass conversion from such specific organs to whole-body burdens may lead to a better picture of the TMF for food webs including top predators (Houde et al., 2006; Miranda et al., 2022). Therefore, the whole-body burdens for porpoises and seals were also calculated, based on the relative volume of different organs and tissues in relation to the whole-body (McLellan et al., 2002; Ahrens et al., 2009a), reported PFOS concentrations in organs and tissues (Van de Vijver et al., 2007; Ahrens et al., 2009a; Fujii et al., 2018), and estimated conversion factors from wet weight to dry weight whole-body burdens (Yang and Miyazaki, 2003; Horn and de la Vega, 2016) (see Supporting information text file for more details).

### 3. Results & discussion

#### 3.1. Food web composition of the Western Scheldt estuary

Stable isotopes analysis was first applied to assess the food web

relations within the Western Scheldt, as backbone for the accumulation and magnification processes. Carbon isotopes ( $\delta^{13}\text{C}$ ) ranged from  $-22.8$  in a seal to  $-16.4$ ‰ in another seal sample, and nitrogen isotopes ( $\delta^{15}\text{N}$ ) from  $13.4$  in a herring to  $22.7$ ‰ in a seal sample (Fig. 2, Table S9). Lowest  $\delta^{15}\text{N}$  values in general were observed in clams ( $13.7$ – $15.3$ ‰) and highest values in seals ( $19.7$ – $22.7$ ‰). The data correspond well with previous values recorded in the Western Scheldt (van Ael et al., 2013). For example, the isotopic composition of lugworms (mean  $\delta^{13}\text{C}$   $18.5$ ‰ and mean  $\delta^{15}\text{N}$   $17.3$ ‰) was similar to the mean  $\delta^{13}\text{C}$  of  $18.1$ ‰ and mean  $\delta^{15}\text{N}$  of  $17.5$ ‰, as reported by van Ael et al. (2013).

Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values showed a spatial gradient along the Western Scheldt, with organisms sampled in the upper estuary being relatively depleted in  $\delta^{13}\text{C}$  and enriched in  $\delta^{15}\text{N}$  compared to the same species sampled in the lower estuary (Fig. 2, Supporting information Figs. S1 and S2). This negative correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between the marine and brackish part of the estuary has been described before in particulate organic matter of the Western Scheldt (Middelburg and Herman, 2007), and is typical for an estuarine system due to varying contributions of fluvial ( $\delta^{13}\text{C}$  depleted) versus marine ( $\delta^{13}\text{C}$  enriched) organic matter as food source (Deegan and Garritt, 1997). This was particularly visible in the invertebrate species (clam, lugworm, crab and shrimp) and in some of the fish species (herring, pouting). It may indicate that these, more pelagic, fish species have a less varied diet than flounders and gobies (demersal fish species) in the Western Scheldt, as the latter group did not show this negative correlation. Such a contrast in feeding strategy between pelagic and demersal fish species was also observed in the temperate estuarine food web of the Gironde, France (Pasquaud et al., 2008). Interestingly, the spatial gradient was also seen in porpoises, but was absent in seals. This may imply that the porpoises that were stranded in the center of the estuary, foraged locally for a prolonged period of time, whereas seals used the estuary more as a resting place and collected their food further offshore. The terns showed a relative depletion in  $\delta^{13}\text{C}$  compared to the other top predators, which may be explained by the terns also using a nearby fresh to brackish water channel for feeding.

Food chain length in the Western Scheldt was estimated at 4.4 trophic levels, which corresponds well with the average for marine ecosystems ( $4.0 \pm 0.5$ , Van der Zanden and Fetzer, 2007), the North Sea ( $\sim 4.0$ , Van der Zanden and Fetzer, 2007), and another European temperate estuarine food web ( $\sim 4.2$ , Munoz et al., 2017). Adding apex predators, such as the seal and porpoise, extended the food web with  $\sim 0.9$  trophic levels. This resulted in the required three trophic levels that are needed to reliably calculate trophic magnification factors for

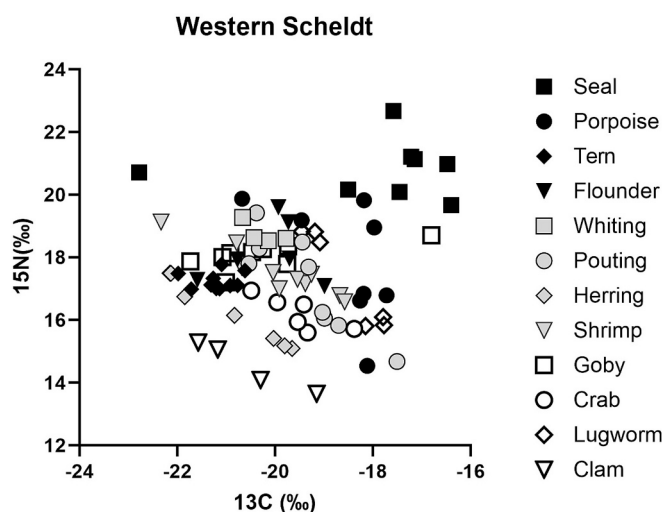


Fig. 2. Food web of the Western Scheldt estuary, based on the  $\delta^{13}\text{C}$  versus  $\delta^{15}\text{N}$  isotope values in the collected species. All samples were collected in 2023, except for porpoises and seals, that were collected between 2018 and 2023.

contaminants in the Western Scheldt food web (Borgå et al., 2012).

### 3.2. PFAS exposure in biota

The Sum-14PFAS concentrations in biota of the Western Scheldt ( $n = 91$ ) ranged from 2.0 to 8.7 ng/g dw in clams to 1613–8243 ng/g dw in seal livers (Table S9). Sum-14PFAS concentrations showed a significant positive correlation with  $\delta^{15}\text{N}$  (Fig. 3, Table S11), depicting transfer and biomagnification of PFAS in the estuarine food web. Even when considering only samples from the center of the Western Scheldt, thus removing the spatial differences in stable isotope values, biomagnification was still observed (Fig. S4).

The Sum-14PFAS concentrations showed substantial variation between porpoise and seal individuals (Fig. 3). This was previously observed in harbor seals from the Dutch Wadden Sea (Van de Vijver et al., 2005). Typically, seals are weaned at a very early age and therefore develop individual foraging and survival strategies (Harvey et al., 2022). Compared to this, the tern (egg) samples showed much lower individual variation. Common terns are predominantly income breeders (Moore et al., 2000). Therefore, the diet of adult females, directly prior to egg laying and complemented by male provisioning, influence both the stable isotope ratios and concentrations of lipophilic and proteonophilic contaminants in the eggs. Furthermore, terns forage on pelagic species in the neighborhood of their breeding colonies, while juvenile porpoises and seals primarily target benthic-pelagic and demersal species in a broader feeding area. This may explain the differences in variability between the terns and marine mammals. Invertebrate and fish samples also showed lower variation between samples. However, each of these samples consisted of multiple individuals, averaging out most of the individual variation. Furthermore, compared to the top predators, these species are more spatially confined to the sampling site and in equilibrium with their surroundings, thus better reflecting the local environment.

PFOS concentrations in biota of the Western Scheldt ranged from 2.0 to 7.7 ng/g dw in clams to 1358–6818 ng/g dw in seal livers, and was the most dominant of all PFAS components in shrimp, fish and top predators from the Western Scheldt (Fig. 4). This is in line with earlier reported data in fish from Dutch and Belgian coastal waters (Zafeiraki et al., 2019; Byns et al., 2022; Jonker, 2024). The PFOS contribution gradually increased up the food chain, starting on average from 34 % in lugworms and 49 % in crabs to >87 % in livers of porpoise and seals (Fig. 4A). The only exception was the PFOS contribution in clams (88 %), which can be explained by a very low total Sum-14PFAS concentration with most PFAS components being below the LoQ (Table S8). In the lugworms, PFOA was found to be more dominant than PFOS. This was also

observed in tidal flat organisms, including lugworms, of the Ariake Sea in Japan (Nakata et al., 2006), and in freshwater mussels from Belgium (Teunen et al., 2021). Some PFAS components were only found in a few individual samples above the LoQ in the Western Scheldt; PFPeA was only observed in goby and pouting, PFHpA in lugworm, PFBS in pouting, and PFDS in porpoise and seal (Table S8). Overall, the concentrations of the majority of the PFAS components were significantly positively correlated with the PFOS concentration in the sample, except for PFOA ( $n = 68$ ) (Table S10). Also, PFPeA and PFDS did not show a correlation, but the number of samples with both components >LoQ were low ( $n = 3$  and  $n = 4$  respectively). Furthermore, all individual PFAS components showed a significant positive correlation with the trophic level indicator  $\delta^{15}\text{N}\%$  of the sample, except for PFOA ( $n = 67$ ) showing no correlation with trophic level (Table S11). Also here, PFPeA ( $n = 3$ ) and PFDS ( $n = 4$ ) were not correlated with  $\delta^{15}\text{N}\%$ .

To allow a comparison of PFOS based on whole-body burden for the entire Western Scheldt food web, the liver concentrations of the marine mammals were converted to estimated whole-body burdens using estimated extrapolation factors (see Supporting information Text file). Whole-body concentrations in the Western Scheldt harbor porpoises were calculated to be  $207 \pm 161$  ng/g dw, which was approximately ten times lower than the measured liver concentrations. Whole-body PFOS burdens for the harbor seals were  $429 \pm 244$  ng/g dw, about seven times lower than the measured liver concentrations. These whole-body-to-liver PFOS concentration ratios were similar to those applied earlier for bottlenose dolphins (*Tursiops truncatus*), which were based on actual measurements in two dolphins (nine times lower; Houde et al., 2006). To the best of our knowledge, this is the first time that such conversion factors for PFOS have been applied for seals and porpoises. To improve the conversion factors from PFAS liver concentrations to whole-body concentrations in marine mammals, it is advised to further develop these based on empirical studies.

### 3.3. Western Scheldt comparison: 2006–2008 vs. 2023

Between 2006 and 2008 field surveys were conducted in the Western Scheldt estuary using a similar approach as the current study (de Vos et al., 2008; Table S16). Among a series of contaminants, several PFAS components were measured in many of the species that were also sampled in 2023. Although the total set of analyzed PFAS substances during the 2006–2008 campaign was somewhat different from the current set of 2023, both datasets included the EFSA-4 PFAS components (PFOS, PFOA, PFNA and PFHxS). This allows a direct comparison between the two data sets based on the Sum-EFSA-4 PFAS concentrations.

In general, PFAS concentrations (Sum-EFSA-4) in biota of the Western Scheldt estuary were lower in 2023 than in 2006–2008 (Fig. 5). Six of the nine species that were sampled in both time periods showed a significant reduction in PFAS (Sum-EFSA-4) concentrations. The highest reductions of 15, 9 and 4 times were observed in lugworm, herring and flounder respectively. No significant change could be detected for clams and shrimp, although for these species most of the concentrations were also lower in 2023 (Fig. 5). For porpoises, only two individuals were sampled in 2006–2008, and although these concentrations were higher than in 2023, the difference was not significant. Decreasing trends in PFAS concentrations in the Western Scheldt estuary were also reported earlier in water and biota (Byns et al., 2022; Jonker, 2024). These are likely a result of measures such as reduced production and use following PFOS's 2009 listing under the Stockholm Convention on Persistent Organic Pollutants (Jonker, 2024) and 3 M's phase-out of PFOS and PFOA (Byns et al., 2022).

### 3.4. Comparison with other regions

Sum-14PFAS concentrations in Wadden Sea biota ( $n = 51$ ) ranged from <LoQ–8.0 ng/g dw in goby to 321.9–863.2 ng/g dw in seal liver. PFAS concentrations in Western Scheldt biota were generally

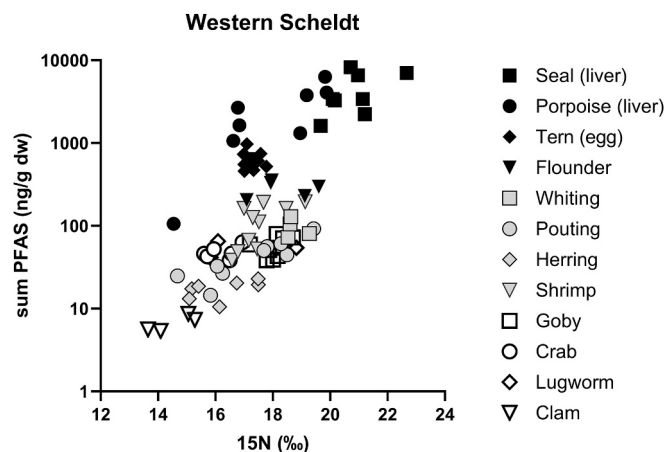


Fig. 3. Nitrogen isotopes versus Sum-14PFAS concentrations (ng/g dw) for all Western Scheldt samples. All samples were collected in 2023, except for porpoises and seals, that were collected between 2018 and 2023.

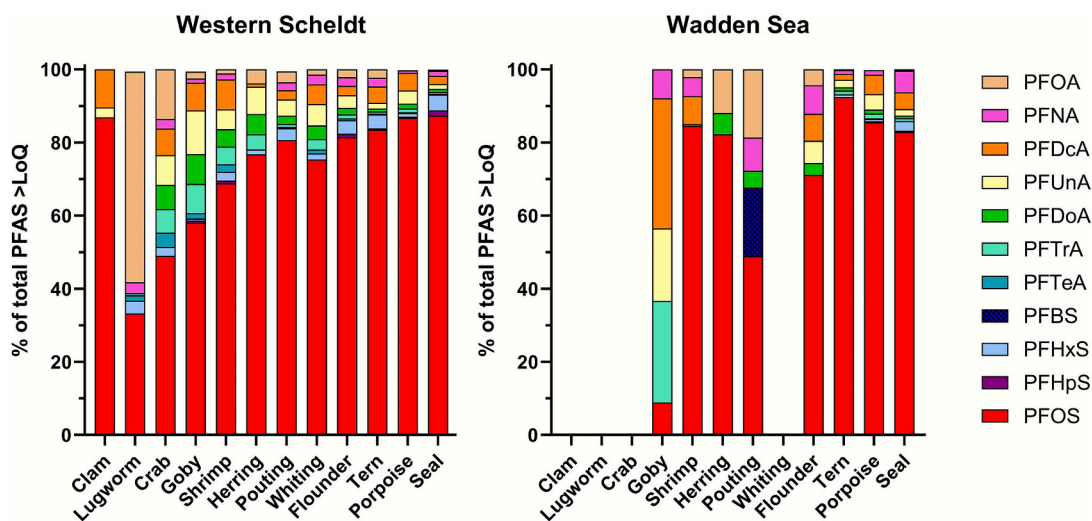


Fig. 4. PFAS profiles in all biota groups for the Western Scheldt estuary (left), and the western Wadden Sea (right). All samples were collected in 2023, except for porpoises and seals, that were collected between 2018 and 2023. Only PFAS components with a contribution of at least 1 % to the Sum-14PFAS are shown, thereby excluding PFPeA, PFHpA and PFDS from this figure.

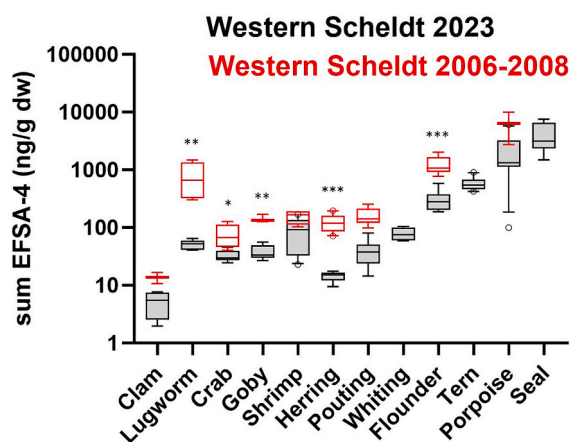


Fig. 5. Tissue concentrations of Sum PFAS (EFSA-4, ng/g dw) in biota from the Western Scheldt collected in 2023 (in black), and 2006–2008 (in red). Box plots showing median, 25–75 percentile (box) and 10–90 percentile (bars). No data were available for whiting, tern and seal in 2006–2008. Statistical significance between years: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (two tailed nonparametric Mann Whitney test). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

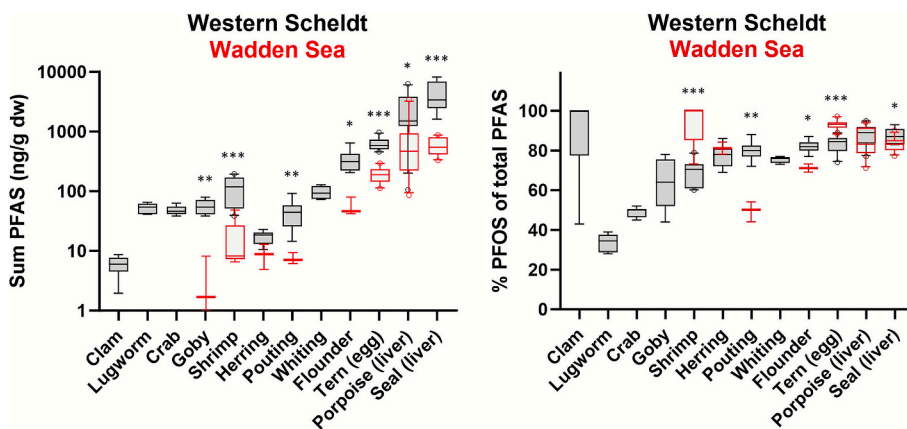
statistically higher than in the same species collected from the Wadden Sea (Fig. 6, Table S12). This further confirms that the Western Scheldt estuary is a hotspot for PFAS in the Dutch coastal waters (Jonker, 2024). The largest differences between the Western Scheldt and Wadden Sea were found in goby, seal (liver), shrimp, flounder and pouting for which Sum-14PFAS concentrations were on average 15.8, 8.0, 7.1, 6.2 and 6.2 times higher in the Western Scheldt estuary than in the Wadden Sea, respectively. For herring ( $n = 2$ ) the sample size in the Wadden Sea was too small to test for differences.

The PFAS profiles also differed between the Western Scheldt and Wadden Sea biota (Figs. 4 and 6). PFBA, PFPeA, PFHxA and PFHpA were not detected >LoQ in Wadden Sea samples, whereas PFBS ( $n = 2$ ), PFHpS ( $n = 4$ ) and PFDS ( $n = 2$ ) were detected in <8 % of the Wadden Sea samples (Table S8). Nonetheless, significant ( $p < 0.006$ ) positive correlations were observed between the PFOS concentrations and several PFAS components (PFTeA, PFDaA, PFUnA, PFTrA, PFNA, PFDcA, PFHxS, PFHpS) that had concentrations above LoQ in the Wadden Sea samples, again except for PFOA (Table S10).

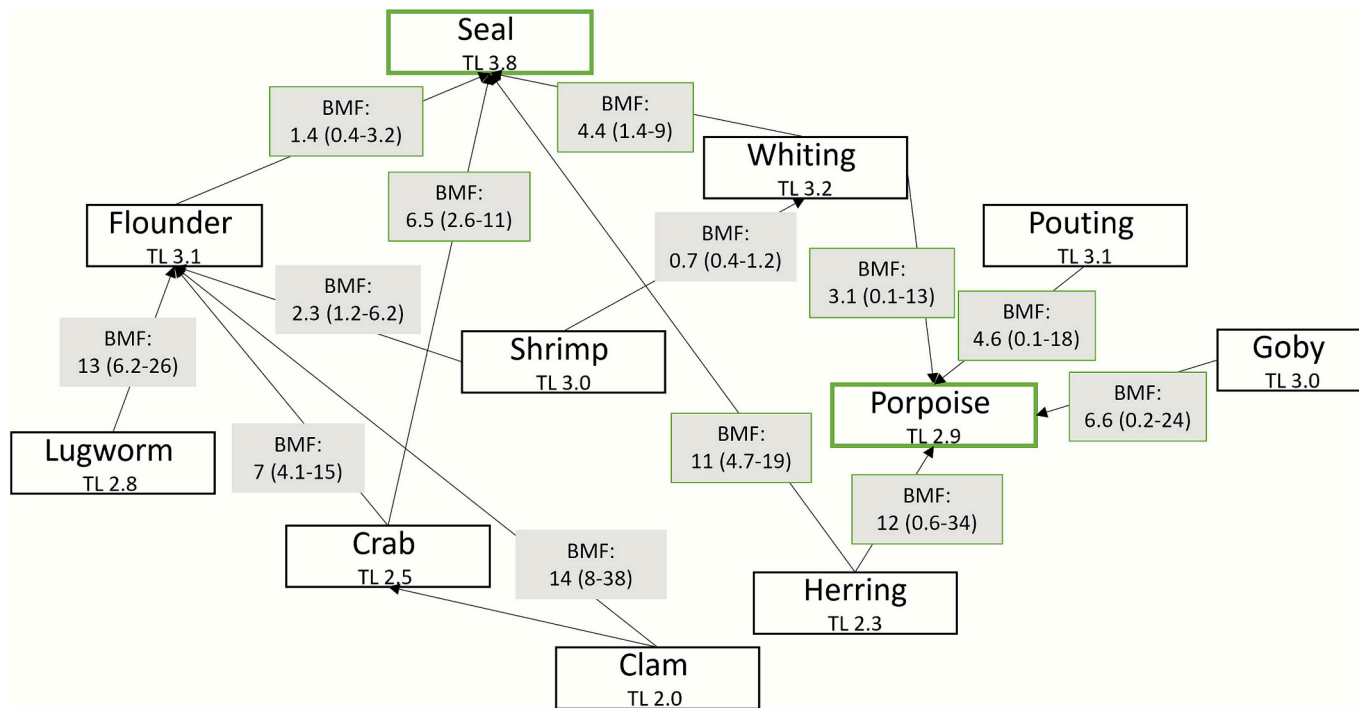
PFOS concentrations in the Western Scheldt estuary were generally at the higher end of concentrations reported elsewhere, and at the lower end for some of the highest concentrations reported 15–20 years ago in some species. PFOS concentrations in shrimp ( $5.2\text{--}33.0$  ng/g ww, mean  $17.6 \pm 10.2$  ng/g ww) were around ten times higher than shrimp collected in the southern North Sea near the entrance of the Western Scheldt ( $0.3\text{--}4.6$  ng/g ww, 2018; Byns et al., 2022). PFOS concentrations in fish from the Western Scheldt ( $1.7\text{--}120$  ng/g ww) were up to four times higher than in fish in the Gironde estuary, southwest France ( $0.3\text{--}27.1$  ng/g ww Sum of branched + linear PFOS, 2012; Munoz et al., 2017). PFOS in common tern eggs from the Western Scheldt ( $84\text{--}190$  ng/g ww) were slightly higher than most reported PFOS concentrations in bird eggs worldwide ( $10\text{--}100$  ng/g ww; Sun et al., 2023), though lower than the highest concentrations observed in eggs from double-crested cormorants (*Phalacrocorax auritus*) in San Francisco Bay, California ( $36.1\text{--}466$  ng/g ww, 2012; Sedlak et al., 2017) and from common guillemots (*Uria aalge*) in the Baltic Sea ( $25\text{--}1324$  ng/g ww, 1968–2003; Holmström et al., 2005). PFOS concentration in harbor porpoise livers from the Western Scheldt ( $24\text{--}1500$  ng/g ww, mean  $536 \pm 417$  ng/g ww) were similar to or a little lower compared with earlier reported concentrations in harbor porpoises from the North Sea ( $53\text{--}1700$  ng/g ww, 1980–2005; Galatius et al., 2011), Baltic Sea ( $159\text{--}2425$  ng/g ww, 1991–2008; Huber et al., 2012) and the United Kingdom ( $357\text{--}2992$  ng/g ww, 2015–2018; Androulakakis et al., 2022). PFOS concentrations in seal livers from the Western Scheldt ( $330\text{--}1800$  ng/g ww, mean  $1015 \pm 579$  ng/g ww) were among the highest reported in seal livers worldwide (Sait et al., 2023) and on the lower end of the highest reported PFOS concentrations in samples from the German Bight in the North Sea, collected 15–20 years ago ( $7.2\text{--}3451$  ng/g ww, 2003–2008; Ahrens et al., 2009b).

### 3.5. Biomagnification of PFOS

Field-derived BMFs for PFOS for seals and porpoises were calculated based on estimated whole-body burdens in these top predators. This allowed a more realistic comparison of PFOS concentrations in the top predator versus the whole-body prey concentrations, and resulted in BMFs for porpoises ranging between 0.1 and 34, and for seals between 0.4 and 18.5 (Fig. 7) (Table S13). Although some BMF values were below 1 for both species, pointing at biodilution, average BMFs were between 3.1 and 11.7 for porpoises and 1.4–11.3 for seals, indicating biomagnification of PFOS in these top predators when based on whole-



**Fig. 6.** Comparison of the Sum-14PFAS concentrations (ng/g dw) (left), and the %PFOS of Sum-14PFAS concentrations (right) in biota of the Western Scheldt (in black) versus the Wadden Sea (in red). All samples were collected in 2023, except for porpoises and seals, that were collected between 2018 and 2023. No data were available for clam, lugworm, crab and whiting in the Wadden Sea. Goby data were excluded in the figure on the right (%PFOS) as only 1 sample out of 6 contained a PFOS concentration above detection limit. Box plots showing median, 25–75 percentile (box) and 10–90 percentile (bars). Statistical significance between locations: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (Two tailed nonparametric Mann Whitney test). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 7.** Field-derived Biomagnification Factors (BMFs) for PFOS in the Western Scheldt estuarine food web, including the harbor porpoise and harbor seal as top predator, in 2023. Placement of the species in the food web was based on the calculated Trophic Level (TL) for each species. PFOS concentrations in the porpoise and seals were converted to whole body concentrations for these BMF values.

body burdens. When BMFs were based on liver PFOS concentrations of seals and porpoises, the average BMFs were up to ten times higher (38–102 for porpoises and 8–56 for seals) (Table S13). This is lower than what has been reported for bottlenose dolphins, where BMFs were up to a 30-fold higher in liver versus calculated whole-body burdens (Houde et al., 2006). The high variation in the observed BMFs were related to the variable trophic differences between predator and prey, as indicated by  $\delta^{15}\text{N}\%$ , as well as large variation in PFOS concentrations in the top predators. This would imply that not all individual top predators that were sampled for this study may have had the same relation with the Western Scheldt food web. In the current study BMFs were not calculated for terns, as these are the product of the adult female, having a

more indirect feeding relation with the food web.

At the lower trophic level, the field-derived BMF for the flounder and its' diet ranged between 1.2 and 38, with the highest BMF value for clam as diet species (8–38) and the lowest for shrimp (1.2–6.2) (Fig. 7) (Table S13). This low bioaccumulation step from shrimp to flounder can be explained by the minimal difference in TL between both species (3.0 in shrimp vs. 3.1 in flounder) and relatively high PFOS concentrations in shrimp. The here calculated field-derived BMFs are somewhat higher than the BMFs found in the temperate estuary of the Gironde (SW France, Munoz et al., 2017). They reported an average  $\text{BMF}_{\text{flounder-clam}}$  of 4.6 for L-PFOS, while here the  $\text{BMF}_{\text{flounder-clam}}$  was 14 for PFOS. Differences in food web composition and/or other environmental and

biological characteristics between the two estuaries may be the cause of this.

Since a suitable standardization method is not available for PFAS, such as basing concentrations on 100 % lipid weight for lipophilic compounds like PCBs, it is important to keep sample types in food webs studies as consistent as possible when assessing biomagnification potential. As determining PFOS whole-body burdens is a practical challenge for larger bodied species such as the top predators, this can be further improved by establishing more empirically based conversion ratios from liver or blood concentrations to whole-body burdens. Similarly, the suitability of eggs as a proxy for PFOS levels in birds and determining conversion rates from eggs to the respective whole-body burden (for adult females) should be further studied. Moreover, it must be kept in mind that a BMF based on field data should always be seen as indicative, as a solid BMF can only be calculated in situations where predators are limited to a single prey.

### 3.6. Trophic magnification (TMF) of PFOS

PFOS showed clear trophic magnification in the Western Scheldt food web (Fig. 3). The trophic magnification (TMF), based on estimated whole-body burdens for seals and porpoises, resulted in a TMF of 5.7 (95 % CI 3.5–9.3) for the entire dataset, a TMF of 23.9 (95 % CI 3.2–178.9) for the spring samples (including the common tern), and a TMF of 6.4 (95 % CI 4.1–9.8) for data collected solely in the fall (Table S14). When based on liver concentrations in seals and porpoises, the TMF was a factor 2.4 higher for the whole dataset (13.6, 95 % CI 7.2–25.6), and also a factor 2.4 for the fall data (15.1, 95 % CI 7.3–31.1) (Table S14). This compares well the factor 2.7 difference between whole-body and liver-based TMFs calculated for marine mammals in an estuary in South Carolina, USA, and the factor 3.8 found in an Arctic marine food web (Houde et al., 2006).

The observed TMFs fall well within the recorded range of 1–20 for PFOS in aquatic ecosystems in general (Miranda et al., 2022). Compared to other studies that include top predators, the Western Scheldt TMFs were higher than found in estuaries in South Carolina, USA ( $1.8 \pm 1.2$ , based on whole-body burden, Houde et al., 2006), and an Arctic marine food web ( $6.5 \pm 4.5$ , based on liver concentrations, Tomy et al., 2004).

The elimination potential of PFOS in air breathing top predators is thought to be lower than elimination of PFOS via the gills of fish, which may result in a higher TMF for those studies that include air-breathing top predators (Byns et al., 2022). When excluding top predators, the TMFs of the Western Scheldt (5.0 - all data, 3.5 - spring data, and 5.0 - fall data) were also higher than those found in other temperate estuarine systems that excluded top predators (1–4 in the Gironde estuary in France (Munoz et al., 2017), 1.6 in the Xiaoqing River estuary in China (Li et al., 2021), and 1.6 in the St. Lawrence estuary in Canada (Munoz et al., 2022)).

Differences in TMF values between the Western Scheldt and other areas can be a consequence of a variety of factors. TMF values for PFAS are influenced by local diets, but also by the way the trophic level is calculated (such as enrichment factor, baseline species, seasonal variation in  $\delta^{15}\text{N}$ ), the expression of the PFAS concentrations in the calculation (dry weight or wet weight or protein content corrected concentrations), and the statistical treatment of the data (e.g. how to include non-detects in a dataset; Miranda et al., 2022). The latter did not affect the current TMF calculation as all Western Scheldt samples had PFOS concentrations above LoQ. However, a clear spatial gradient was visible along the Western Scheldt estuary for both  $\delta^{15}\text{N}$  and PFOS, implying that sampling location in an estuary affect the TMF (Figs. S2 and S3). Given that PFOS concentrations were consistently higher in species with higher trophic levels, also at single sample location (Fig. S4), we argue that PFOS magnified throughout the Western Scheldt food web, despite the spatial gradient in nitrogen isotopes and PFOS concentrations in the estuary.

### 3.7. Potential health implications for biota

#### 3.7.1. European Water Framework Directive (WFD)

In the EU Guidance Document no.32 (EU, 2014), Ecological Quality Standards for biota (EQS<sub>biota</sub>) are described for a selection of contaminants that apply for the European Water Framework Directive. These EQS values are derived for two protection goals: 1) protection against effects of accumulation and secondary poisoning of substances in the food chain, especially for top predators such as birds and mammals (QS<sub>secpois</sub>), and 2) safeguarding human health from contaminated food (QS<sub>hhfood</sub>). For PFOS the QS<sub>secpois</sub> and QS<sub>hhfood</sub> are set at 33 and 9.1 ng/g respectively. The Ecological Quality Standard (EQS<sub>biota</sub>) is set as the lowest of these QS values (9.1 ng/g).

In the Western Scheldt most of the studied fish had concentrations around (goby, pouting) or well above this threshold (whiting, flounder), except for herring (Fig. 8). The concentrations in most fish of the Wadden Sea were below this threshold, except for one flounder sample (1/3).

#### 3.7.2. Internal effect concentrations

Insight in critical tissue concentrations, especially for those PFAS components that bioaccumulate, are essential to understand whether they pose a risk in wild organisms. Reported effects after PFAS exposure include affected liver, kidney and thyroid functioning in humans based on metadata epidemiological studies (Cakmak et al., 2022); effects on the immune system in mice (DeWitt et al., 2012), humans (Ayuk et al., 2025), and mussels (Liu and Gin, 2018); and fatty liver disease and adverse effects on reproduction in fish (Cheng et al., 2016; Zhang et al., 2016). Reported effects of PFAS at environmentally relevant concentrations, however, are scarce (Sinclair et al., 2020). Furthermore, information linking PFAS tissue concentrations in marine biota to biological or health effects is limited, especially for invertebrates. (Table S15). Despite this, a first comparison was conducted to enable a preliminary assessment of potential impacts of PFAS on biota in the Western Scheldt estuary based on the available information.

Effects on the immune system of invertebrates have been reported for green mussels (*Perna viridis*), at tissue concentrations of 2–5 ng/g ww PFOS (Liu and Gin, 2018). The lower boundary of this effect level is a factor ten higher than the PFOS concentrations that were measured in the clam of the Western Scheldt (0.2–0.8 ng/g ww). This could indicate a potential risk for subtle effects on shellfish in the Western Scheldt, warranting further investigations.

Experiments with fish revealed endocrine and growth effects at internal PFOS concentrations of 7700 ng/g ww (Wang et al., 2011), being 64 times higher than the highest PFOS concentration of 120 ng/g ww

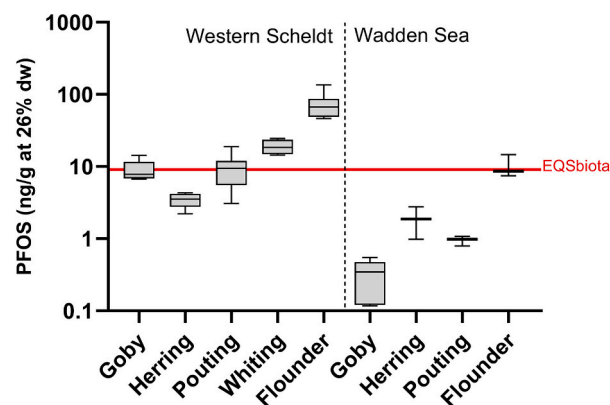


Fig. 8. PFOS concentrations (ng/g, 26 % dw) in whole fish from the Western Scheldt estuary and Wadden Sea, expressed as a standard fish with 26 % dry weight, according to the guideline of the European Water Framework Directive. The solid horizontal line indicates the EQS-biota of PFOS, based on the QS for human health (QS<sub>h</sub>: 9.1 ng/g).

that was observed in fish (flounder) from the Western Scheldt. Information on whether other physiological effects, like immunotoxicity, may occur at these concentrations has not been found.

For birds, effects on hatchability have been reported for leghorns when eggs were injected with PFOS dosed as high as 1,000,000 ng/g egg weight (Molina et al., 2006). Much lower effect concentrations were found in a field study with great tits (*Parus major*) in which reduced shell thickness was found at 5111 ng PFOS/g egg weight, and changes in blood protein and triglyceride levels at 19 ng PFOS/g egg weight (Parolini et al., 2022). In the eggs of the common terns from the Western Scheldt, PFOS concentrations between 84 and 190 ng/g ww were observed, thus well below the levels where embryo mortality could be expected. However, assuming a similar sensitivity as in the great tit, sublethal effects on blood proteins and triglyceride cannot be excluded, pointing at a need for further studies in birds.

For small cetaceans, first estimated tentative critical concentrations (TCCs) of 677–775 ng/g ww were established for PFOS, based on rat and monkey toxicity data (Lam et al., 2016). Comparison of the liver concentrations of the porpoises and seals from the Western Scheldt with these TCCs, showed that 60 % of the porpoises (6/10) and 60 % of the seals (5/8) contained levels above these, pointing at potential adverse effects of PFOS.

#### 4. Conclusions

This study further illustrates the biomagnification potential of PFAS in estuarine food webs, with both Sum-14PFAS and PFOS concentrations being positively and significantly correlated with the trophic level. Both stable isotopes and PFAS concentrations showed a spatial gradient in the estuary. Furthermore, the contribution of PFOS to the Sum-14PFAS pointed at enhanced bioaccumulation of PFOS, with the Sum-14PFAS concentrations consisting of >80 % of PFOS in all predator samples. PFAS concentrations in the Western Scheldt food web were significantly lower in 2023 than in 2006–2008 for most biota. PFAS concentrations were significantly higher than the reference location, Wadden Sea, and generally high in comparison with other coastal areas worldwide.

To the best of our knowledge, this is the first time that whole-body burdens for harbor porpoises and harbor seals were extrapolated from liver concentrations, applying estimated extrapolation factors, to allow further comparison between trophic levels. Based on this, field-derived BMF values for PFOS ranged from 0.1 to 34 in these marine mammals. Furthermore, this is the first study to assess the TMF for PFOS in an estuarine system that includes top predators (based on whole-body burdens), resulting in a TMF of 5.7.

The threshold level of the WFD (EQS<sub>biota</sub>) for PFOS was exceeded in most of the fish samples from the Western Scheldt. The observed PFOS concentrations in clams, terns and marine mammals of the Western Scheldt may indicate potential risks of sublethal effects.

All things considered, it is highly recommended to develop more knowledge on subtle, physiological effects in a range of marine species after (chronic) exposure to PFAS, in studies that include reporting the critical body concentrations. This will allow an improved future assessment of the impact of PFAS whole-body concentrations in field situations (Ankley et al., 2021), which is essential for assessing the effects of PFAS in multi-stress conditions in a changing environment.

#### CRedit authorship contribution statement

**Martine J. van den Heuvel-Greve:** Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Jildou Schotanus:** Writing – review & editing, Methodology, Investigation. **Michiel J.J. Kotterman:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Christiaan J.A.F. Kwadijk:** Writing – review & editing, Methodology, Formal analysis. **Sophie M.J.M. Bresseur:** Writing – review & editing, Conceptualization. **Mardik Leopold:** Writing – review & editing. **Jetze**

**van Zwol:** Writing – review & editing, Investigation. **Douwe van den Ende:** Writing – review & editing, Investigation. **Suzanne A. Cornelisse:** Writing – review & editing, Investigation. **Evert de Froe:** Writing – review & editing, Formal analysis. **Edwin M. Foekema:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

#### 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.marpolbul.2025.119053>.

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