

Bioaccumulation, Ecological Effects, and Monitoring of PFAS in Aquatic Ecosystems

Bioaccumulatie, Ecologische Effecten en Monitoring van PFAS in Aquatische Ecosystemen

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From a young age, I was captivated by the life thriving in aquatic environments, which led me to pursue a master in marine and lacustrine biology. But beyond fascination, there was also heartbreak. As a child, I remember feeling devastated when I saw images of birds, seals, and otters covered in thick layers of oil after spills. I was horrified by the sight of marine mammals entangled in debris, trapped in a disaster they had no part in creating. The sheer scale of human impact on nature felt overwhelming, yet it also planted a seed, a deep sense of urgency and responsibility. That early heartbreak became the driving force behind my decision to dedicate my career to studying pollution and its effects on ecosystems. This motivation ultimately led me to my PhD research on PFAS pollution. I am profoundly grateful for the opportunity to contribute to this critical field and for the many people who have supported me along the way. This journey would not have been possible without their guidance, encouragement, and generosity, and I would like to take this moment to express my deepest appreciation.

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Summary

In 1938, Dr. Roy Plunkett, a young chemist, accidentally discovered the world's first fluoropolymer, later identified as polytetrafluoroethylene (PTFE), which became widely known under its market name, Teflon. This breakthrough paved the way for the development of a vast class of chemicals collectively referred to as per- and polyfluoroalkyl substances (PFAS). According to the most recent definition, PFAS encompass any chemical containing at least one fully fluorinated methyl ($-CF_3$) or methylene group ($-CF_2-$). Under this definition, an estimated 7 million substances are classified as PFAS, highlighting their extensive diversity. PFAS, once regarded as industrial marvels for their exceptional durability, amphiphilic properties, and resistance to heat and chemicals, have found widespread use in various industrial and commercial applications. However, the very qualities that make PFAS so valuable also render them a significant environmental challenge. Their persistence in the environment, tendency to bioaccumulate, and potential toxicity have raised serious concerns. PFAS contamination originates from numerous direct and indirect sources, including industrial processes, wastewater discharge, and everyday consumer products. These pollutants have become ubiquitous in aquatic ecosystems, posing severe ecological and public health risks. This highlights an urgent need for more comprehensive monitoring programs and regulatory frameworks.

In Europe, current regulations and biota quality standards primarily focus on safeguarding human health and protecting top predators from secondary poisoning. However, there is a notable lack of regulations aimed at preserving the ecological integrity of entire ecosystems. Additionally, our understanding of PFAS impacts remains limited, primarily due to the predominance of laboratory-based assessments conducted under idealized conditions and the relative scarcity of field-based effect studies. This imbalance creates significant knowledge gaps, particularly regarding the ecotoxicity of PFAS under real-world environmental conditions.

In June 2021, environmental PFAS contamination gained widespread attention in Belgium after the discovery of high PFAS levels in soil samples during construction activities near a perfluorochemical plant in Antwerp. This incident underscored the severity of PFAS pollution and sparked broad media coverage, particularly raising questions regarding environmental and health implications. Despite increased awareness, critical gaps persist in understanding PFAS behavior, bioavailability, and distribution in aquatic systems. These unresolved challenges and knowledge gaps provided the foundation for this work, emphasizing the need for research to address the ecological, monitoring and regulatory deficiencies associated with PFAS contamination.

Therefore, the general aim of this work is to assess the bioaccumulation, distribution, and ecological effects of PFAS in aquatic ecosystems. The study encompassed a range of environments, from small inland rivers and streams to the Scheldt estuarine system and the Belgian North Sea. By exploring these diverse ecosystems, this research assessed the risks of PFAS bioaccumulation across multiple levels of biological organization. Furthermore, various monitoring techniques are implemented and assessed throughout the chapters.

The first study (**Chapter II**) is focusing on the distribution, bioaccumulation and trophic transfer of PFAS in marine species from the Belgian North Sea. Across 10 locations, seven fish and two crustacean species were sampled. Results reveal that PFOS predominates in marine biota, with long-chain PFAS frequently detected in liver and muscle tissues. The ecological risks of PFAS, particularly PFOS, were evident, as concentrations in some species exceeded regulatory safety thresholds for secondary poisoning. Moreover, human health risks were identified, with consumption of certain species, such as European plaice, sole, whiting and shrimps, potentially posing hazards if intake exceeds specified guidelines. This chapter highlights the urgent need for comprehensive marine monitoring programs to safeguard human and environmental health.

Chapter III focuses on the Scheldt River, a critical ecological and industrial corridor, to investigate the spatial distribution of PFAS bioaccumulation in fish and invertebrates. This study integrates ecotoxicological data with ecological tracers, including stable isotope compositions of carbon, nitrogen, and sulfur, to elucidate PFAS bioaccumulation pathways in relation to feeding ecology. The results reveal distinct patterns of PFAS distribution. Legacy compounds, such as PFOS and long-chain PFCAs, were found to be evenly distributed along the river,

indicating historical pollution. In contrast, alternative PFAS, including FBSA, were concentrated near Antwerp, suggesting recent industrial discharges. Stable isotope analyses provided crucial insights into bioaccumulation pathways. Pelagic feeders, such as Atlantic herring and European smelt, exhibited bioaccumulation profiles dominated by alternative PFAS, including FBSA, 6:2 FTS, and NaDONA, reflecting water-mediated uptake due to the high solubility of these substances. Conversely, benthic feeders, such as European eels, predominantly accumulated legacy compounds like long-chain PFCAs, which preferentially adsorb onto sediments. These findings highlight the significant role of ecological traits in shaping PFAS contamination profiles.

To overcome the limitations of passive biomonitoring, **Chapter IV** investigates the use of Asian clams for active biomonitoring in the Scheldt river. PFAS concentrations were analyzed in clams, water, and sediment samples collected from six sites along the river. To evaluate the physiological and molecular impacts of PFAS exposure under field conditions, oxidative stress biomarkers, as well as the condition index and respiration rates of the clams, were assessed in relation to bioaccumulated PFAS concentrations. The Asian clams demonstrated significant bioaccumulation of PFAS, including some that were undetectable in water and sediment samples. This underscores the critical role of biological monitoring in identifying contaminants that may be overlooked in abiotic matrices. Biomarker analysis revealed that PFAS exposure might induce oxidative stress, as evidenced by significant positive correlations between malondialdehyde (MDA) levels and various PFAS, suggesting lipid peroxidation as a response to PFAS-induced damage. The condition index of the clams showed significant negative correlations with PFAS concentrations, indicating potential adverse effects of PFAS exposure on overall physiological health. However, no significant effects on respiration rates were observed. These findings highlight the value of Asian clams as active biomonitors for PFAS contamination and provide evidence of physiological and molecular responses to PFAS exposure. Further research, both in situ and under controlled laboratory conditions is essential to validate these results.

Chapter V studies benthic macroinvertebrate communities in 30 small streams and rivers in Flanders, where ecological water quality was assessed calculating the Multimetric Macroinvertebrate Index of Flanders (MMIF). Two complementary biomonitoring approaches were employed: passive biomonitoring, which involved measuring PFAS concentrations in three resident macroinvertebrate taxa (*Gammarus* sp., *Asellus* sp., and *Chironomus* sp.), and active biomonitoring, which utilized translocated zebra mussels. The study examined whether

bioaccumulated PFAS concentrations were associated with ecological responses, as reflected by changes in the macroinvertebrate community structure. Threshold body burdens, protective of ecological damage were also estimated. These body burdens refer to the concentration limit of a pollutant accumulated within an organism, below which no noticeable ecological impact is observed. Results indicated that long-chain perfluorinated carboxylic acids (PFCAs) were the predominant PFAS detected in both resident macroinvertebrates and translocated zebra mussels. Moreover, a significant relationship was observed between increasing PFAS concentrations in *Gammarus* sp. and zebra mussels and a decline in ecological water quality. These findings suggest that both species are reliable biomonitors, serving two key purposes: (i) monitoring PFAS bioavailability and (ii) predicting ecological responses of macroinvertebrate communities to PFAS pollution. This research provides the first evidence linking accumulated PFAS concentrations in benthic macroinvertebrates to reduced ecological water quality. Additionally, it represents the first documentation of threshold body burdens for specific PFAS in aquatic invertebrates. Further validation of these threshold values could lead to their application in regulatory contexts.

In the final chapter (**Chapter VI**), comprehensive comparison of the findings from all preceding chapters is conducted in relation to both existing and newly proposed Biota Quality Standards. The results of this thesis demonstrate the pervasive presence and bioaccumulation of PFAS in aquatic environments, emphasizing their ecological implications. These findings not only underscore the urgent need for effective regulatory measures but also highlight new research questions and opportunities for further investigation into the mechanisms of PFAS bioaccumulation, species-specific responses, and the development of robust biomonitoring frameworks.

Samenvatting

In 1938 ontdekte Dr. Roy Plunkett, een jonge scheikundige, toevallig 's werelds eerste fluorpolymeer, later polytetrafluorethyleen (PTFE) genoemd, dat algemeen bekend werd onder de marktnaam Teflon. Deze doorbraak maakte de weg vrij voor de ontwikkeling van een grote groep chemische stoffen die gezamenlijk per- en polyfluoralkylstoffen (PFAS) worden genoemd. Volgens de meest recente definitie omvatten PFAS alle chemische stoffen die ten minste één volledig gefluoreerde methylgroep (-CF₃) of methyleengroep (-CF₂-) bevatten. Volgens deze definitie worden naar schatting 7 miljoen stoffen geclassificeerd als PFAS, wat hun grote diversiteit benadrukt. PFAS, ooit beschouwd als industriële wonderen vanwege hun uitzonderlijke duurzaamheid, amfifiele eigenschappen en weerstand tegen hitte en chemicaliën, kennen een wijdverspreid gebruik in verschillende industriële en commerciële toepassingen. Maar juist de eigenschappen die PFAS zo waardevol maken, maken ze ook tot een belangrijke ecologische bedreiging. Hun persistentie in het milieu, neiging tot bioaccumulatie en potentiële toxiciteit hebben tot ernstige bezorgdheid geleid. PFAS-verontreiniging is afkomstig van talloze directe en indirecte bronnen, waaronder industriële processen, lozing van afvalwater en alledaagse consumentenproducten. Deze verontreinigende stoffen zijn alomtegenwoordig geworden in aquatische ecosystemen en vormen een ernstig risico voor het milieu en de volksgezondheid. Dit maakt duidelijk dat er dringend behoefte is aan uitgebreidere monitoringprogramma's en regelgevende richtlijnen.

In Europa zijn de huidige regelgevingen en kwaliteitsnormen voor biota vooral gericht op het beschermen van de menselijke gezondheid en het beschermen van toppredatoren tegen secundaire vergiftiging. Er is echter een opvallend gebrek aan regelgeving die gericht is op het behoud van de ecologische integriteit van gehele ecosystemen. Bovendien blijft ons inzicht in de ecotoxiciteit van PFAS onder reële milieuomstandigheden beperkt door het gebrek aan veldstudies.

In juni 2021 kreeg de PFAS-verontreiniging in België veel aandacht na de ontdekking van hoge PFAS-concentraties in bodemstalen genomen tijdens bouwwerkzaamheden in de buurt van een perfluorchemische fabriek in Antwerpen. Dit incident onderstreepte de ernst van PFAS-verontreiniging en leidde tot veel media-aandacht, met name voor de gevolgen voor het milieu en de gezondheid. Ondanks de toegenomen bewustwording blijft er een gebrek aan kennis bestaan in het begrijpen van het gedrag, de biobeschikbaarheid en de verspreiding van PFAS in aquatische systemen. Deze onopgeloste uitdagingen vormden de basis voor dit werk en benadrukken de nood aan onderzoek om de ecologische, monitoring- en regelgevende tekortkomingen in verband met PFAS-verontreiniging aan te pakken.

Het algemene doel van dit werk is daarom om de bioaccumulatie, verspreiding en ecologische effecten van PFAS in aquatische ecosystemen te beoordelen. De studie omvatte een reeks aquatische ecosystemen, variërend van kleine rivieren en beken tot het Schelde-estuarium en de Belgische Noordzee. Door deze diverse ecosystemen te onderzoeken, evalueerde dit onderzoek de risico's van PFAS bioaccumulatie op meerdere niveaus van biologische organisatie. Bovendien worden in de verschillende hoofdstukken diverse monitoringstechnieken toegepast en geëvalueerd.

De eerste studie (**Hoofdstuk II**) richt zich op de verspreiding, bioaccumulatie en trofische transfer van PFAS in mariene soorten uit de Belgische Noordzee. Op 10 locaties werden zeven vissoorten en twee invertebratensoorten bemonsterd. De resultaten tonen aan dat PFOS overheerst in mariene biota, waarbij ook lange PFAS ketens vaak gedetecteerd worden in lever- en spierweefsels. Er werden ecologische risico's van PFAS, met name voor PFOS, vastgesteld aangezien de concentraties in sommige soorten de gereguleerde biotanormen voor secundaire vergiftiging overschreden. Bovendien werden er risico's voor de menselijke gezondheid vastgesteld, waarbij de consumptie van bepaalde soorten, zoals schol, wijting, garnaal en tong, gevaar kan opleveren als de inname consumptierichtlijnen overschrijdt. Het hoofdstuk benadrukt de dringende behoefte aan uitgebreide mariene monitoringprogramma's om de gezondheid van mens en milieu te beschermen.

Hoofdstuk III concentreert zich op de Schelde, een vitale ecologische en industriële corridor. De studie onderzoekt de verspreiding van PFAS in vissen en ongewervelde dieren op verschillende locaties in de rivier. Door ecotoxicologische data te integreren met ecologische tracers, waaronder stabiele isotopen van koolstof, stikstof en zwavel, wordt de rol van soort-

specifieke voedingsecologie in PFAS bioaccumulatie benadrukt. De bevindingen onthullen verschillende patronen in de verspreiding van PFAS. Reeds lang bestaande verbindingen, zoals PFOS en lange-keten PFCA's, bleken uniform verspreid te zijn langs de rivier, wat duidt op historische vervuiling. Daarentegen waren alternatieve, nieuwe PFAS, waaronder FBSA en 6:2 FTS, geconcentreerd in de buurt van Antwerpen, wat wijst op recente industriële lozingen. Stabiele isotopenanalyses leverden cruciale inzichten in de bioaccumulatielroutes. Pelagische soorten, zoals haring en spiering, vertoonden bioaccumulatieprofielen die werden gedomineerd door nieuwe PFAS-verbindingen, waaronder FBSA, 6:2 FTS en NaDONA, wat duidt op opname via het water vanwege de hoge oplosbaarheid van deze stoffen. Daarentegen vertoonden bentische soorten, zoals paling, bioaccumulatieprofielen die voornamelijk bestonden uit oude verbindingen zoals lange-keten PFCA's, die bij voorkeur adsorberen aan sedimenten. Deze bevindingen onderstrepen het belang van ecologische eigenschappen bij het vormen van PFAS-contaminatieprofielen.

In **hoofdstuk IV** worden Aziatische korfmosselen gebruikt als actieve biomonitors in de Schelde. PFAS concentraties werden geanalyseerd in korfmosselen, water- en sedimentstalen, verzameld op zes locaties langs de rivier. Om de fysiologische en moleculaire effecten van blootstelling aan PFAS onder milieuomstandigheden te evalueren, werden biomerkers voor oxidatieve stress, de conditie-index en het zuurstofverbruik van de korfmosselen onderzocht in relatie tot de bioaccumulatie van PFAS. De Aziatische korfmosselen vertoonden een uitgebreide bioaccumulatie van PFAS componenten, waaronder sommige stoffen die niet detecteerbaar waren in water- en sedimentstalen. Dit onderstreept de cruciale rol van biologische monitoring bij het identificeren van PFAS die mogelijk over het hoofd worden gezien in abiotische matrices. Biomerkeranalyse toonde aan dat blootstelling aan PFAS oxidatieve stress kan veroorzaken, zoals blijkt uit significante positieve correlaties tussen malondialdehyde (MDA)-niveaus en verschillende PFAS. De conditie-index van de korfmosselen vertoonde significante negatieve correlaties met PFAS concentraties, wat wijst op mogelijke nadelige effecten van blootstelling aan PFAS op de algehele fysiologische gezondheid. Er werden echter geen significante effecten op de ademhalingssnelheid waargenomen. Deze bevindingen benadrukken de waarde van Aziatische korfmosselen als actieve biomonitors voor PFAS-verontreiniging en leveren preliminair bewijs voor fysiologische en moleculaire effecten ten gevolge van PFAS-blootstelling. Verder onderzoek, zowel in situ als onder gecontroleerde laboratoriumomstandigheden, is essentieel om deze resultaten te valideren en de

mechanismen die ten grondslag liggen aan PFAS-geïnduceerde stress en gezondheidseffecten in aquatische organismen beter te begrijpen.

Hoofdstuk V richt zich op benthische macroinvertebratengemeenschappen in 30 kleine beken en rivieren in Vlaanderen. De ecologische waterkwaliteit werd bepaald door de Multimetrische Macro-invertebraten Index van Vlaanderen (MMIF) te berekenen. Er werden twee complementaire biomonitoringstechnieken gebruikt: passieve biomonitoring, waarbij PFAS-concentraties werden gemeten in drie residente macroinvertebraten (*Gammarus* sp., *Asellus* sp. en *Chironomus* sp.), en actieve biomonitoring, waarbij getransloceerde zebramosselen werden gebruikt. De studie onderzocht of geaccumuleerde PFAS-concentraties geassocieerd werden met ecologische effecten, zoals weerspiegeld door veranderingen in de structuur van de macroinvertebratengemeenschap. Er werden ook PFAS drempelwaardes voor de lichaamsconcentraties afgeleid ter bescherming van de ecologische integriteit. De resultaten gaven aan dat geperfluoreerde carbonzuren met een lange keten (PFCAs) de voornaamste PFAS waren die werden gedetecteerd in zowel de residente macroinvertebraten als in de getransloceerde zebramosselen. Bovendien werd er een significante relatie waargenomen tussen toenemende PFAS-concentraties in *Gammarus* sp. en zebramosselen en een afname van de ecologische waterkwaliteit. Deze bevindingen suggereren dat beide soorten betrouwbare biomonitors zijn, die twee belangrijke doelen dienen: (i) het monitoren van de biologische beschikbaarheid van PFAS en (ii) het voorspellen van ecologische reacties van macroinvertebratengemeenschappen op PFAS-verontreiniging. Dit onderzoek legt een verband tussen geaccumuleerde PFAS-concentraties in benthische macro-invertebraten en een verminderde ecologische waterkwaliteit. Bovendien is dit de eerste studie die drempelwaarden afleidt voor verschillende PFAS componenten in ongewervelde waterdieren. Verdere validatie van deze drempelwaarden kan leiden tot hun toepassing in regelgevende contexten.

In het laatste hoofdstuk (**hoofdstuk VI**) worden de resultaten uit alle voorgaande hoofdstukken uitgebreid vergeleken met zowel bestaande als nieuw voorgestelde kwaliteitsnormen voor biota. De resultaten van dit proefschrift tonen de alomtegenwoordigheid en bioaccumulatie van PFAS aan in aquatische milieus en benadrukken hun ecologische implicaties. Deze bevindingen onderstrepen niet alleen de dringende nood aan doeltreffende regelgevende maatregelen, maar vestigen ook de aandacht op nieuwe onderzoeksvragen en mogelijkheden voor verder onderzoek naar de mechanismen van PFAS bioaccumulatie, soortspecifieke implicaties en de ontwikkeling van robuuste kaders voor biomonitoring.

List of Abbreviations

abbreviation	Meaning
11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid
3.6-OFHpA	Perfluoro-3,6-dioxaheptanoic acid
4:2 FTS	4:2 Fluorotelomer Sulfonic Acid
6:2 FTS	6:2 Fluorotelomer Sulfonic Acid
8:2 FTS	8:2 Fluorotelomer Sulfonic Acid
9CL-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane--sulfonic acid
ABM	Active biomonitoring
ACN	Acetonitrile
AFFFs	Aqueous film-forming foams
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
BQS	Biota Quality Standard
BSAF	Biota Sediment Accumulation Factors
EFSA	European Food Safety Authority
EPT-taxa	Ephemeroptera, Plecoptera, Trichoptera taxa
EU	European Union
FABP	Fatty acid binding proteins
FASAs	Fluorinated alkane sulfonamides
FBSA	Perfluorobutane sulfonamide
FOSA	Perfluorooctane sulfonamide
FPPPs	Fluorinated Plant Protection Products
FRAP	Ferric Reducing Ability of Plasma
FTOHs	Fluorotelomer alcohols
HFPO-DA	Hexafluoropropylene oxide dimer acid
IAEA	International Atomic Energy Agency
K_d	Sediment – water partition coefficient
LOQ	Limit of Quantification
MDA	Malondialdehyde
MLs	Maximum contamination Levels
MMIF	Multimetric Macroinvertebrate Index Flanders
MTS	Mean Tolerance Score

MXR	Multixenobiotic Resistance
NaDONA	Sodium dodecafluoro-3H-4,8-dioxanonoate
NST	Number of Sensitive Taxa
OATs	Organic anion transporters
PBM	Passive biomonitoring
PF4OPeA	Perfluoro-4-oxapentanoic acid
PF5OHxA	Perfluoro-5-oxahexanoic acid
PFAAs	Perfluoroalkyl acids
PFAS	Per- and polyfluorinated alkyl substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFCAs	Perfluoroalkyl carboxylic acids
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFDS	Perfluorodecanesulfonic acid
PFECAs	Perfluoroether carboxylic acids
PFESAs	Potassium perfluoro(2-ethoxyethane)sulfonate
PFESAs	Perfluoroether sulfonic acids
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptanesulfonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFPeA	Perfluoropentanoic acid
PFPeS	Perfluoropentanesulfonic acid
PFSAs	Perfluoroalkyl sulfonic acids
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFUnDA	Perfluoroundecanoic acid
POPs	Persistent organic pollutants
PTFE	Polytetrafluoroethylene
QS	Quality Standard
REACH	Registration, Evaluation, Authorization, and Restriction of Chemicals
RPF	Relative Potency Factor
SCHEER	Scientific Committee on Health, Environmental and Emerging Risks

SD	Standard Deviation
SEA_c	Corrected Standard Ellipse Area
SWD	Shannon-Wiener Diversity index
TAC	Total Antioxidant Capacity
TAX	Taxonomic richness
TMF	Trophic Magnification factor
TOC	Total Organic Carbon
TPC	Total Polyphenols Content
TWI	Tolerable Weekly Intake
WWTPs	Wastewater Treatment Plants

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Chapter I

General Introduction

1.1. PFAS, A Chemical Wonder

1.1.1. The Accidental Invention

In the aftermath of the First World War, the global economy was in a state of depression (Romer, 1988). This period was characterised by intense pressure on scientists to identify new materials to support the development of new technologies and accommodate population growth. The field of polymer science, in particular, witnessed a rapid expansion at both the academic and industrial level (Ebnesajjad, 2013). In 1938, a young chemist named Dr. Roy Plunkett was engaged in the development of a novel project within the laboratories of DuPont's Jackson Laboratory in New Jersey (Science History Institute, 2017). His objective was to develop a novel refrigerant gas to replace the existing, hazardous alternatives. While chlorofluorocarbons (CFCs) represented a significant improvement, there was a persistent drive to identify a superior alternative. Therefore, Dr. Plunkett was engaged in experimentation with tetrafluoroethylene (TFE) gas (Norton, 2021). The gas was stored in small cylinders and prepared for reaction with hydrochloric acid. However, upon opening one of the cylinders, no gas was observed to emerge. Perplexed by this outcome, Plunkett proceeded to weigh the cylinder, which he found to be heavier than anticipated, as if the gas had undergone a phase transition and solidified. Dr. Plunkett was intrigued by this phenomenon and proceeded to cut open the cylinder, thereby revealing a slippery, waxy white powder within. In collaboration with his laboratory assistant, Jack Rebok, Plunkett initiated an investigation into the nature of the substance. The material demonstrated exceptional resistance to heat, chemical inertness, and a low coefficient of friction. It was identified as polytetrafluoroethylene (PTFE), the world's first fluoropolymer (Garrett, 1962; Ebnesajjad, 2013). A series of tests was conducted by Plunkett and his team in order to ascertain the potential of this new material. The non-reactivity and heat resistance of PTFE made it suitable for a variety of applications (Gaines, 2023). However, its true potential was in its hydrophobic properties, which rendered it highly slippery. This property resulted in the development of a revolutionary product which was patented by Dupont in 1994. The initial deployment of PTFE was in a military context, namely the Manhattan project, which aimed to develop an atomic bomb (Dams & Hintzer, 2016; Norton, 2021). Therefore, PTFE was utilised for the coating of valves and seals due to its capacity to withstand the corrosive impact of uranium hexafluoride. After the Second World War, PTFE was

introduced to the market under the trade name Teflon. Initially, its primary application was as a non-stick coating for cookware, marking a significant shift from laboratory research to widespread domestic use. Following this, the development of additional fluorinated compounds, including perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), expanded the range of applications into various industrial and commercial sectors (Gaines, 2023). For example, these compounds are utilized as surfactants in the mining and oil and gas industries, where they enhance metal recovery from ores and improve oil or gas extraction from wells (Murphy & Hewat, 2008; UNEP, 2011; Xu et al., 2017). Due to their properties, such as reducing surface tension, enhancing penetration, and improving rinse-off and uniform surface coverage, Per- and polyfluoroalkyl substances (PFAS) are also employed in numerous cleaning products, as well as coatings such as waxes, paints, inks, and in aqueous film-forming foams (AFFFs) used in firefighting (Kissa, 1994; Knepper & Lange, 2012; UNEP, 2011; Wang et al., 2014). Additionally, PFAS are present in consumer products frequently used by the public, serving as emulsifiers in cosmetics and as water-resistant agents in food packaging (Gaines, 2023; Glenn et al., 2021). Despite the wide array of PFAS applications, it remains difficult to determine which specific compounds are employed and in what quantities across various industries (Gaines, 2023).

1.1.2. Terminology, Classification and Properties

The terminology surrounding PFAS has evolved significantly over the years, reflecting both advances in chemical synthesis and growing awareness of their environmental and health impacts. Initially, terms such as "perfluorinated chemicals," "perfluorinated organics," and "highly fluorinated compounds" were used inconsistently, leading to confusion among scientists and regulators (Wang et al. 2021). These terms often referred to different groups of fluorinated substances, without clear distinctions between them. In 2011, a milestone in PFAS terminology was achieved with the publication of a comprehensive classification system by Buck et al. (2011). This work provided a clear structural definition of PFAS and established a standardized set of names and acronyms for over 200 individual PFAS. Buck et al. (2011) defined PFAS as aliphatic substances that contain one or more carbon atoms on which all hydrogen substituents have been replaced by fluorine atoms, resulting in the presence of a perfluoroalkyl moiety ($C_nF_{2n+1}-$). This definition was widely adopted and became the foundation for PFAS classification and regulation in subsequent years. However, as scientific research progressed, it

became clear that this definition did not fully capture the diversity of PFAS. Many newly identified substances, including those with complex structures and functional groups, did not fit into this definition. Furthermore, advancements in analytical techniques revealed the presence of previously unknown PFAS in environmental and product samples, prompting the need for a more inclusive definition. Recognizing these limitations, Wang et al. (2021) proposed a revised definition of PFAS that encompasses a broader range of compounds. According to this new definition, PFAS are "fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any hydrogen, chlorine, bromine, or iodine atom attached to it)" (Wang et al., 2021). This definition includes any chemical with at least a perfluorinated methyl group ($-\text{CF}_3$) or a perfluorinated methylene group ($-\text{CF}_2-$), with few exceptions. The rationale behind this revision is to create a coherent and consistent definition that can be easily understood and implemented across different sectors, from academic research to regulatory enforcement. In addition, this publication has led to an increased awareness of the number of PFAS that are generally known.

Aside from this updated definition, the framework proposed by Buck et al. (2011) remains the most commonly used for the classification of PFAS. PFAS can be classified in a number of ways, according to their chemical structure, production processes, and environmental properties. Initially, the PFAS family is divided into two principal categories: non-polymers and polymers. The polymers category encompasses fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers, which are constituted by the linkage of monomer units in a repeating pattern, resulting in the formation of large molecules (Buck et al., 2011). In literature, polymeric PFAS are generally described as water-insoluble, non-bioaccumulative, non-toxic and not prone to long-range environmental transport (Henry et al., 2018; Korzeniowski et al., 2023). Consequently, they are typically classified as 'polymers of low environmental concern' and were not further addressed in this work. However, a recent review questions these assumptions, highlighting significant and widespread environmental contamination by polymeric PFAS (Lohmann and Letcher, 2023). This finding underscores the urgent need to prioritize this group of chemicals in future scientific research. The non-polymer PFAS encompass two major subclasses: perfluoroalkyl and polyfluoroalkyl substances (Figure 1.1). The perfluoroalkyl substances are characterized by the replacement of all hydrogen atoms with fluor atoms, except for hydrogens whose substitution would modify the nature of any functional group. In contrast, polyfluoroalkyl substances are compounds for which all

hydrogens on at least one (but not all) carbons have been replaced by fluor atoms (Buck et al., 2011).

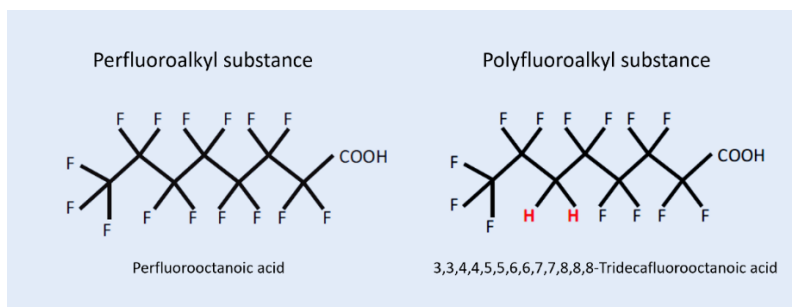


Figure 1.1: Examples of perfluoroalkyl and polyfluoroalkyl substances (PFAS) (modified from Buck et al., 2011).

Both classes are each further subdivided in many groups and subgroups. The perfluoroalkyl acids (PFAAs) family encompasses perfluoroalkyl carboxylic, sulfonic, sulfinic, phosphonic, and phosphinic acids. Among these, perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAAs) are identified as the two primary groups of environmental significance (Buck et al., 2011). These compounds, with the general chemical formulas $C_nF_{2n+1}COOH$ and $C_nF_{2n+1}SO_3H$, are distinguished by a fluorinated tail with low polarizability, leading to pronounced hydrophobic behavior (Leung et al., 2023). When combined with a hydrophilic polar head, this structure gives rise to the amphiphilic nature of PFAAs (Johnson et al., 2022). Furthermore, the high electronegativity of fluorine creates a very strong bond with carbon, making these compounds chemically stable. This chemical stability means that PFAS are not easily broken down by natural processes such as hydrolysis, photolysis, or microbial activity. PFAAs are often referred to as either "long-chain" or "short-chain" compounds. It is important to note that the definition of chain length differs between PFCAs and PFSAAs. For PFCAs, "long-chain" compounds have eight or more carbon atoms, whereas for PFSAAs, "long-chain" refers to compounds with six or more carbon atoms (Buck et al., 2011). Two of the most extensively studied "long-chain" compounds are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), which were phased out in 2002 due to their extreme persistence, bioaccumulation capacity, and toxicity (Houde et al., 2006; Wang et al., 2013). Consequently, another subgroup within the PFAAs family, frequently overlooked, includes perfluoroether carboxylic acids (PFECAs) and perfluoroether sulfonic acids (PFESAAs). This group comprises alternative compounds, such as NaDONA and GenX, which have been developed with the intention of

replacing PFOS and PFOA (Munoz et al., 2019; Wang et al., 2017). Moreover, the phase-out of PFOS and PFOA has prompted a shift in production from long-chain to short-chain PFCAs and PFSA, given that short-chain PFAS are regarded as suitable substitutes because they are assumed to be less bioaccumulative and toxic (Ateia et al., 2019). At last the family of polyfluoroalkyl substances consists of two primary subgroups: perfluoroalkane sulfonamido substances and fluorotelomer substances (Buck et al., 2011). Unlike fully fluorinated compounds, polyfluoroalkyl substances have weaker carbon-hydrogen bonds, making them more vulnerable to biotic and abiotic transformation. As a result, polyfluoroalkyl substances containing a perfluoroalkyl C_nF_{2n+1} moiety are considered potential precursor compounds that can be transformed into PFAAs (Butt et al., 2014; Liu and Avendaño, 2013). Figure 1.2 offers a schematic overview of the PFAS groups and subgroups with examples of their chemical structures. It should be noted that this figure is not exhaustive and is primarily intended to highlight the compounds that form the primary focus of this work.

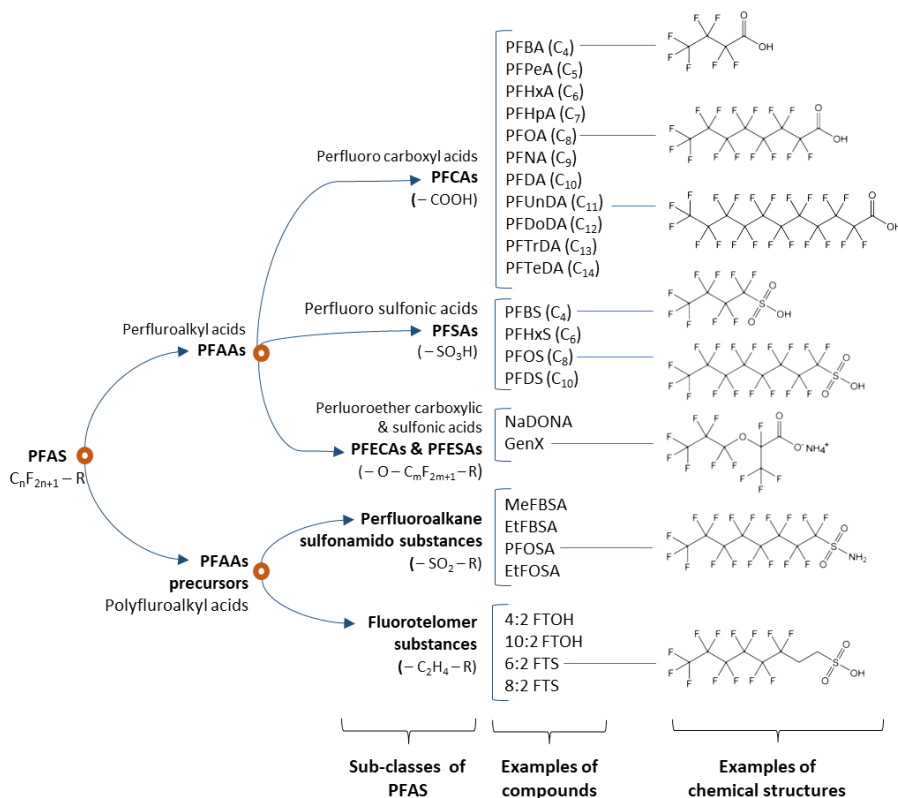


Figure 1.2: PFAS simplified family tree.

1.2. PFAS, Here, There, Everywhere

1.2.1. Contamination Sources and Long-range Transport

PFAS have become a significant environmental concern due to their widespread contamination and persistent nature. Their release into the environment happens through numerous direct and indirect pathways, leading to their ubiquitous presence globally (Kurwadkar et al., 2022; Khan et al., 2023).

Manufacturing facilities that produce or use PFAS in various products, such as non-stick coatings, stain repellents, and firefighting foams, are considered major direct sources of PFAS to the environment (Prevedouros et al., 2006). For example, the textile and leather industries, paper mills, and chemical manufacturing plants release PFAS directly into the air, water, and soil through emissions and waste disposal practices (Dasu et al., 2022). The environments surrounding of such chemical plants are often identified as contamination hotspot (Groffen et al., 2017; Menegatto et al., 2022). Another significant direct source of PFAS contamination is the use of aqueous film-forming foams (AFFF) in firefighting, particularly at airports, military installations, and fire-training sites (Barzen-Hanson et al., 2017). Several studies have documented the long-lasting impact of firefighting foam use on water quality, highlighting PFAS concentrations far above regulatory limits (Anderson et al., 2018, 2019; Filipovic et al., 2015; Houtz et al., 2013). Wastewater treatment plants (WWTPs) play a crucial role in the indirect dissemination of PFAS. Although WWTPs are designed to remove contaminants, PFAS often resist degradation during the treatment process or are not completely retained in the sludge, and are released into water bodies along with treated effluent (Lenka et al., 2021). A number of studies have documented higher PFAS concentrations in effluents than in influents, with biodegradation identified as the primary mechanism responsible for the transformation of PFAS precursors to PFAAs (Barisci and Suri, 2021; Eriksson et al., 2017; Lee et al., 2010). Furthermore, the sewage sludge from WWTPs were in the past commonly applied as fertilizer on agricultural land, which served as another vector for spreading PFAS into soils, contributing to their indirect environmental release (Fredriksson et al., 2022). Another significant indirect source of PFAS is the leachate from landfills due to the disposal and destruction of PFAS-containing commercial products (Huset et al., 2011; Lang et al., 2017).

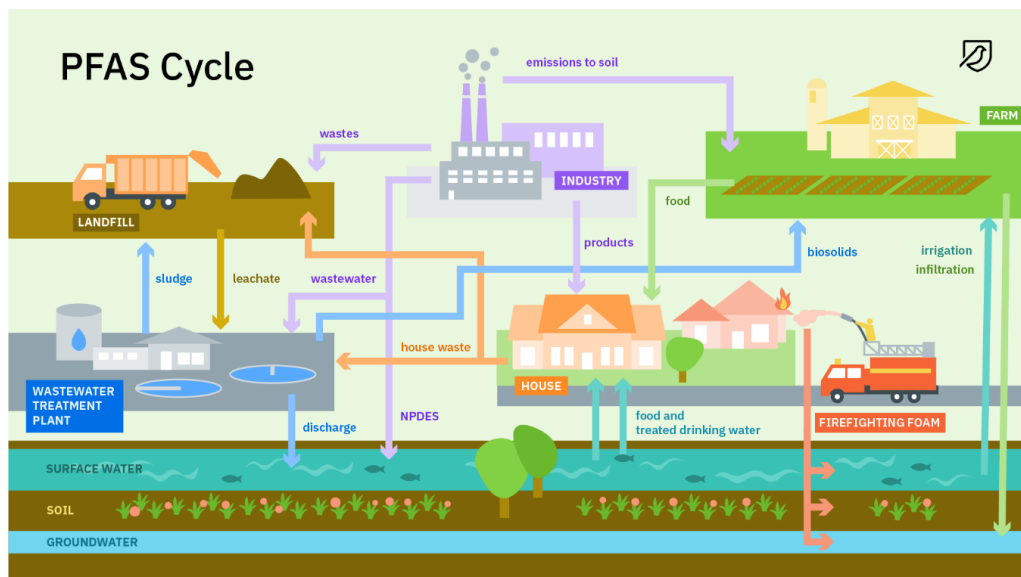


Figure 1.3: PFAS cycle, sources and distribution. Image sourced from www.ecoonline.com.

Once released into the environment, PFAS do not remain localized but can travel vast distances, resulting in their global distribution (Muir and Miaz, 2021; Yamashita et al., 2005). Their long-range transport is facilitated by both atmospheric and oceanic processes (Armitage et al., 2009). Volatile PFAS precursors, such as fluorotelomer alcohols (FTOHs), can enter the atmosphere and undergo long-range transport through atmospheric circulation (Stock et al., 2007; Zhao et al., 2012). During atmospheric transport, these precursors degrade into more stable PFCAs and PFSAs, which eventually can deposit in regions far from their original source (Wallington et al., 2006). For instance, air sampling studies have shown significant levels of PFAS in the Arctic, suggesting that atmospheric transport plays a critical role in their global spread (Stock et al., 2007; Young et al., 2007; Zhao et al., 2012). Ocean currents further contribute to the widespread distribution of PFAS (Muir and Miaz, 2021). Several studies have detected PFAS in remote marine environments, far from industrialized areas (Armitage et al., 2009; Prevedouros et al., 2006). A study by Yamashita et al. (2005) found detectable levels of PFOS and PFOA in the deep waters of the Pacific and Arctic Oceans, underscoring the ability of oceanic currents to transport PFAS across hemispheres. Furthermore, PFAS concentrations in sea-spray aerosols were found to be significantly higher than in seawater, with enrichment factors ranging from 522 to 4690 (Casas et al., 2020). This suggests that sea-spray aerosols may play a crucial role as a vector, facilitating the transition of PFAS from oceanic transport to long-range atmospheric transport. The relative importance of atmospheric and oceanic transport

remains unclear, but oceanic transport is currently regarded as the most significant pathway (Ahrens et al., 2023; Boucher et al., 2019). In contrast, Yeung et al. (2017) estimated that atmospheric inputs of PFOA in the Arctic mixed ocean layer accounted for a range of 34 to 59%.

1.2.2. Trends in the Abiotic Environment

PFAS contamination is not restricted to specific environments; rather it affects a wide array of environmental matrices including surface waters, groundwater, and sediments. Water bodies, in particular, serve as significant reservoirs for PFAS, with detectable levels reported across the globe (Kurwadkar et al., 2022).

Numerous studies have explored PFAS concentrations in both freshwater and marine environments, providing insights into the spatial and temporal trends of these pollutants. In freshwater ecosystems, PFAS contamination is frequently linked to industrial and urban activities (Baabish et al., 2021; Dauchy et al., 2012; Liu et al., 2016). In Western Europe, PFAS levels detected in surface waters are generally higher compared to the Northern Scandinavian waters (Mclachlan et al., 2007). In a European study on the discharge of PFCAs in 14 major European rivers, the Po river, in Southern Europe, Italy, was identified as the predominant source of PFOA in Europe, accounting for two-thirds of the total PFOA discharge among all the rivers analyzed (Mclachlan et al., 2007). With maximum PFOA concentrations up to 337 ng/L, the contamination of the Po river is likely coming from local industrial sources (Loos et al., 2008). Also in other European rivers such as the Rhone and Danube, PFOA and PFOS were identified as predominant compounds in surface waters. In Germany, a comprehensive, long-term study was conducted to monitor the overall trends in PFAS occurrence in the surface water of the Hesse region from 1999 to 2018 (Janousek et al., 2019). The results indicated a decline in both the detection frequency and maximum concentrations of legacy compounds such as PFOA over the course of the study. Conversely, there was a notable increase in the detection frequency of short-chained PFAS, particularly after 2014. This is likely to be linked to the production shift towards short-chain compounds. In the study by Muir & Miaz (2021), riverine discharges of PFAS were estimated over a time period from 2000 to 2019 in coastal waters worldwide. A general decrease in the discharge of long-chain PFCAs was observed in the Mediterranean Sea, the North Sea, and the North Atlantic. In contrast, discharges into the Bohai Sea, Yellow Sea, and South China Sea either increased or remained stable. This trend is likely

attributable to the continued use of legacy compounds such as PFOS and PFOA in several industries in China, despite their phase-out in many other countries. Also in open oceans, including the North and South Atlantic, Indian Ocean, Arctic Ocean, and North Pacific, Muir & Miaz (2021) reported a general increase in the ratios of perfluorobutane sulfonate (PFBS)/ Σ PFSA and Σ short-chain PFCAs/ Σ long-chain PFCAs. Although these short-chain alternatives are presumed to have lower bioaccumulation potential and lower toxicity, research has shown that they exhibit distinct yet equally concerning properties compared to their long-chain counterparts (Bowman, 2015; Gomis et al., 2018; Wang et al., 2017). Specifically, short-chain PFAS remain highly persistent and demonstrate greater water solubility, leading to an increased environmental mobility (Brendel et al., 2018). Recent studies have further indicated that short-chain PFAS tend to predominate in the water phase, while long-chain PFAS are more commonly found in sediments (Zhao et al., 2016, Bai & Son, 2021).

Considering that certain PFAS tend to adsorb to sediments, these sediments may serve as substantial sinks and reservoirs for contaminants, thereby playing a critical role in the distribution, transport, and bioavailability of pollutants within aquatic ecosystems, particularly when they are disturbed or resuspended (Munoz et al., 2015; Reif et al., 2022).

The bioavailability of PFAS refers to the fraction of the chemical that is accessible for uptake by an organism and capable of interacting with biological systems, such as tissues or membranes (Gourlay-Francé & Tusseau-Vuillemin, 2013). Importantly, the total concentration of a contaminant in a system does not necessarily reflect the concentration to which organisms are exposed. For example, when PFAS are adsorbed onto sediments, they may not be bioavailable to organisms that primarily feed in the water column. To assess the environmental behavior of PFAS, the sediment-water partition coefficient (K_d) is often employed (Johansson et al., 2001; Zhang et al. 2013; Zhao et al. 2016). The K_d value describes the equilibrium distribution of a substance between sediment or soil and water. A low K_d implies the substance exists predominantly in the dissolved phase, whereas a high K_d indicates stronger adsorption to particulate matter, making it more likely to accumulate in sediments. Several factors influence K_d values, including the chemical properties of PFAS, such as carbon chain length and functional groups, as well as environmental factors including sediment organic content, salinity, and pH (Li et al., 2018; Munoz et al., 2017a; Navarro et al. 2022). The primary mechanisms driving PFAS sorption to particles are hydrophobic and electrostatic interactions (Alves et al., 2020; Leung et al. 2023). The hydrophobic interactions are driven by the repulsion of the hydrophobic

fluorinated tails from water and their attraction to non-polar, organic rich regions of sediments (Li et al., 2018). Electrostatic attractions are formed between the negatively charged functional head of PFAS and the positive charges on the organic phases of the sediment (Li et al., 2018). Research has demonstrated that the sorption of PFAS to organic matter increases with chain length, a trend attributed to the enhanced hydrophobicity of longer-chain compounds (Ahrens & Bundschuh, 2014; Groffen et al., 2019b; Joerss et al., 2019). Nevertheless, field research has indicated that this chain-length dependency trend is predominantly observed in long-chain PFCAs and PFSA, but does not necessarily extend to short-chain compounds (Habibullah-Al-Mamun et al. 2016; Joerss et al., 2019; Zhu et al. 2014). These findings indicate that alternative mechanisms may influence the water-sediment partitioning of short-chain PFAS. For example, ion exchange or interactions with sorption sites, which are inaccessible to larger molecules due to steric hindrance, may play a significant role in this process (Guelfo & Higgins, 2013). The functional groups attached to PFAS molecules also play a critical role in determining their hydrophobicity and, consequently, their sorption behavior. For instance, PFSA are generally more hydrophobic and exhibit stronger sorption than PFCAs (Gagliano et al., 2020; Milinovic et al., 2015).

Sediment or soil organic carbon has been identified as the primary environmental factor influencing PFAS sorption to sediments and soils (Higgins and Luthy 2006; Liu and Lee 2005). Consequently, PFAS tend to accumulate in environments with high organic carbon content (Higgins & Luthy, 2006; You et al., 2010). This relationship is particularly strong for long-chained PFAS, which have been correlated with the total organic carbon (TOC) content (Gagliano et al., 2020; Joerss et al., 2019; Zhao et al., 2016). However, PFAS sorption cannot be accurately predicted based solely on organic carbon content (Li et al., 2018). Salinity represents a further critical abiotic factor influencing the distribution and fate of PFAS, particularly in aquatic environments exhibiting salinity gradients, such as estuaries (Miranda et al., 2021; Munoz et al., 2017a). Studies indicate that increasing concentrations of cations, such as Ca^{2+} , Na^+ , and Mg^{2+} , enhance PFAS sorption to sediment through multiple mechanisms (You et al., 2010; Navarro et al., 2022). The cations will neutralize the negative charge on the sediment surface, and thereby decrease the repulsion with the negatively charged PFAS heads. The sorption is further enhanced due to the salting-out effect, which occurs because the presence of dissolved ions disrupts the normal structure of water molecules, making the solvent environment less favorable for the dissolution of non-polar compounds (Turner & Rawling, 2001). Additionally,

divalent cations can form bridges between the anionic functional groups of PFAS and the negatively charged soil or sediment surface, further enhancing sorption (Li et al., 2018). The pH of the water is another factor that can influence the electrostatic interactions within sediment or soil (Oliver et al., 2019). Generally, lower pH levels result in increased K_d values, indicating greater sorption to sediment and a net surface charge closer to neutral (less negative) (Higgins & Luthy, 2006; Oliver et al., 2019). Research on the influence of temperature on the sediment-water partitioning of PFAS is limited; however, a study by Wang et al. (2023) observed a decrease in K_d values for PFAS with increasing temperatures. This behavior was explained by the exothermic nature of PFAS sorption to sediment, where increasing temperatures reduce the affinity of PFAS for sediment, favoring their transfer from sediment to water.

It is important to note that abiotic factors alone are unlikely to be the sole determinants of PFAS sorption. Instead, it is the interactions between these factors and the PFAS themselves that play a crucial role. This interplay represents a significant knowledge gap that must be addressed to achieve a more comprehensive understanding of the behavior and fate of PFAS in aquatic environments.

1.2.3. PFAS Bioaccumulation and Biomagnification in Aquatic Systems

PFAS are not only persistent in abiotic environments but also exhibit bioaccumulative properties, leading to their accumulation in living organisms. The bioaccumulation of PFAS has been extensively studied in both freshwater and marine ecosystems, revealing significant concerns about the transfer of these chemicals through food webs and their potential impacts on wildlife and human health (Houde et al., 2011).

Before delving into the bioaccumulation of PFAS, it is essential to define key terms relevant to this research, including bioconcentration, bioaccumulation, and biomagnification. **Bioconcentration** refers to the direct uptake of PFAS by an organism from the surrounding abiotic environment, quantified by the bioconcentration factor (BCF) (Kowalska et al., 2024). **Bioaccumulation** encompasses the total amount of PFAS accumulated in an organism over time, including both via bioconcentration and dietary intake, and is expressed using the bioaccumulation factor (BAF) (Burkhard, 2021). **Biomagnification** describes the increase in PFAS concentrations in organisms at higher trophic levels within a food chain, based on predator-prey relationships, and is typically measured using the biomagnification factor (BMF)

(Cheng et al., 2022). Additionally, the trophic magnification factor (TMF) is used to describe biomagnification, based on field measurements of contaminant concentrations across relative trophic levels (Borgå et al., 2012; Miranda et al., 2022). Another important metric is the biota-sediment accumulation factor (BSAF), which quantifies the accumulation of a compound in an organism through sediment exposure (Abercrombie et al., 2021). However, the use of these metrics in the literature varies widely and is influenced by environmental factors, leading to significant discrepancies in reported values and complicating the comparison of metrics across different systems.

The bioaccumulation of PFAS in biota is largely attributed to their strong binding affinities to proteins rather than passive diffusion across cell membranes. Due to their amphiphilic and ionizable nature, many PFAS do not readily diffuse through cell membranes at environmentally relevant pH values (Bangma et al., 2022). Instead, PFAS primarily enter and distribute within organisms through protein-mediated transport mechanisms. Key proteins include serum proteins such as albumin, which binds PFAS in blood plasma and facilitates systemic circulation (D'eon et al., 2010), fatty acid binding proteins (FABP) that aid intracellular transport (Luebker et al., 2002), and organic anion transporters (OATs), which actively mediate PFAS uptake into cells (Nakagawa et al., 2008). Bioaccumulation models on fish have indicated that PFAS tend to accumulate through dietary and gill uptake. The bioaccumulation model presented by Liang et al. (2022) demonstrates that the uptake and elimination of PFAS in fish are strongly influenced by molecular chain length. The model indicates that shorter-chain PFAS (C6–C8) are primarily absorbed through the gills and rapidly eliminated via respiration. As the chain length increases, dietary intake becomes the predominant route of PFAS uptake, while gill absorption plays a diminished role (Liang et al., 2022). Simultaneously, the elimination of longer-chain PFAS occurs at a slower rate, primarily through fecal excretion, with higher retention observed in fish with elevated protein content (Liang et al., 2022). Very long-chain PFAS (C12–C16) present significant challenges for excretion, leading to substantial bioaccumulation in fish tissues. Recent research has also identified dermal exposure as a significant additional uptake route for PFAS in fish (Xiong & Li, 2024). The bioaccumulation model indicates that 46–75% of the total short-chain PFAS (C6–C7) uptake occurs through dermal absorption, while for long-chain PFAS (C8–C12), dermal exposure accounts for 2–27% of the total uptake. Furthermore, renal elimination has been identified as an additional pathway for PFAS excretion, particularly for shorter-chain compounds (Sun et al., 2022). The model indicates that fish with more efficient

renal clearance exhibit significantly lower bioaccumulation levels, as the kidneys play a crucial role in removing PFAS from the bloodstream. The mechanisms governing the uptake and elimination of PFAS in aquatic invertebrates have received comparatively less attention than those in fish, likely due to the substantial variability in these processes across species. Such differences arise from variations in habitat, feeding behavior, respiratory mechanisms, and metabolic capacity. A comparative study on worms, snails, and mussels has demonstrated species-specific bioaccumulation patterns, with worms exhibiting the highest PFAS accumulation (Yun et al., 2023). This is primarily attributed to sediment ingestion as their dominant uptake pathway, coupled with their relatively limited metabolic capacity for contaminant elimination (Liang et al., 2022; Higgins et al., 2009). In contrast, mussels and snails, which primarily rely on gill respiration, possess an additional elimination mechanism via gill exchange, a pathway absent in worms, potentially reducing their PFAS accumulation rates (Yun et al., 2023). Furthermore, in certain mussel species, such as the zebra mussel (*Dreissena polymorpha*) and the California mussel (*Mytilus californianus*), an additional mechanism known as the multixenobiotic resistance (MXR) system has been identified as actively facilitating PFAS elimination (Fernández-Sanjuan et al., 2013; Stevenson et al., 2006). These species-specific disparities highlight the complexity of PFAS bioaccumulation.

The bioaccumulation of PFAS in aquatic ecosystems starts at the lowest trophic levels, such as biofilms and aquatic plants. Notably, few studies have focused on PFAS bioaccumulation within biofilms. PFAS can adsorb to the surface of biofilms, be incorporated into extracellular polymeric substances (EPS), or accumulate intracellularly (Gerbersdorf et al., 2015). Munoz et al. (2018) reported high detection frequencies, particularly for long-chain PFAS, in freshwater biofilms. Additionally, the composition of biofilms may significantly influence the extent of bioaccumulation. For example, in bacteria, PFAS can be incorporated both intracellularly and within cell membranes (Fitzgerald et al., 2018; Zhang et al., 2013). A comparative study has shown that biofilms tend to accumulate a broader range of PFAS compared to planktonic algae (Penland et al., 2020). Overall, biofilms likely play a significant role in enhancing the bioavailability of PFAS to their consumers. However, further research is needed to better understand their contribution to the PFAS transfer to higher trophic levels. Furthermore, the accumulation of PFAS in aquatic plants has also not been thoroughly studied. In general, plant species and habitat characteristics, such as whether the plant is submerged, emergent, sediment-rooting, floating-leaved, or free-floating, appear to be important factors influencing

the bioaccumulation (Zhang et al., 2020). For example, submerged plants have been found to have a higher bioaccumulation potential than emergent or free-floating species, suggesting that the uptake occurs primarily through the water column (Wang et al., 2019). Furthermore, a distinct partitioning has been observed within plant parts, with long-chain PFAS predominantly retained in the roots, while short-chain PFAS exhibit greater mobility and are thus translocated to the shoots (Pi et al., 2017; Wang et al., 2019).

As we move further up the food chain, invertebrates represent the first trophic level exposed to PFAS not only through water and sediment but also through their diet. Nevertheless, research on the accumulation of PFAS in invertebrates, both in marine and freshwater environments, remains scarce. In the context of freshwater macroinvertebrates, foraging mode (e.g. grazers, gatherers, shredders, filterers, and predators) is regarded as a pivotal factor in determining the specific PFAS to which macroinvertebrates are exposed and subsequently bioaccumulate (Bertin et al., 2014, 2016; Lewis et al., 2022; Prosser et al., 2016). Furthermore, research has indicated that the bioaccumulation of PFAS in freshwater macroinvertebrates is influenced by a number of environmental factors, including dissolved organic matter (DOM), dissolved cations and sediment composition as these factors can alter the bioavailability of PFAS (Lasier et al., 2011; Wen et al., 2016; Xia et al., 2015). In marine crustaceans, diet and feeding strategy have also been identified as key factors driving species-specific differences in PFAS bioaccumulation, particularly between benthic and pelagic invertebrates (Khan et al., 2023). Among marine invertebrates, crustaceans and mollusks are the most extensively studied. A diverse array of PFCAs and PFSA has been documented to bioaccumulate, with the highest concentrations observed in bivalves (Khan et al., 2023). Notably, bioconcentration factors for PFOA have been reported to be higher in bivalves than in fish (Quinete et al., 2009). This can be attributed to the lack of advanced excretory organs, such as kidneys and liver, in bivalves, resulting in slower elimination of PFOA (Quinete et al., 2009). Additionally, their limited enzymatic metabolism compared to fish and efficient uptake through filter feeding further contribute to higher accumulation.

Data on PFAS bioaccumulation in fish, both in marine and freshwater environments, is more abundant, allowing for clearer observation of trends. This extensive research in fish is likely due to the fact that fish consumption is recognized as a primary pathway for human exposure to PFAS (Berger et al., 2009; Zhao et al., 2011; Shi et al., 2012; Augustsson et al., 2021). Consequently, PFAS levels are frequently reported in fish muscle tissue, despite the much

higher accumulation affinity in other tissues such as the liver and blood (Martin et al., 2003; Chen et al., 2021; Hoa et al., 2022; Melake et al., 2022). This tissue partitioning of PFAS in fish has been widely documented in both freshwater and marine species and is attributed to the high binding affinity of PFAS to proteins such as serum albumin and fatty acid-binding proteins, which are more prevalent in liver tissue (Conder et al., 2008; Shi et al., 2012). As a result, certain fish species with lower concentrations in muscle may not be considered a risk to food safety for humans. However, elevated concentrations in their liver tissue may indicate overall poor fish health and represent a significant pathway for biomagnification when consumed by predator species (Khan et al., 2023). Furthermore, research on PFAS bioaccumulation in fish has revealed that BCFs and BAFs are generally higher for PFSAAs compared to PFCAs of the same chain length (Khan et al., 2023; Lewis et al., 2022). This can be attributed to the stronger binding affinity of the sulfonic acid group in PFSAAs to proteins, particularly albumin, in comparison to the carboxyl group in PFCAs (Zhao et al., 2023). A number of studies have reported differences in the bioaccumulation of PFAS between carnivorous and herbivorous fish in freshwater environments. The findings indicate that long-chain PFAS are present in higher proportions in carnivorous fish, while short-chain PFAS are more abundant in herbivorous and omnivorous fish (Lam et al., 2014; Ahrens et al., 2016; Liu et al., 2019; Lee et al., 2020). In marine environments, a distinction has been observed between cartilaginous and bony fish, with long-chain PFCAs being more accumulated in the liver and muscle tissues of cartilaginous fish compared to bony fish (Khan et al., 2023). These differences are likely attributable to variations in feeding strategies, trophic positions, and physiological characteristics; however, further research is needed to confirm these assumptions. In both marine and freshwater environments, the biotransformation of precursor compounds, such as perfluorooctane sulfonamide (FOSA) and FTOHs, has been identified as a significant source of variability in PFAS bioaccumulation among species, alongside environmental and ecological factors (Martin et al., 2010). Particularly in marine environments, higher trophic-level species have been observed to possess a greater capacity for transforming PFOS precursors into PFOS (Kannan et al., 2005; Galatius et al., 2011). This confounding effect of biotransformation can complicate the study of PFAS biomagnification in aquatic biota and should be carefully considered when reporting biomagnification factors (Simonnet-Laprade et al., 2019; Tomy et al., 2004).

Despite the global variation in metrics used to assess the trophic transfer of PFAS, the trophic magnification factor (TMF) is widely recognized as the most reliable measure for evaluating

biomagnification. A global review on the trophic magnification of PFAS in aquatic systems identified PFOS as the most extensively studied compound, frequently shown to biomagnify across various food webs (Miranda et al., 2022). While biomagnification of other long-chain carboxylates has also been observed, TMF values for these compounds vary significantly among studies. In addition to differences in sampling methodologies, tissues analyzed, and proximity to PFAS sources, the observed variations in TMF values can also be attributed to the length of the food web studied. For instance, an Arctic food web reported a high TMF for PFAS, as it encompassed a broad range of trophic levels, from algae to marine mammals (Tomy et al., 2009). In Arctic ecosystems, certain trophic levels are often occupied by a limited number of species, resulting in a long and narrow food web (Hobson et al., 2002). In contrast, tropical food webs typically feature a wide range of species at each trophic level, leading to a shorter and broader food web (Borgå et al., 2012; Zhao et al., 2018). This broader structure can dilute energy flow and, consequently, reduce the transfer of contaminants among species. Especially food webs ending with air-breathing animals, such as marine mammals, were found to have particularly high PFOS TMFs (Miranda et al., 2022). This is likely due to the negligible elimination of PFAS through lung air exchange in comparison to the more efficient respiratory elimination via gills in water-breathing organisms, leading to greater accumulation of these contaminants in air-breathing animals (De Silva et al., 2021). The biomagnification potential of short-chain carboxylates and sulfonates has been less frequently studied, with some research indicating biodilution of these compounds (Miranda et al., 2022). This phenomenon is likely due to the higher solubility of short-chain compounds compared to their long-chain counterparts. However, some studies suggest that short-chain PFAS may compete with long-chain PFAS for binding sites (Wen et al., 2017). Given the increasing global use of short-chain compounds, there is potential for these substances to outcompete banned long-chain PFAS in the future. Therefore, further research and monitoring are recommended to better understand their environmental impact.

1.3. Stable Isotope Analysis in Ecotoxicology

Stable isotope analysis has become a valuable tool for ecotoxicologists to study dietary and habitat exposure, as well as biomagnification of contaminants in wild species and populations (Crawford et al., 2008; Trueman et al., 2012). Stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$ or $\delta^{13}\text{C}$),

nitrogen ($^{15}\text{N}/^{14}\text{N}$ or $\delta^{15}\text{N}$), sulfur ($^{34}\text{S}/^{32}\text{S}$ or $\delta^{34}\text{S}$), hydrogen ($^2\text{H}/^1\text{H}$ or $\delta^2\text{H}$) and oxygen ($^{18}\text{O}/^{16}\text{O}$ or $\delta^{18}\text{O}$) are naturally occurring and are expressed as isotopic ratios in delta notation. This delta notation quantifies the relative difference between the ratio of heavy to light isotopes in a sample compared to a standard reference material. This difference is represented by the formula (Werner & Brand, 2001):

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

Where X represents the heavier isotope (e.g. ^{13}C), R_{sample} refers to the ratio of the heavy to light isotope in the sample, and R_{standard} is the corresponding ratio in the standard reference material. The delta notation is reported in per mil (‰). In this thesis, only carbon, nitrogen, and sulfur isotopes were utilized; therefore, hydrogen and oxygen isotopes will not be further addressed.

The application of stable isotopes in ecological studies is founded on two primary principles: (1) consumers typically exhibit enriched stable isotope ratios relative to their diet, and (2) differences in isotopic ratios among basal food sources are mirrored in the isotopic composition of consumers (Jardine et al., 2006). The first principle primarily arises from the enrichment of nitrogen stable isotope ratios across trophic levels. Research indicates that $\delta^{15}\text{N}$ values in consumers are approximately 3-4 ‰ higher than those of their diet (Deniro & Epstein, 1981; Peterson & Fry, 1987; Post, 2002). This significant enrichment of $\delta^{15}\text{N}$ between trophic levels results from the preferential excretion of the lighter nitrogen isotope, ^{14}N (Post, 2002). Consequently, $\delta^{15}\text{N}$ serves as an estimator for relative Trophic Level (TL) and facilitates the examination of contaminant accumulation along food chains (Post, 2002). Numerous studies in the literature investigating PFAS biomagnification employ $\delta^{15}\text{N}$ measurements for this purpose (Mazzoni et al., 2020; Miranda et al., 2022; Munoz et al., 2022). In contrast to the observed enrichment of $\delta^{15}\text{N}$ values across trophic levels, carbon isotope ratios ($\delta^{13}\text{C}$) exhibit minimal shifts throughout the food chain. This relative constancy allows for the effective tracing of primary carbon sources for organisms, particularly when these sources exhibit distinct isotopic signatures, which aligns with the second foundational principle (Fry & Sherr, 1989). Isotopic fractionation is a significant process that occurs during the fixation of carbon by primary producers. For example, C3 plants, which utilize the Calvin cycle, demonstrate a higher degree of discrimination against the heavier ^{13}C isotope, resulting in more negative $\delta^{13}\text{C}$ values (O'Leary, 1988). In contrast, C4 plants, which undergo an initial carbon fixation step,

demonstrate reduced discrimination, resulting in relatively enriched $\delta^{13}\text{C}$ values (O'Leary, 1988). In aquatic ecosystems, $\delta^{13}\text{C}$ is a valuable tool for distinguishing between offshore and littoral feeding, as well as between benthic and pelagic foraging behaviors (France, 1995; Fry & Sherr, 1989; Hobson, 1999). Carbon sources in benthic and coastal areas are typically more enriched due to the prevalence of several primary producers and carbon sources (benthic algae, detritus, seagrasses, etc.) compared to the less productive offshore pelagic regions, which are primarily dominated by phytoplankton. Consequently, offshore food webs typically exhibit higher (less negative) $\delta^{13}\text{C}$ values compared to nearshore areas (Fry & Sherr, 1989). In addition to carbon isotopes, sulfur isotopes ($\delta^{34}\text{S}$) can be used as a further tracer to identify food sources by differentiating the contributions of various primary producers (Connolly et al., 2004). Similarly, sulfur isotopes remain stable throughout trophic levels, thus serving as reliable markers in determining food sources. This characteristic is of particular value in complex estuarine environments that are influenced by inputs from terrestrial, marine, and riverine sources, where sulfur isotopes can discern sources that carbon and nitrogen isotopes may not distinguish (Connolly et al., 2004). In accordance with the findings of Fry et al. (1982), four principal sulfur sources are available to plants in an estuarine system. The predominant form of sulfur in the water column is sulfate, which is typically enriched around +20 ‰. In the absence of oxygen, bacterial reduction of sulfates results in the formation of sedimentary sulfides, which exhibit an isotopic composition that is approximately 24‰ lighter. Porewater sulfates exhibit greater variability. They may be enriched in comparison to water column sulfates (up to +60‰) or depleted, contingent on processes such as the reoxidation of sedimentary sulfides. Furthermore, rainwater sulfates, with values ranging from +2 to +16 ‰, can serve as an additional sulfur source, particularly for emergent or intertidal plants. Consequently, primary producers that rely on different sulfur sources exhibit distinct isotopic signatures, which allows to distinguish between, for instance, benthic and pelagic producers, such as saltmarsh plants and phytoplankton, in estuarine ecosystems.

1.4. Ecological Risk Assessment

Bioaccumulation studies reveal the widespread occurrence of PFAS in aquatic environments. However, the effect of this exposure and potential ecological consequences, remain challenging research fields.

1.4.1. Prioritizing PFAS

Prioritizing PFAS for ecotoxicity research is a primary challenge given the vast number of these synthetic chemicals, which includes over 6000 individual compounds (Glüge et al., 2020). Schymanski et al., (2023) suggest that this number could potentially increase to over 7 million when considering all possible PFAS variants. This prioritization is crucial because, in many cases, these chemicals may continue to find widespread use until sufficient evidence of their harmful environmental impacts triggers regulatory action. Currently, ecotoxicity research has been conducted on only 135 PFAS, representing a mere 2.3% of the total PFAS identified (Gkika et al., 2023). Of these, PFOS and PFOA have been the most extensively studied, accounting for 45% of the available ecotoxicity data. Given these limitations, selecting PFAS for further ecotoxicity research involves several key considerations. A primary question is which compounds are present in the environment but remain understudied. According to Gkika et al. (2023), environmental occurrence data are currently available for only 41 PFAS, primarily legacy PFAS, which account for less than 1% of the 6000 identified PFAS. This issue is underscored by the use of non-target screening and total oxidizable precursor assays, highlighting the importance of broadening the range of PFAS currently quantified in environmental matrices (Göckener et al. 2021; Hensema et al., 2021). Continuous optimization of analytical methods is essential to improve the detection and assessment of a wider array of PFAS, ensuring that environmental monitoring and risk assessment efforts keep pace with the diversity of chemicals in use. Secondly, the physicochemical properties of PFAS, such as persistence, solubility, and potential for bioaccumulation, are critical factors in determining their environmental behaviour and impact. Compounds with high persistence and bioaccumulation potential are more likely to pose long-term risks to ecosystems. Thirdly, the production and usage volumes of PFAS can serve as a proxy for exposure potential. Compounds that are produced or used in large quantities are more likely to be released into the environment, making them higher priorities for risk assessment (Kurwadkar et al., 2022). Lastly, the specific applications of PFAS can influence their potential environmental impacts. For example, compounds used in products with direct environmental exposure pathways, such as firefighting foams, may warrant more immediate attention than those used in less accessible applications. Prioritizing PFAS for ecotoxicity research is a complex but essential task. By focusing on those compounds with the greatest potential for adverse environmental effects, researchers can more effectively allocate resources to address the most pressing ecological risks associated with PFAS. This approach not

only helps to protect ecosystems but also informs regulatory actions that may be necessary to mitigate the impact of these persistent and potentially harmful chemicals.

1.4.2. Current Ecotoxicity Knowledge

The current knowledge on the toxicity of PFAS to aquatic invertebrates and fish is built on a limited but growing body of research. The studies available reveal important insights into how these compounds affect aquatic life, but also highlight significant gaps that need to be addressed.

According to Ankley et al. (2021), research on the ecotoxicity of PFAS to aquatic invertebrates includes 47 different compounds grouped into six categories: fluorotelomers, PFCAs, PFSAs, fluorocyclohexanes, fluorinated alkane sulfonamides (FASAs), and fluorinated plant protection products (FPPPs). Despite the range of compounds tested, the vast majority of data (95%) focus on just three categories: PFCAs, PFSAs, and FPPPs. PFOA and PFOS are the most extensively studied compounds, making up 21% and 39% of the available data, respectively. Crustaceans, such as *Daphnia magna* and larvae of non-biting midges (*Chironomus sp.*), are among the two most frequently tested groups. Nevertheless, the variability in test conditions complicates an evaluation of interspecies sensitivity based on the available data. While more research has been conducted on fish, the toxicity of PFAS has been studied across 29 different compounds, which is a smaller number than that of invertebrates. Similar to invertebrates, PFOA and PFOS dominate the research accounting for 62% of the fish related data. Most studies have focused on freshwater species, especially zebrafish (*Danio rerio*) and fathead minnows (*Pimephales promelas*), although some data exist for marine species such as mullet (*Mugil spp.*) and flounder (*Platichthys spp.*). In general, it is observed that toxicity often increases with the length of the fluorocarbon chain, and PFSAs tend to be more toxic than PFCAs (Ankley et al., 2021).

Toxicity can be evaluated at various biological levels, beginning at the molecular scale and triggering a cascade of downstream responses that may ultimately disrupt the complex functioning of entire ecosystems. For instance, PFAS exposure to fish has been shown to activate nuclear receptors—proteins responsible for sensing specific molecules such as steroids and hormones—which in turn can disrupt diverse metabolic pathways (Lee et al. 2020). One such pathway involves the alteration of thyroid-related genes, leading to tissue and organ-level effects, such as thyroid malformation (Deng et al., 2018; Hagenaaers et al., 2013; Zhang et al.,

2018). Since the thyroid plays a crucial role in growth, development, and metabolism, its disruption is associated with a range of disorders at the organism level, including reduced fecundity, neural damage, and developmental abnormalities (Lee et al. 2020; Liu et al. 2019; Mahoney et al. 2022). In addition to nuclear receptor activation, oxidative stress and membrane interactions have been identified as key molecular events triggered by PFAS exposure which eventually lead to tissue and organ-level effects, resulting in reproductive, growth, and developmental abnormalities, neurobehavioral impairments, and other disorders (Lee et al. 2020; Mahoney et al. 2022). PFAS-caused reproductive toxicity has been reported by multiple studies in both fish and invertebrates and is observed by diverse mechanisms such as decreases in larval survival rate, gonadal developmental delay, larval malformation, sex ratio change, sperm number decrease, ovary degeneration, etc., which may eventually lead to a decrease in population growth (Ankley et al. 2005; Han and Fang 2010; Lee et al. 2020; Suski et al. 2021). Also PFAS-induced reduced organism growth and developmental issues are often reported in multigenerational studies for both fish and invertebrates (Gust et al. 2024; Haimbaugh et al. 2022; Marziali et al. 2019). Furthermore, neurobehavioral changes are observed in fish due to PFAS-induced adverse effects such as reversed light-dark responses, increased or either decreased swimming behaviors (distinct toxicity for different compounds) and reduced aggression behavior (Hamed et al. 2024; Jantzen et al. 2016; Wang et al. 2022).

Most of the toxicological findings discussed above primarily pertain to legacy PFAS, such as PFOS and PFOA, whereas emerging PFAS have been less extensively studied. However, despite their lower bioaccumulation potential, this does not necessarily imply that these compounds are safer or exhibit lower toxicity. Recent research has demonstrated that certain alternative PFAS can exhibit toxicity levels comparable to those of PFOA and PFOS (Espartero et al. 2022; Pietropoli et al. 2024; Rericha et al. 2021). Due to their increased mobility, short-chain PFAS also demonstrate a higher efficiency of placental transfer compared to legacy compounds (Cai et al. 2020; Gao et al. 2019). Moreover, studies examining the toxicological effects of compounds such as perfluorobutanoic acid (PFBA) and PFBS in fish have reported disruptions to the endocrine system (specifically thyroid and reproductive functions), impairments in swim bladder development, and even behavioral abnormalities (Ivantsova et al. 2024). These findings underscore the need to expand our understanding of the potential hazards and underlying mechanisms associated with alternative PFAS (Ateia et al. 2019).

A notable limitation in the field of ecotoxicity research is that the majority of toxicological findings are derived from controlled experimental testing. In laboratory settings, an idealized and simplified environment is created for test organisms, whereby they are often exposed to a single stressor or compound. These conditions do not accurately reflect real-world environmental conditions, in which organisms are subjected to a multiplicity of stressors, including temperature fluctuations, a mixture of pollutants, predation, and other ecological pressures. Moreover, the concentrations of PFAS employed in laboratory toxicity tests are often considerably higher than those observed in natural environments, which may result in an overestimation of adverse effects (Sinclair et al., 2020). There is a lack of studies investigating the impact of PFAS on free-living organisms in natural, field-based conditions, particularly in aquatic ecosystems. As noted by Ankley et al. (2021), only three field studies had been conducted on fish and invertebrate species. In a study conducted by Bangma et al. (2018), no adverse relationship was observed between liver PFAS concentrations and reproductive outcomes in the striped mullet (*Mugil cephalus*). However, in a separate study on striped bass (*Morone saxatilis*) from the Cape Fear River (USA), notable associations were identified between serum PFAS concentrations and health-related parameters, as well as biomarkers in the liver and immune system (Guillette et al., 2020). In the case of invertebrates, Rusconi et al. (2015) examined macrobenthic invertebrate communities situated both upstream and downstream of a fluorotelomer plant in Northern Italy. The upstream site was found to support a greater diversity of more sensitive taxa than the downstream location. Furthermore, genetic diversity measurements in a trichopteran species indicated a 6.8% variation between the two sites, which suggests the occurrence of genetic drift in the downstream population.

The limited number of field studies is likely due to the logistical and financial challenges associated with fieldwork, as it is labor-intensive and costly. Moreover, interpreting field study results is inherently complex, given the multitude of environmental factors that may contribute to observed effects. The potential for unidentified stressors to confound the results makes it challenging to isolate the impact of PFAS. Thus, a critical consideration in such research is the selection of appropriate endpoints for study. Despite these challenges, there is an urgent need for field-based effect studies in aquatic ecosystems in order to gain a deeper understanding of the broader impact of PFAS contamination. In order to address the current knowledge gap regarding the effects of PFAS on individuals and populations under environmentally realistic conditions, it is essential to integrate field-based research with mechanistic studies at the

molecular, cellular, and tissue levels. Such an integrated approach will facilitate a more comprehensive understanding of the impact of PFAS on aquatic ecosystems.

1.5. Navigating the Regulatory Maze

Regulatory measures for PFAS in Europe have been progressively developed due to growing concerns over their environmental persistence and health risks. These measures span several legislative frameworks targeting various exposure pathways, such as water, food, consumer products, and industrial uses. The REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) regulation (EC/1907/2006) is one of the primary frameworks for regulating PFAS in Europe. Both PFOS (2008) and PFOA (2017) and their precursors have been subjected to restrictions under REACH and in 2021 around 200 long-chain PFCAs were added to the list. In 2010 and 2020, PFOS and PFOA were also added to the POP (Persistent Organic Pollutants) regulation (EC 850/2004) which aims to eliminate or restrict the production and use of chemicals that are persistent in the environment. Furthermore there is the European Chemicals Agency (ECHA), which is currently working on a comprehensive restriction proposal that may ban all non-essential uses of PFAS throughout the EU, which was proposed by five EU member states (ECHA, 2023). To safeguard drinking water, thresholds for PFAS were in 2020 included under the Drinking Water directive (EU 2020/2184). It introduced limits for the sum of 20 selected PFAS at 100 ng/L and a total PFAS concentration limit of 500 ng/L (Tsaridou & Karabelas, 2021). To continue safeguarding public health, maximum contaminant levels (ML) were set in certain food categories, including fish, eggs, meat, and dairy products (Commission Regulation EU 2022/2388). These limits were set for four compounds separately, i.e. PFOS, PFOA, PFNA and PFHxS, as well as for their sum (EU, 2022). The MLs most relevant for this work are shown in Table 1.1. Furthermore, in 2020, the European Food Safety Authority (EFSA) recommend a new Tolerable Weekly Intake (TWI) of 4.4 ng/kg body weight for the sum of these four PFAS (PFOA, PFOS, PFNA, PFHxS). The TWI refers to the estimated amount of a contaminant unintentionally present in food or drinking water that can be safely consumed on a weekly basis throughout a lifetime without posing a significant health risk (EFSA, 2020). The EFSA guideline is not necessarily a legally binding threshold, but it is frequently used as a reference for implementing regulatory decisions aimed at protecting human health. To establish this threshold, immune system effects were considered the most critical for risk

assessment, with a particular focus on a human study that observed immunosuppression in one-year-old children resulting from long-term maternal exposure (Abraham et al., 2020).

Table 1.1: Maximum contaminant levels (ML) in muscle meat of fish in µg/kg ww

	PFOS	PFOA	PFNA	PFHxS	Σ4PFAS
Fish category 1					
All fish, except those listed under category 2 and 3	2.0	0.20	0.50	0.20	2.0
Fish category 2					
e.g.: seabass, flounder, Baltic herring, plaice, etc.	7.0	1.0	2.5	0.20	8.0
Fish category 3					
e.g.: anchovy, perch, whitefish, eel, bream, etc.	35	8.0	8.0	1.5	45

Furthermore, thresholds for environmental matrices were also derived under the Water Framework Directive (2000/60/EC), particularly for water and biota. These thresholds are referred to as Environmental Quality standards (EQS) and are used to assess and monitor the status of European water bodies. Currently, the annual average concentration EQS for surface water (AA-EQS = 0.65 ng/L), as well as the EQS for biota (EQS_{biota} = 9.1 µg/kg ww) were derived for only one compound which is PFOS. Both thresholds were established to protect human health from fish consumption and are therefore based on the EFSA's TWI. However, it is important to note that these standards are still based on the TWI from 2008 (15 ng/kg/day), which is nearly 240 times higher than the updated TWI set in 2020 (EFSA, 2008). The previously discussed threshold values are primarily focused on protecting human health, which is why the EQS biota specifically refers to PFAS concentrations in fish muscle tissue. For the protection of the environment, only one threshold has been established: the EQS for the protection of top predators against secondary poisoning, set at 33 µg PFOS/kg (European Union, 2014). Since aquatic top predators are likely to consume entire prey specimens, it is more appropriate to account for PFAS accumulation in whole fish, rather than limiting the assessment to muscle tissue alone.

Belgium adheres to the same regulatory framework as broader European legislation. However, Flemish legislation has introduced adjustments to the Environmental Quality Standards (EQS) for surface waters, distinguishing between freshwater environments (e.g., rivers and lakes) and estuarine and marine environments (VLAREM Bijlage 2.3.1). For freshwater environments, an annual average PFOS concentration of 0.65 ng/L and a maximum concentration of 36 µg PFOS/L

apply. In contrast, stricter limits are imposed on estuarine and marine environments, with an annual average of 0.13 ng PFOS/L and a maximum concentration of 7.2 µg PFOS/L. EU Member States have the authority to implement more stringent national standards based on local environmental conditions and risk assessments. However, the specific reasoning behind the stricter limits for estuarine and marine environments has not been explicitly stated.

In conclusion, current regulations predominantly prioritize human health protection, while thresholds aimed at safeguarding ecosystem health are limited and in need of revision. Moreover, the transparency regarding the derivation and implementation of these thresholds is often lacking, making it challenging for operators, regulators, scientists, and other stakeholders to accurately interpret and apply the standards. This lack of clarity can lead to confusion and inconsistency in their application. Additionally, the use and emission of short-chain compounds, such as PFBA and PFBS, as well as alternative substances like NaDONA and GenX, remain entirely unregulated.

1.6. PFAS Pollution: a Case Study in Belgium

Research on PFAS contamination in Belgium, particularly in Flanders, has been ongoing since the early 2000s. Especially the 3M site in Antwerp has been a key focus of biomonitoring efforts, as it houses one of the main PFAS manufacturing facilities. Studies conducted on the surrounding terrestrial environment have reported some of the highest PFAS concentrations ever observed in wildlife, identifying this area as a significant PFAS hotspot (Dauwe et al., 2007; D'Hollander et al., 2014; Groffen et al., 2019a; Hoff et al., 2004, 2005). Research on the adjacent aquatic environment, particularly in the Scheldt River, also revealed substantial bioaccumulation of PFOS in fish in the early 2000s (Hoff et al., 2003; Van De Vijver et al., 2003). However, it was up until June 2021, that the pollution of PFAS in Belgium came to light, sparking a widespread concern and media attention. This was after high levels of PFAS were discovered in soil samples during construction work on the Oosterweel Link, a major infrastructure project near the 3M site (Decré & Baert, 2021). This discovery led to broader investigations, which pointed to the 3M chemical plant as the main source of the contamination. The public's concern grew as it became clear that PFAS pollution had likely been ongoing for decades, impacting not only the immediate surroundings of the 3M plant but also the broader region. Local residents,

particularly those living within a 10 km radius of the factory, were warned not to consume produce, fish, or animal products from the area due to the risk of contamination (De Standaard, 2021). Local health authorities also conducted blood tests on residents, which revealed elevated levels of PFAS in their bloodstreams, further fueling public anxiety (VITO, 2022). These tests indicated that some residents had been exposed to PFAS levels far exceeding what is considered safe, raising concerns about potential long-term health effects. This, combined with fears of soil and water contamination, led to widespread criticism of both 3M and the Belgian and Flemish government for failing to act earlier. In response, the Flemish government took several decisive actions to mitigate the crisis. One of the first steps was the establishment of a parliamentary investigation committee, composed of environmental experts, scientists, and policymakers. This task force was tasked with coordinating the government's response, overseeing environmental monitoring programs, and advising on new regulatory measures to address the contamination. An extensive monitoring program was launched to assess PFAS levels in soil, water and air, not only in Zwijndrecht but across other parts of Flanders as well. It quickly became evident that the PFAS contamination was not confined to a single area but was widespread across Belgium with various sources being identified (Figure 1.4) (Dorjbayar et al., 2021). To inform the public, researchers, and policymakers about the production, discharge, and presence of PFAS in Flanders, an online platform called 'PFAS Verkenner' (<https://www.vlaanderen.be/pfas-vervuiling/pfas-verkenner-voor-professionele-gebruikers>) was developed. This platform integrates data from various national and local databases, presenting the information through interactive maps and graphs for better accessibility and analysis. Drinking water sources were rigorously tested to verify compliance with safety standards, and results confirmed that they met the required criteria (Vlaamse Milieumaatschappij, 2022). Furthermore, the Flemish government introduced stricter regulations on industries that use or produce PFAS, tightening the permitting process and enforcing stronger controls on emissions and waste disposal. This move aimed to prevent further contamination and hold industries accountable for their environmental impact. The government also increased funding for PFAS research, supporting scientific studies on the health and environmental effects of these chemicals. Collaborating with universities and research institutes, the government aimed to improve understanding of PFAS behavior in the environment and explore new technologies for detecting and cleaning up PFAS contamination. To address the contamination and fund remediation efforts, the Flemish government reached

a settlement with 3M in 2022. This settlement was designated for cleaning up the contaminated areas, conducting health assessments, and supporting long-term monitoring programs. However, legal proceedings against 3M continue, with environmental groups and affected residents pushing for stricter accountability and compensation.

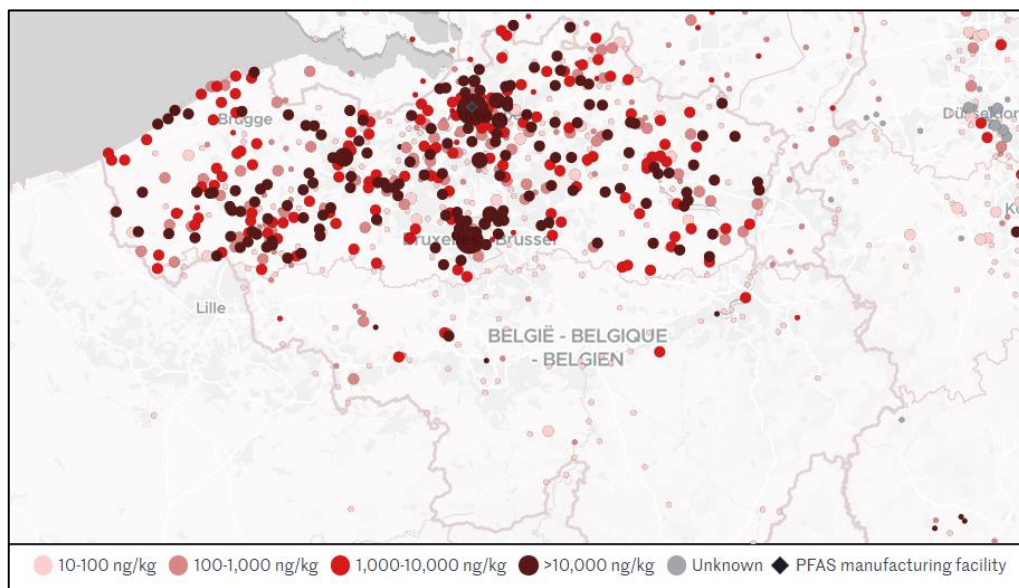


Figure 1.4: Widespread PFAS contamination across Flanders. PFAS were monitored in water, soil or organisms. For concentrations in water, 1 ng/L and 1 ng/kg are considered as equivalent. The map was sourced from the Forever Pollution Project (www.lemonde.fr).

1.7. Research Aims and Thesis Outline

The central aim of this thesis was to evaluate the bioaccumulation, distribution, and effects of PFAS in aquatic ecosystems across Flanders, Belgium. The scope of the study spans from the smallest inland rivers and streams to the Scheldt estuarine system and ultimately, the Belgian North Sea. These diverse systems were investigated to assess the risks of PFAS bioaccumulation at multiple levels. In freshwater ecosystems, the focus was on ecological responses at the community level, while in marine environments, the emphasis was on organismal and human health risks. The estuarine environment, in contrast, enabled the exploration of feeding behavior and its impact on bioaccumulation, as well as molecular-level effects. Throughout the chapters, various monitoring techniques were employed and compared. Notably, all studies

were conducted in the field to expand our understanding of PFAS bioaccumulation and its consequences under real-world conditions.

Chapter II examines the bioaccumulation of PFAS in the liver and muscle tissues of seven fish species and the whole-body tissues of two crustacean species. Samples were collected from 10 sites within the Belgian North Sea, spanning estuarine, coastal, and deeper water environments. The study aims to assess the distribution of PFAS across these zones, with a particular focus on tissue-specific accumulation patterns in fish. Additionally, stable isotope analysis was conducted to investigate trophic transfer and bioaccumulation differences among species. It is hypothesized that PFAS concentrations vary by species, tissue type, and environmental zone, with higher bioaccumulation expected in estuarine and coastal areas due to greater pollution inputs. Moreover, stable isotope analysis may reveal trophic-level-dependent PFAS transfer, with higher concentrations in species at higher trophic levels. Furthermore, the research evaluates the ecological and human health risks associated with consuming contaminated marine species, using existing human health and ecological risk benchmarks as references.

Chapter III investigates the bioaccumulation of PFAS in 12 fish and invertebrate species along the Scheldt river, with a focus on spatial distribution and potential contamination sources. It is hypothesized that PFAS bioaccumulation varies spatially, with higher concentrations occurring near industrial areas. Additionally, ecological factors, such as feeding ecology, are expected to influence PFAS exposure routes and bioaccumulation patterns across different species. To explore these dynamics, ecotoxicological data were integrated with stable isotope analysis, providing deeper insights into the ecological processes governing PFAS contamination within the estuarine system.

Chapter IV aims to evaluate the potential of Asian clams as active biomonitoring species for PFAS contamination, with the Scheldt river as a case study. Since PFAS profiles are expected to differ between biotic (clams) and abiotic (water and sediment) environments, bioaccumulated PFAS concentrations in clams are compared with those in water and sediment samples. This comparison assesses the clams' relevance as biomonitors of PFAS pollution and helps characterize the spatial distribution of PFAS contamination within the river system. Furthermore, as laboratory experiments have demonstrated that PFAS exposure induces biological responses in clams, this study investigates whether similar effects can be observed

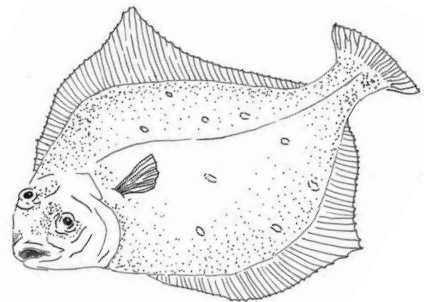
under field conditions. Specifically, the relationship between PFAS bioaccumulation in exposed clams and biological responses—such as oxidative stress biomarkers, condition index, and respiration rates—is examined to determine the extent of PFAS-related physiological impacts in natural environments.

To gain a more nuanced understanding of the ecological impact of PFAS, the research scope was expanded to include freshwater environments. **Chapter V** examined the bioaccumulation of PFAS in resident aquatic macroinvertebrates and translocated zebra mussels in small streams and rivers. It was hypothesized that PFAS contamination could have significant ecological effects at the community level. Therefore, the relationship between bioaccumulated PFAS and the macroinvertebrate community structure was assessed using the Multimetric Macroinvertebrate Index Flanders (MMIF). Furthermore, the study aimed to estimate PFAS threshold values that are safe for ecological health to inform future regulations. Zebra mussels were also hypothesized to be effective biomonitoring tools for PFAS pollution, with their bioaccumulation profiles expected to be comparable to those of resident macroinvertebrate species. This would confirm their reliability for long-term environmental monitoring.

Finally, in **Chapter VI**, an overall comparison of the results from all chapters was summarized and discussed. This chapter integrates the findings to provide a comprehensive understanding of PFAS bioaccumulation patterns, ecological impacts, and potential human health risks, drawing connections between the various ecosystems and monitoring techniques investigated throughout the presented studies.

Chapter II

Bioaccumulation and Trophic Transfer of PFAS in Marine Biota from the Belgian North Sea: Distribution and Human Health Risk Implications



Adapted from:

Byns, C., Teunen, L., Groffen, T., Lasters, R., & Bervoets, L. (2022). Bioaccumulation and trophic transfer of perfluorinated alkyl substances (PFAS) in marine biota from the Belgian North Sea: Distribution and human health risk implications. *Environmental Pollution*, 311, 119907. <https://doi.org/10.1016/J.ENVPOL.2022.119907>

Abstract

Per- and polyfluorinated alkyl substances (PFAS) are highly persistent environmental pollutants that pose potential risks to aquatic ecosystems due to their bioaccumulative behavior and toxicological effects. While the global distribution of PFAS in marine environments has been extensively studied, limited data exist on their contamination in the southern North Sea. This study investigates the bioaccumulation and trophic transfer of PFAS in marine biota from the Belgian North Sea, analyzing liver and muscle tissues of seven fish species and whole-body tissues of two crustacean species collected from 10 sites. Furthermore, potential ecological and human health risks associated with PFAS exposure were assessed.

PFOS was identified as the predominant PFAS across all sample matrices, with other long-chain PFAS frequently detected. PFOS concentrations ranged from below the limit of quantification (LOQ) to 107 ng/g wet weight (ww) in fish liver, <LOQ to 24 ng/g ww in fish muscle, and from 0.29 to 5.6 ng/g ww in crustaceans. Elevated concentrations of PFTrDA were observed in fish liver from estuarine and coastal regions (<LOQ–116 ng/g ww), suggesting a specific point source contamination. Stable isotope analysis did not reveal distinct trophic transfer patterns, as the trophic range of the studied species was too limited to draw definitive conclusions. Risk assessment based on dietary intake limits revealed that consumption of commercially relevant species such as common shrimp (*Crangon crangon*), European plaice (*Pleuronecta platessa*), common sole (*Solea solea*), and whiting (*Merlangius merlangus*) could pose potential health risks if intake exceeds 17 g/day, 18 g/day, 26 g/day, and 43 g/day, respectively. While most PFOS concentrations in muscle tissue remained below the $QS_{\text{biota,hh}}$ threshold of 9.1 ng/g ww for human health protection, 43% of European plaice samples and 28% of common sole samples, based on the combined concentrations in muscle and liver tissue, exceeded the 33 ng/g ww PFOS benchmark for wildlife protection against secondary poisoning. These findings highlight the need for ongoing monitoring and risk assessment of PFAS contamination in the Belgian North Sea to protect both marine biodiversity and public health.

2.1. Introduction

Perfluorinated 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 are 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 effluents 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). The accumulation of PFAS in aquatic biota, such as invertebrates, fish, marine mammals and birds, has been reported worldwide (Houde et al., 2011). Higher accumulated PFAS concentrations in marine organisms with a higher trophic level, including top predators, suggests trophic transfer of PFAS in marine food webs (Haukås et al., 2007; Kelly et al., 2009). Biomagnification of PFAS provokes an increasing concern regarding their risks of secondary poisoning in top predators. 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 report potential adverse effects of PFAS on the reproductive-, developmental-, endocrine- and immune system of both aquatic and terrestrial organisms, including humans (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 (Carloni, 2009).

Furthermore, PFOS and perfluorooctanoic acid (PFOA) were also 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 as well as 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 due to the location of most fluorochemical plants 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 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, since elevated PFAS concentrations were also detected in other 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 3M 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, in these studies only PFOS was targeted 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., 2009). 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 PFAS 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.

2.2. Materials and Methods

2.2.1. Sample Collection

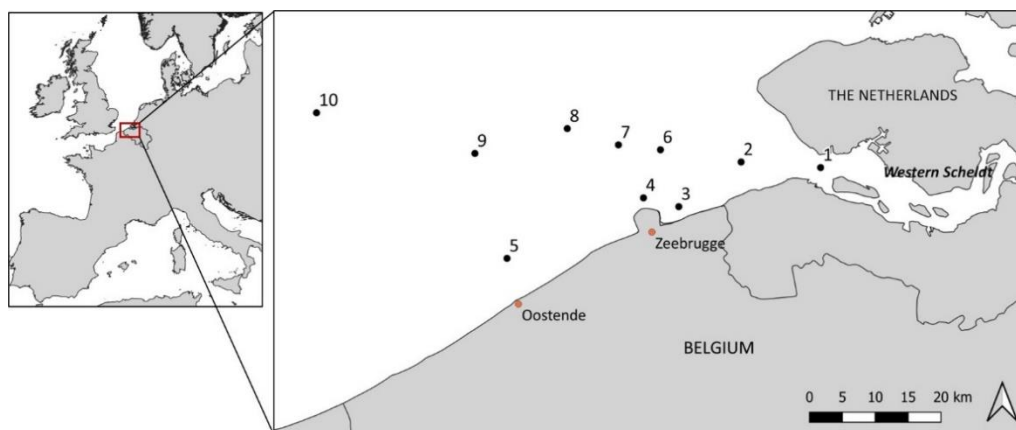


Figure 2.1. Overview of the Belgian North Sea and the sampling sites, ranging from the mouth of the Scheldt estuary towards open sea.

In total, 10 sites were sampled in the Belgian part of the North Sea and the Western Scheldt estuary (Figure 2.1.). 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 (Supplementary Information Table A.1-A.2). 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 A.1). 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.

2.2.2. 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 hours. Hereafter, samples were centrifuged (Eppendorf 5804R) for 10 min at 1037 x 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 one minute. After centrifugation (10 min, 9279 x 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).

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 13mm Syringe Filter with 0.2 µm Supor polyethersulfone (PES) Membrane was attached.

2.2.3. 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 PFAS. Target analytes were perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acid (PFHpA), PFOA, PFOS, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), Perfluorodecane sulfonate (PFDS), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA).

Analytes were separated on an ACQUITY BEH C18 column (2.1×50 mm; 1.7 µm, Waters, USA). An ACQUITY BEH C18-pre-column (2.1 x 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 µL/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 A.3) (Groffen et al., 2019b).

2.2.4. 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 A.4) (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 A.5). During PFAS analysis, ACN was injected on a regular basis, as instrumental blank, to prevent the carry-over effect to the next sample.

2.2.5. Stable Isotope Analysis

Individual fish (muscle) and whole invertebrate specimens were pooled per species and per location (Table A.6). The samples were freeze-dried at -55°C for three days and homogenised into fine powder. Approximately 0.5 mg of the fish samples was weighed and encapsulated into a 5 x 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 x 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):

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

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).

2.2.6. 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 Environmental Quality Standards for biota (EQS_{biota}) which were set by the European Commission under the Water Framework Directive (European Union, 2013). The human health-based threshold ($EQS_{biota, hh}$) of 9.1 μg PFOS/kg ww was compared with the accumulated PFOS concentrations in muscle tissue. To test for compliance with the EQS that was specifically derived for the protection of top predators against secondary poisoning ($EQS_{biota, secpois} = 33 \mu\text{g}$ PFOS/kg ww), the sum of the accumulated PFOS concentrations in muscle and liver tissue was used (European Union, 2014).

2.2.7. 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 A.5-A.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_{pearson}) 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_{Spearman}) 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).

2.3. Results

2.3.1. Spatial PFAS Distribution and Profiles

A summary of the PFAS concentrations in fish and crustacean species at each site is given in Tables A.7 – A.9. 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 species that were collected at ≥ 7 sampling sites, i.e., *P. platessa*, *S. solea* and *M. merlangus*, *C. crangon* and *L. holsatus* (Figure 2.2). In liver tissue, PFTrDA 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*: $p < 0.001$) (Table A.10). For *M. merlangus*, PFTrDA was also significantly higher at location 2 compared to all other sites, except for location 4 ($p < 0.001$) (Table A.5). 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* $p = 0.011$). 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. holtsatus* at location 7 compared to location 1 ($p=0.021$) and 3 ($p=0.021$).

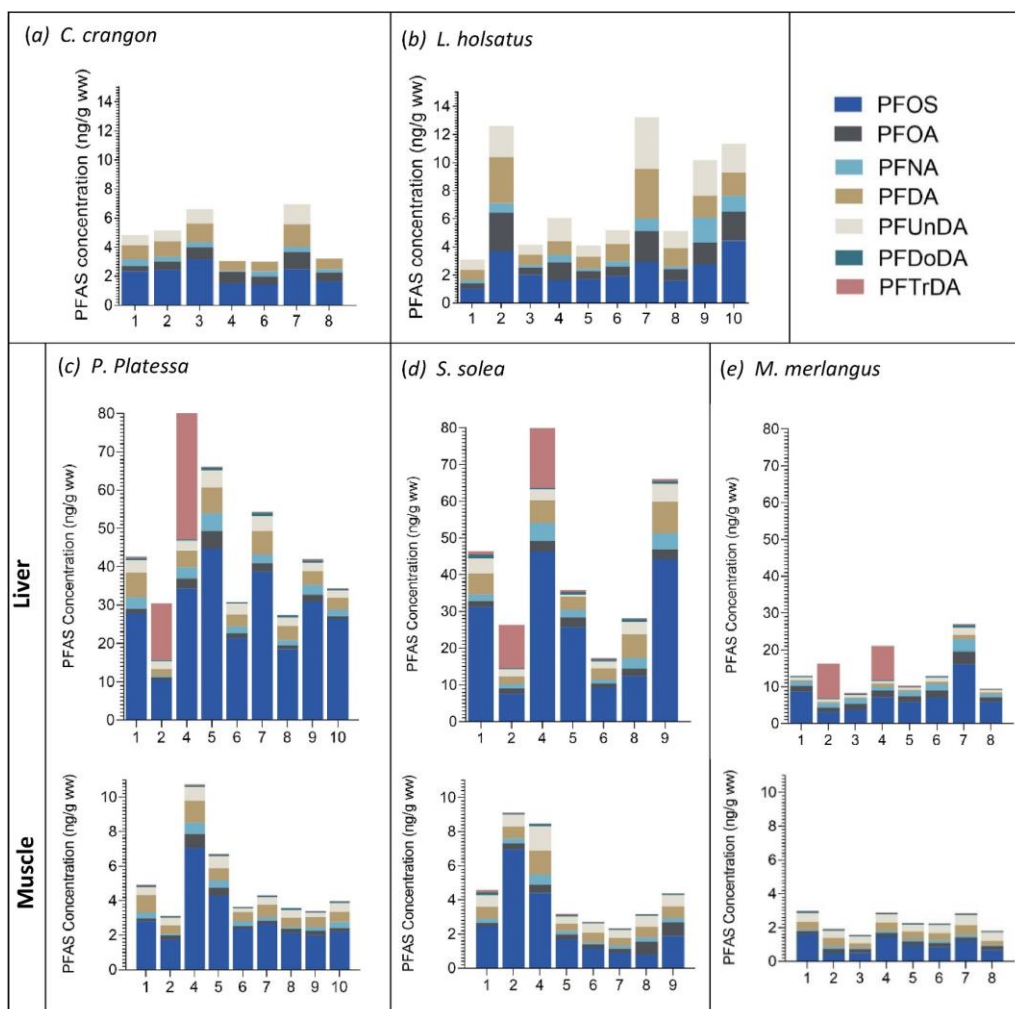


Figure 2.2: Spatial distribution of PFAS profiles and concentrations of 2 invertebrate species (a) *C. crangon*, (b) *L. holtsatus* and three fish species (c) *P. platessa*, (d) *S. solea* and (e) *M. merlangus* in liver and muscle tissue ($N=3-4$ replicates per species per location).

2.3.2. 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 PFTTrDA (30%) in liver tissue and for PFUnDA (22%) in muscle tissue. In liver tissue (Figure 2.3), 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 PFTrDA 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 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 (Figure 2.3), 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 and 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* ($p < 0.001$). 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 A.11 – A.12. No significant differences were found between the crustacean species, but mean Σ PFAS concentration was 7.60 ng/g for *L. holtsatus* and 4.74 ng/g for *C. crangon*.

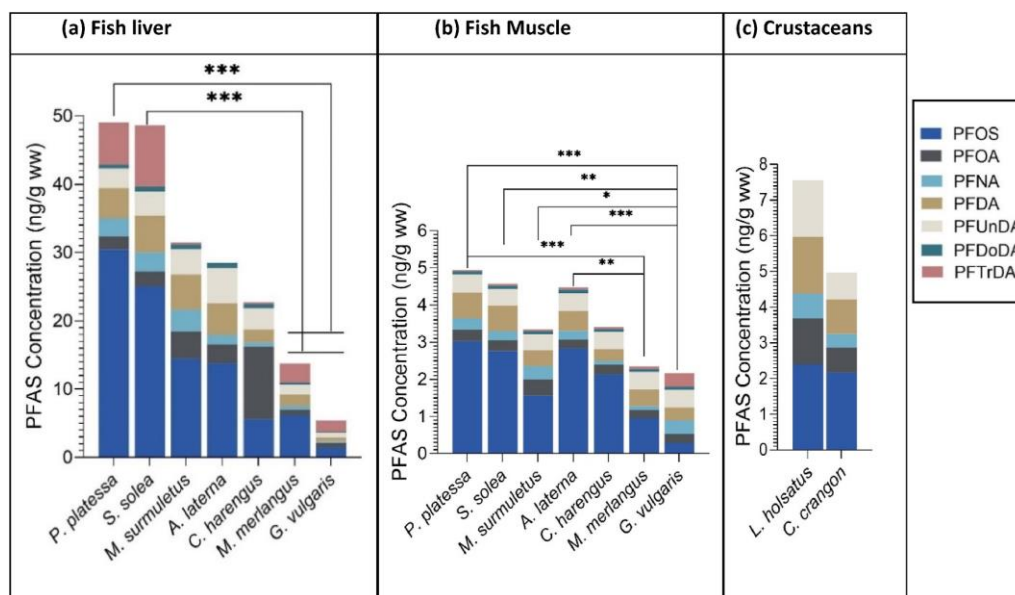


Figure 2.3: PFAS profiles and concentrations (ng/g ww) in liver (a) and muscle (b) tissue of seven fish species, *P. platessa* (N=29), *S. solea* (N=23), *M. surmuletus* (N=9), *M. merlangus* (N=26), *A. laterna* (N=9), *C. harengus* (N=10), *G. vulgaris* (N=11) and two invertebrate species, *L. holtsatus* (N=29) and *C. crangon* (N=20). Significant differences (sign.) are indicated by asterisks, with '*': $p < 0.05$, '**': $p < 0.01$, '***': $p < 0.001$.

2.3.3. Stable Isotope Analysis: Trophic Transfer 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 Tables A.13 – A.15. Mean relative trophic levels range from 2.7 for *L. holtsatus* up to 3.6 for *M. merlangus* (Figure 2.4). *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 *M. surmuletus* ($p = 0.022$), *P. platessa* ($p < 0.001$) and *L. holtsatus* ($p < 0.001$). For *L. holtsatus*, 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. holtsatus* (Figure 2.5). The corrected standard ellipse area (SEA_c), which indicates the size of the isotopic niche, was the largest for *L. holtsatus* (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).

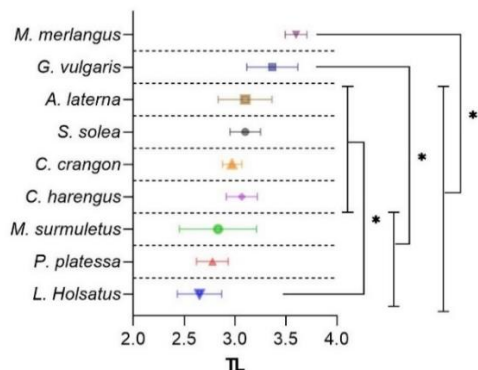


Figure 2.4: Mean Relative Trophic levels with standard deviations (SD) of seven fish species, *P. platessa* (N=9), *S. solea* (N=9), *M. surmuletus* (N=3), *M. merlangus* (N=8), *A. laterna* (N=3), *C. harengus* (N=3), *G. vulgaris* (N=3) and two crustacean species, *L. holsatus* (N=10) and *C. crangon* (N=7). Significant differences are indicated by '*’.

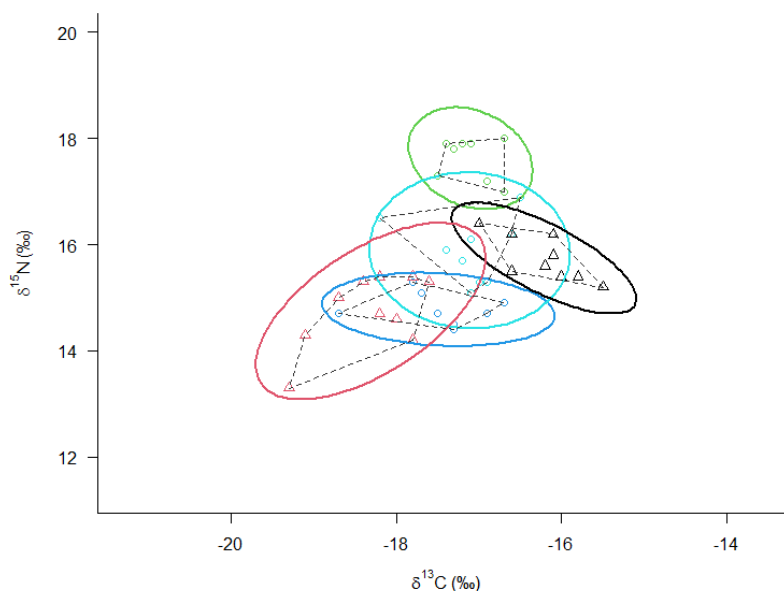


Figure 2.5: Isotopic niche overlap among *M. merlangus* (green ellipse, N=8), *P. platessa* (dark blue ellipse, N=8), *S. solea* (light blue ellipse, (N=9), *C. crangon* (black ellipse, N=9) and *L. holsatus* (red ellipse, N=10).

The trophic transfer of PFAS through biota was studied by looking at the relationship between the TLs from all species and the accumulated 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 significant 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 ($r_{\text{Pearson}} = -0.81$, $p=0.025$) (Figure 2.6). Significantly negative correlations were also found with PFUnDA at location 5 ($r_{\text{Pearson}} = -0.90$, $p=0.038$) (Figure 2.6). At location 8, significantly positive correlations were found between TL and PFTrDA ($r_{\text{Pearson}} = 0.90$, $p=0.037$) (Figure 2.6).

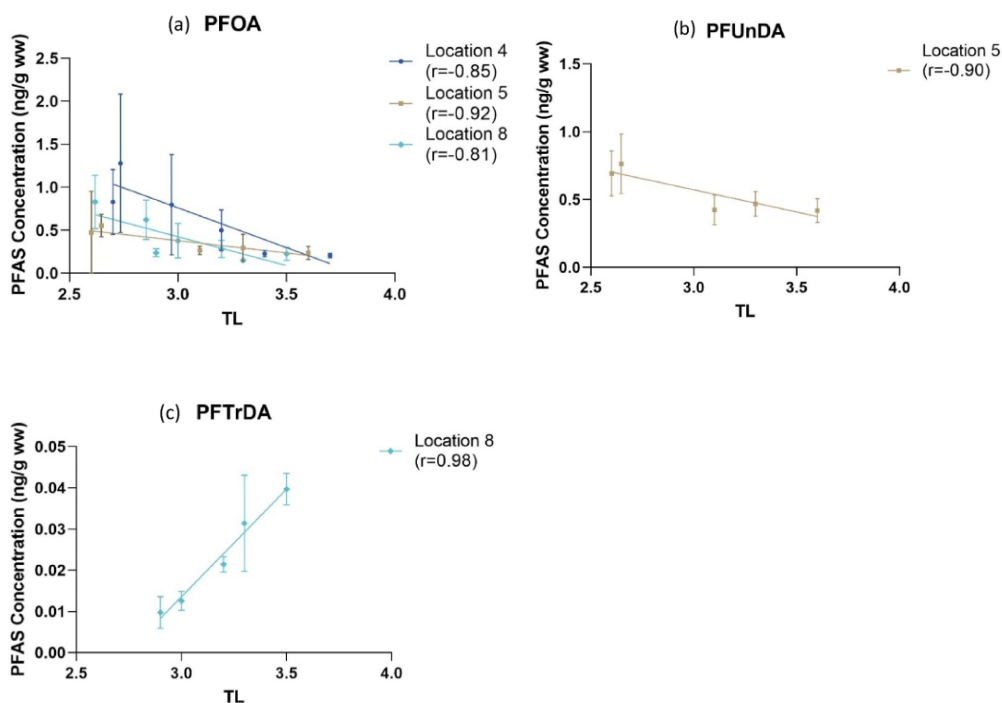


Figure 2.6: Mean PFAS concentrations (ng/g ww) with SD in muscle tissue of fish species and whole body tissue of crustaceans correlated with the relative trophic levels for the corresponding species for (a) PFOA, (b) PFUnDA, (c) PFTrDA.

2.3.4. 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 2.1. 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.

Table 2.1: 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.

	ATSDR		EFSA
	PFOS	PFOA	PFOA + PFOS + PFNA + PFHxS
MRL:	2 ng/kg/day	3 ng/kg/day	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. holisatus</i>	110 (25 – 464)	334 (48 – 1640)	18 (4 – 63)
<i>C. crangon</i>	102 (31 – 479)	434 (133 – 1591)	17 (7 – 44)

2.4. Discussion

2.4.1. 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 (Figure A.1). 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 Σ PFAS 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 19.8-28.8 ng/g ww in fish muscle tissue of 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.

By comparing PFOS concentrations of the present study with concentrations that were found in 2003, we can conclude that actual PFOS concentrations are lower in both fish (*P. platessa*) and crustaceans (*C. crangon* and crab sp.) compared to 2003 (Table 2.2). The most probable explanation is the phase-out of PFOS and PFOA in 2000 by the perfluorochemical manufacturer 3M (Carloni, 2009). Several temporal studies report a decrease in PFOS concentrations in biota over time (Houde et al., 2011; Ahrens et al., 2009). For example, PFOS levels in European eel sampled in 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 (Munschy 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 (FASAAs), sulfonamides (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.

*Table 2.2: 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)

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 – 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 <0.03–1.1 ng/g to 0.27–22 ng/g (Pan et al., 2014). In the study of Munsch 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 ΣC7-C12 towards ΣC4-

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 – 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 50-60 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. 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.

2.4.2. Species-specific PFAS Profiles

Overall, similar contamination profiles were found among the 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 Dahlmstrom, 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).

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. However, species might also be affected by other exposure routes due to differences in spawning and nursery behaviour. For example, 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.

2.4.3. Trophic Transfer of PFAS

The negative correlations between TL and PFOA and PFUnDA concentrations in fish muscle and crustaceans, at locations 4, 5 and 8, suggests biodilution of these compounds, while the significant positive correlation with PFTrDA at location 8 suggests biomagnification of this compound throughout the North Sea food web. However, we must stress that at most locations and for most compounds, no significant correlations were found which indicates 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 1999-2002: 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 PFAS in gill breathing organisms appears to be lower compared to air breathing organisms (De Silvia et al., 2021). This is due to the greater respiratory elimination of PFAS via gills, facilitated by the high water throughput, compared to the elimination via lungs to air (De Silvia et al., 2021). Therefore, the present study implies that PFAS bioconcentration from the surrounding abiotic environment is most likely dominating over biomagnification in the North Sea food web. 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 likely 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 indicates 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. 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). In general, species with a higher trophic level are found to have a higher metabolic capacity to transform precursors such as perfluorooctane sulfonamides (FOSA) into PFOS (Gebbink et al., 2016; Galatius et al., 2013). However, in the Gironde estuary (France) no significant differences in mean FOSA-to-PFOS ratios were found between invertebrates and fish (Munoz et al., 2017b). 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.

2.4.4. 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 that is 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 A.1). As only large adult fish are available on commercial markets, the effects of both biomagnification and tissue dilution on PFOS concentrations in adult fish should be studied. 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 & Parrish, 1965; De Veen, 1978; Henderson & Holmes., 1989; Koutsikopoulos et al., 1995). Hence, the accumulated PFAS concentrations and the subsequent potential risks reported in this study are also crucial for international fisheries of 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, an Environmental Quality Standard (EQS) 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 (EQS_{biota, hh}) was derived. In the present study, the EQS_{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 EQS_{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 EQS_{biota, hh} that is still based on the

EFSA MRL from 2008 (15 ng/kg/day) which is almost 240 times higher compared the updated MRL from 2020 (0.063 ng/kg/day) (EFSA, 2008; EFSA, 2020).

Additionally, a specific EQS of 33 µg PFOS/kg was derived for the protection of top predators against secondary poisoning (EQS_{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 EQS_{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 EQS_{biota, secpois} was found. These findings indicate that the consumption of juvenile *P. platessa* and *S. solea* from the southern North Sea 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 potential risks for secondary poisoning need further clarification but also the potential effects of PFAS bioaccumulation on the population dynamics of these species must be investigated.

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 to 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, field monitoring and toxicity testing of a broad range of PFAS is of critical importance to elaborate existing risk assessment programs.

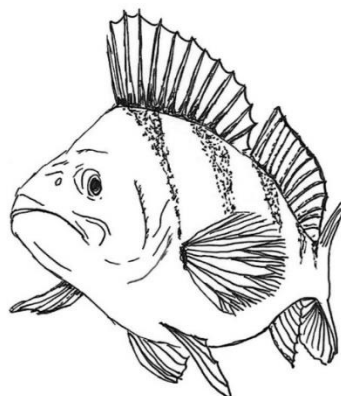
2.5. Conclusions

The present study shows that PFOS is still the predominant PFAS 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 PFTTrDA 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 Σ PFAS concentrations were found for *P. platessa* in liver tissue (52.5 ng/g ww) and the lowest mean Σ PFAS 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 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. 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 of secondary poisoning were identified for *P. platessa* and *S. solea* based on the combined PFAS concentrations in muscle and liver tissue, exceeding the EQS_{biota,secpois} threshold of 33 ng/g ww. However, no risks were observed when considering PFOS concentrations in muscle tissue alone, as they remained below the EQS_{biota,hh} threshold of 9.1 ng/g ww set for human health protection. This indicates the importance to interpret and discuss both standards in future studies.

Chapter III

Novel Insights into PFAS Bioaccumulation in the Scheldt River: The Role of Feeding Ecology using Stable Isotope Analysis



Manuscript under preparation

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Abstract

This study examines the spatial bioaccumulation patterns of per- and polyfluoroalkyl substances (PFAS) in fish and invertebrates along the Scheldt River. By integrating ecotoxicological data with stable isotope analysis of carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), and sulfur ($\delta^{34}\text{S}$), this study examines how ecological factors, such as feeding habitat preference, influence species-specific bioaccumulation patterns of PFAS.

The bioaccumulation of legacy PFAS was relatively evenly distributed along the river, suggesting historical pollution as the primary source. In contrast, alternative PFAS, such as FBSA and 6:2 FTS, showed a more localized distribution, indicating recent pollution from specific sources. Sulfur isotope analysis effectively distinguished pelagic and benthic foraging behaviors, revealing that pelagic feeders, such as Atlantic herring (*Clupea harengus*) and European smelt (*Osmerus eperlanus*), accumulated primarily water-soluble alternative PFAS, while benthic feeders, such as European eel (*Anguilla anguilla*), predominantly accumulated sediment-associated legacy compounds.

This study also reports some of the highest recorded concentrations of alternative PFAS in aquatic biota, including FBSA (71 ng/g ww in pike-perch (*Sander lucioperca*) muscle, 91 ng/g ww in European eel liver), NaDONA (18 ng/g ww in Atlantic herring muscle, 72 ng/g ww in European smelt liver), 6:2 FTS (170 ng/g ww in bream (*Abramis brama*) muscle), and PFBS (91 ng/g ww in European eel liver). These findings highlight the urgent need for continued biomonitoring of alternative PFAS, despite their presumed lower bioaccumulation potential.

3.1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are pervasive contaminants in aquatic environments, where their persistence, mobility, and bioaccumulative properties pose significant risks to ecosystems and organisms. These compounds comprise thousands of synthetic chemicals characterized by at least one fully fluorinated methyl or methylene carbon atom. Since the 1940s, PFAS have been extensively utilized in industrial and commercial applications due to their exceptional stability and amphiphilic nature (Buck et al., 2011; Wang et al., 2021). However, these same properties contribute to their environmental persistence, resistance to degradation, and potential toxicity, making them a major environmental concern (Ankley et al., 2021; Cousins et al., 2020; Dickman & Aga, 2022). PFAS enter the environment through multiple pathways, including industrial emissions, wastewater treatment effluents, and the leaching of consumer products from landfills (Ahrens & Bundschuh, 2014; Clara et al., 2008; Manojkumar et al., 2023; Möller et al., 2010). Once introduced, these chemicals are transported via riverine and atmospheric processes, facilitating their widespread distribution and global presence in both biotic and abiotic matrices (Khan et al., 2023; Kurwadkar et al., 2022).

The Scheldt river is a unique system for studying the bioaccumulation patterns of PFAS, as one of Europe's largest producers of PFAS is located in Antwerp on the banks of the river. A number of studies have documented the bioaccumulation of PFAS in fish and invertebrate species sampled from the Scheldt river, its estuary and its plume extending into the North Sea (Byns et al., 2022; de Vos et al., 2008; Groffen et al., 2024; Hoff et al., 2003; Teunen et al., 2021; Van De Vijver et al., 2003). However, these studies mainly focus on a limited number of compounds and solely examine the bioaccumulation in one or a few aquatic species. A notable temporal decrease in perfluorooctane sulfonate (PFOS) concentrations in fish and crustaceans sampled from the Scheldt estuary between 2003 and 2018 was observed, underlining the effectiveness of regulatory measures (Byns et al., 2022). In 2000, the PFAS manufacturer 3M initiated the phase-out of PFOS and perfluorooctanoic acid (PFOA) in response to the rising concerns regarding human and environmental health (Carloni, 2009). This action was followed by the introduction of further regulations on long-chain PFAS over the years, which resulted in a global production shift from long-chain to short-chain PFAS (Ateia et al., 2019). Initially, short-chain PFAS were considered suitable substitutes, based on the assumption that they have a lower

bioaccumulation potential and reduced toxicity (Bowman, 2015; Buck et al., 2011). However, the use of unregulated short-chain compounds has been subject to significant criticism due to their distinct, yet equally concerning, properties (Wang et al., 2017). Many short-chain compounds remain highly persistent, and their increased solubility in water leads to greater environmental mobility, as evidenced by their widespread environmental distribution (Brendel et al., 2018; Li et al., 2020).

The distinctive properties of PFAS may result in variations in their environmental fate, which in turn may lead to disparate bioaccumulation patterns. In addition, characteristics such as feeding behaviour, feeding strategy and habitat use can influence the exposure route and, consequently, explain the differences in bioaccumulation observed among species (Semerád et al., 2022). For instance, it can be hypothesized that pelagic feeders may be more exposed to short-chain PFAS in the water column, whereas benthic feeders are more likely to encounter long-chain PFAS that adsorb onto sediments. Consequently, the exposure risks faced by aquatic organisms may vary depending on their respective ecologies.

Therefore, the present study seeks to integrate ecotoxicological data with ecological tracers, such as stable isotope ratios. The nitrogen isotope ratio ($^{15}\text{N}/^{14}\text{N}$, hereafter $\delta^{15}\text{N}$) is a commonly used method for assessing the dietary nitrogen sources, but also for estimating the relative trophic position of a species. This is due to the progressive increase of $\delta^{15}\text{N}$ in the food chain due to the preferential release by animals of ^{14}N in excretion products (Pinnegar et al., 2002; Post, 2002). In contrast, carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$, hereafter $\delta^{13}\text{C}$) undergo less alteration during trophic transfer. The use of carbon isotope ratios allows for the identification of primary carbon sources that support the food chain. In the Scheldt Estuary, Middelburg and Nieuwenhuize (1998) have shown that four major pools of organic matter were recognized: riverine, estuarine, marine and terrestrial materials. Terrestrial organic matter ($\delta^{13}\text{C}$: -26‰, $\delta^{15}\text{N}$: 3.5‰, C:N = 21) is important for the sedimentary pool, but suspended matter is dominated by a mixture of marine ($\delta^{13}\text{C}$: -18‰, $\delta^{15}\text{N}$: 9‰, C:N = 8), riverine ($\delta^{13}\text{C}$: -30‰, $\delta^{15}\text{N}$: 9‰, C:N = 7.5) and estuarine ($\delta^{13}\text{C}$: -29‰, $\delta^{15}\text{N}$: 15‰, C:N = 8) organic matter. It is also a mixture of living phytoplankton and detrital organic particles. These contributions varied both spatially (along the salinity gradient) and temporally (across tidal cycles and seasons), highlighting the complexity of organic matter dynamics in the river (Cnudde et al., 2015; Moens et al., 2005). Sulfur isotopes serve as an additional tracer for organic matter sources, independent of the commonly used carbon isotope distribution (Herzka, 2005; Peterson & Howarth, 1987).

Although less frequently used, research indicates that sulfur isotopes are likely to refine input sources that cannot be clearly separated using the traditional dual nitrogen-carbon isotope approach due to the large differences in isotopic composition of seawater sulfate and sediment sulfides (Connolly et al., 2004; Peterson & Howarth, 1987). Stable isotope data can be visualized in so called isotopic niches which is a proxy of the ecological niche of a certain species at a certain region (Jackson et al., 2011). The combination of contamination profiles with species feeding ecology using stable isotopes has been found to be important to understand bioaccumulation pathways and species conservation (Coat et al., 2011; Miranda et al., 2021). However, for PFAS, such studies are generally lacking.

Therefore, this study aims to investigate the influence of habitat use and trophic position, as inferred from stable isotope analysis, on PFAS bioaccumulation patterns along the Scheldt River. Firstly, we examine the bioaccumulation of PFAS in different fish and invertebrate species, with a particular emphasis on their spatial distribution, along a salinity gradient, and with the identification of potential point sources along the river. Secondly, by measuring stable isotope ratios of C, N and S, we aim to explain differences in bioaccumulation patterns among species.

3.2. Materials and Methods

3.2.1. Sample Collection and Preparation

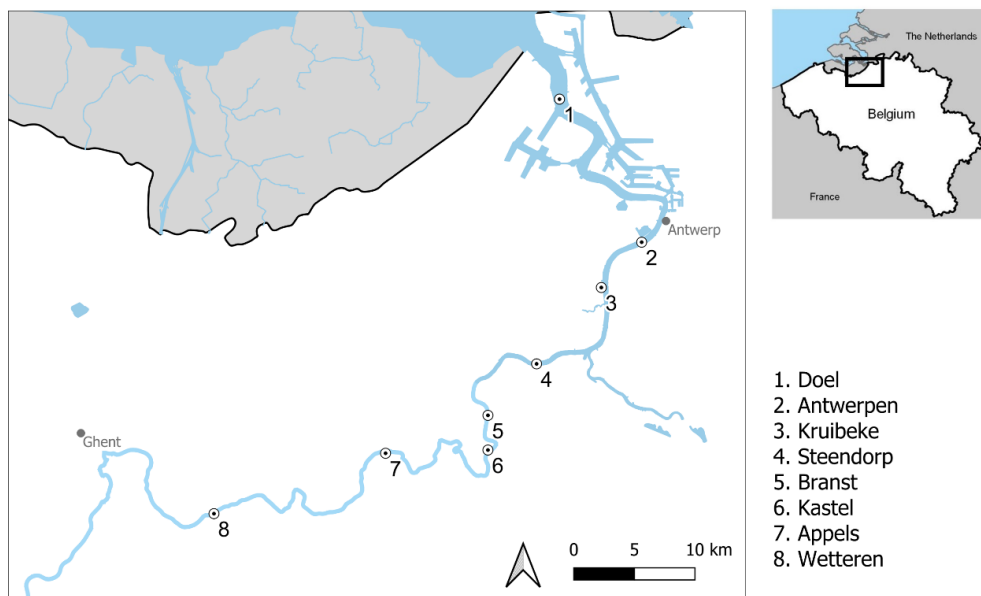


Figure 3.1: Sampling stations along the Zeeschelde

In September and October 2020, fish and invertebrate species were sampled at 8 stations along the Zeeschelde in collaboration with the Institute for Nature and Forest Research (INBO) (Figure 3.1). One station was located in the mesohaline zone (Doel), three in the oligohaline zone (Antwerp, Kruibeke and Steendorp) and four in the freshwater zone (Branst, Kastel, Appels and Wetteren). Detailed information on the coordinates and sampling dates can be found in Table B.1 in the Supplementary Information. The sample collection was done using cone shaped fish traps which were placed at the low water line and emptied after 24 hours. At each station, fish and invertebrates were counted and measured for monitoring purposes (Breine et al., 2021). Then, maximum 10 specimens per species were collected and euthanized using clove oil. The remaining organisms were released in the water. The selected specimens were stored in polyethylene bags in the freezer (-20 °C) until further dissection and analysis. Additionally, at 7 out of 8 sampling stations, INBO measured abiotic water characteristics such as temperature, oxygen, pH, turbidity, salinity, and conductivity at several timepoints (Table B.2).

From the stored fish and invertebrate specimen, four species, sampled at a minimum of five distinct sites, were selected for dissection and extraction analysis. This was done in order to study the spatial distribution of PFAS. Furthermore, in order to facilitate a comparison of species, all species collected in Antwerp were also subjected to dissection, given that this location exhibited the highest species diversity. This diversity encompassed nine fish species and three invertebrate species. For each fish and shrimp specimen the total length and bodyweight was measured and for the crabs, both carapax length and width were measured (Table B.7 and Figure B.1). The dissection was conducted in the lab and the dissection material was rinsed with ethanol (70%), acetonitrile (ACN, Acros Organics B.V.) and MilliQ (MQ; pore size: 0,22 μm , Merck Millipore, Darmstadt, Germany) in-between specimens to avoid cross-contamination. For all specimens muscle and liver (only for fish) tissue were dissected, homogenized and stored in the freezer (-20 °C) until further extraction.

3.2.2. PFAS Extraction Procedure

The PFAS extraction procedure was adapted from Powley et al. (2005) with minor modifications. Approximately 0.3 g wet weight (ww) of homogenized tissue was weighed and spiked with 10 ng of isotopically mass-labelled internal standard (ISTD, mPFAC-MXA, Wellington Laboratories, Guelph, Canada). Ten millilitres of ACN was then added, and the samples were vortexed and sonicated three times for 10 minutes each, with vortexing between sonication sessions. After incubating overnight on a shaking plate at room temperature, the samples were centrifuged at 4 °C for 10 minutes at 2400 rpm (Eppendorf 5804 R with A-4-44 rotor), and the supernatants were concentrated to approximately 0.5 mL using a vacuum concentrator (Martin Christ, RVC 2-25, Osterode am Harz, Germany). The extract was transferred to a 1.5 mL Eppendorf tube containing 50 mg of graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Belgium), which was activated with 50 μL of glacial acetic acid. The original tubes were rinsed twice with 250 μL of ACN to ensure that any remaining extract was transferred to the Eppendorf tubes. Each Eppendorf tube was vortexed for at least one minute and then centrifuged at 4 °C for 10 minutes at 10,000 rpm (Eppendorf 5415 R with F-45-24-11 rotor). The supernatant was subsequently dried using a vacuum concentrator. Finally, 200 μL of a 2% ammonium hydroxide solution was added to reconstitute the supernatants, which were then filtered through a 13 mm ion chromatography Acrodisc syringe filter into polypropylene injector vials.

3.2.3. UPLC-MS/MS analysis

A total of 28 PFAS were assessed using Ultra Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) (Table B.3). For comprehensive details on the analytes and instrumental settings, refer to (Groffen et al., 2021). To maintain quality control, a procedural blank consisting of 10 mL of ACN was utilized for every 10 samples during extraction. The limit of quantification (LOQ) for each analyte was calculated within the matrix based on the integrated peak area of a low-concentration signal achieving a signal-to-noise ratio of 10. To avoid cross-sample contamination between injections, ACN was periodically injected as an instrumental blank throughout the PFAS analysis.

3.2.4. Stable Isotope Analysis

The muscle tissue of the analysed fish and invertebrate specimens was freeze-dried for 48 h and homogenized into powder. However, as tissue samples from European eel (*Anguilla anguilla*) were not fully dried due to the high lipid content, these samples were several times washed in a 2:1 chloroform/methanol solution to extract the lipids (Folch et al., 1957). Stable isotopes ratios were analysed using an isotopic ratio mass spectrometer (PrecisION, Elementar, UK) coupled in continuous flow with an elemental analyser (VarioMicro cube, Elementar, Germany). Stable isotope ratios of carbon, nitrogen and sulfur were expressed in δ notation ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ respectively) and in ‰ relative to international references: Vienna Pee Dee Belemnite (vPBD) for carbon, Atmospheric Air for nitrogen and Vienna Canyon Diablo Troilite (vCDT) for sulfur. Furthermore, certified substance from IAEA (International Atomic Energy Agency), namely sucrose IAEA-C₆ (mean $\delta^{13}\text{C}$ =-10.8 ± SD 0.5‰), ammonium sulfate IAEA-N₂ (mean $\delta^{15}\text{N}$ =20.3 ± SD 0.2‰) and silver sulfide IAEA-S₁ (mean $\delta^{34}\text{S}$ =-0.3‰) were used as primary analytical standards, and sulfanilic acid (mean ± SD: $\delta^{13}\text{C}$ =-25.6 ± 0.4‰; $\delta^{15}\text{N}$ =-0.1 ± 0.5‰; $\delta^{34}\text{S}$ =5.9‰ ± 0.5‰) as secondary standard. For samples with a C:N ratio > 3.5 a correction was applied as $\delta^{13}\text{C}$ values in these samples may be influenced due to a high percentage of fat content (Post et al., 2007). This was done using the normalization equation of Post et al. (2007): $\delta^{13}\text{C}_{\text{normalised}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times (\text{C:N})$.

3.2.5. Statistical analysis

Statistical analyses were performed using Rstudio (version 2022.12.0+353) and GraphPad Prism (version 10.2.2 397) with the level of significance set at $p \leq 0.05$. Before conducting statistical analyses, the Maximum likelihood estimation (MLE) was used to assign replacement concentrations for PFAS measurements below the LOQ (Villanueva, 2005). This method assumes that the data follow a normal distribution; therefore, only compounds with a detection frequency of at least 30% were included in the statistical analysis (Helsel, 2006). To compare the PFAS concentrations among sampling sites of one particular species, two-way ANOVA analyses were performed. Post-hoc comparisons were performed using Tukey's honestly significant difference (HSD) adjusted post-hoc test or the non-parametric equivalent Dunn's post-hoc test when model assumptions (homogeneity of variances and normality of residuals) were not met. Correlation analyses were performed between the stable isotopes and the specimen lengths to determine if a correction for length variation was necessary. However, since only one correlation was found for the Chinese mitten crab versus $\delta^{15}\text{N}$ ($r=-0.77$, $p<0.05$), no length correction was applied. To link the spatial variability in stable isotopes with PFAS concentrations, correlation analysis were performed per species and for all sampling sites combined. Furthermore, the stable isotope analyses were explored using the Stable Isotopes Bayesian Ellipses Package (SIBER) in R (Jackson et al., 2011). The statistics of SIBER analysis are based on Bayesian inference and multivariate ellipse-based metrics to estimate isotopic niche width and overlap among species or groups. The isotopic niches ($\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ vs. $\delta^{34}\text{S}$, $\delta^{13}\text{C}$ vs. $\delta^{34}\text{S}$) and contamination niches (ΣPFAS vs. $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$) were examined for seven fish species and three invertebrate species. It should be noted that insufficient data were available for bream (*Abramis brama*) ($N=3$) and European perch (*Perca fluviatilis*) ($N=3$) to be included in the modelling of their ellipses (less than five individuals). The standard ellipse area (SEA) represents the geometric representation of a niche in a SIBER model, incorporating 40% of the studied individuals. This can be considered an equivalent to the standard deviation.

3.3. Results

3.3.1. PFAS Bioaccumulation Patterns: Spatial Distribution

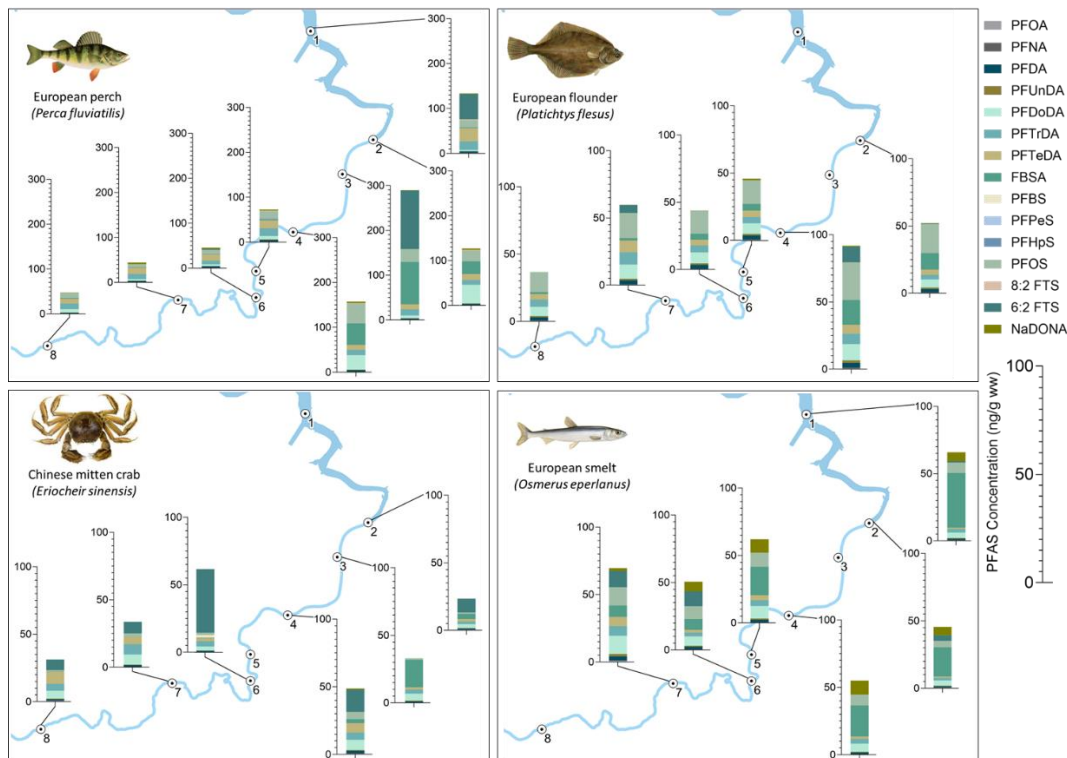


Figure 3.2: Spatial distribution pattern of PFAS profiles for four species sampled at different sampling sites along the Scheldt river: European perch, European flounder, Chinese mitten crab and European smelt. PFAS concentrations are expressed in ng/g ww. Site 1 = Doel, 2 = Antwerp, 3 = Kruibeke, 4 = Steendorp, 5 = Branst, 6 = Kastel, 7 = Appels, 8 = Wetteren.

The spatial distribution of PFAS contamination along the Scheldt River was investigated in muscle tissue for four species: European perch, European flounder (*Platichthys flesus*), Chinese mitten crab (*Eriocheir sinensis*) and European smelt (*Osmerus eperlanus*), which were collected from at least five sampling sites (Figure 3.2, Table B.4).

For the European perch (*Perca fluviatilis*), significantly elevated concentrations of perfluorododecanoic acid (PFDoDA) were detected at site two (Antwerp) when compared to all other sampling locations (p -values <0.01), with the exception of site four (Steendorp). Additionally, at Steendorp, PFDoDA levels were significantly higher than those measured at sites seven (Appels; $p=0.004$) and six (Kastel; $p=0.02$). Site three (Kruibeke) showed significantly

higher perfluorobutane sulfonamide (FBSA) concentrations than any other site (all p-values <0.001). Moreover, elevated FBSA concentrations were also recorded at site four (Steendorp) relative to sites eight (Wetteren), seven (Appels), six (Kastel), five (Branst), and one (Doel) (all p-values <0.001), and at site two (Antwerp) in comparison to sites seven (Appels), six (Kastel), and five (Branst) (p-values <0.05). PFOS concentrations at site four (Steendorp) were also found to be significantly higher compared to all other sampling sites (p<0.05), with the exception of site two and three. In terms of 6:2-fluorotelomersulfonic acid (6:2 FTS), significantly higher concentrations were identified at sites three (Kruibeke) and one (Doel) compared to all other sites (all p-values <0.001).

In the European flounder (*Platichthys flesus*), significant higher bioaccumulation of FBSA and PFOS were observed at site four (Steendorp) compared to all other sites (p<0.001). Notably, this site also presented significantly higher concentrations of 6:2 FTS compared to site three (Antwerp; p=0.01). Meanwhile, 6:2 FTS was not detected at sites eight (Wetteren), six (Kastel), and five (Branst).

In the Chinese mitten crab (*Eriocheir sinensis*), significantly higher concentrations of FBSA were found at site three (Kruibeke) relative to sites seven (Appels), six (Kastel), four (Steendorp), and two (Antwerp) (all p-values <0.01). Additionally, site six (Kastel) demonstrated higher concentrations of 6:2 FTS compared to all other sampling sites (p<0.001).

For European smelt (*Osmerus eperlanus*), markedly higher concentrations of FBSA were documented at site one (Doel) in comparison to all other sites (p<0.001). Furthermore, reduced FBSA concentrations were observed at sites seven (Appels) and six (Kastel) compared to sites five (Branst), four (Steendorp), and two (Antwerp) (all p-values <0.01).

Considering the sum of PFAS, in European perch, significant higher concentrations were detected at site three (Kruibeke) when compared to site eight (Wetteren), seven (Appels), six (Kastel) and five (Branst) (all p-values <0.001). Additionally, a higher concentration was noted at site four (Steendorp) compared to site seven (Appels) (p=0.04). For European flounder higher Σ PFAS concentrations were detected at site four (Steendorp) compared to site eight (Wetteren), seven (Appels), six (Kastel) and five (Branst) (p-values <0.05). For Chinese mitten crab and European smelt no significant differences were found.

3.3.2. Spatial Variability of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$

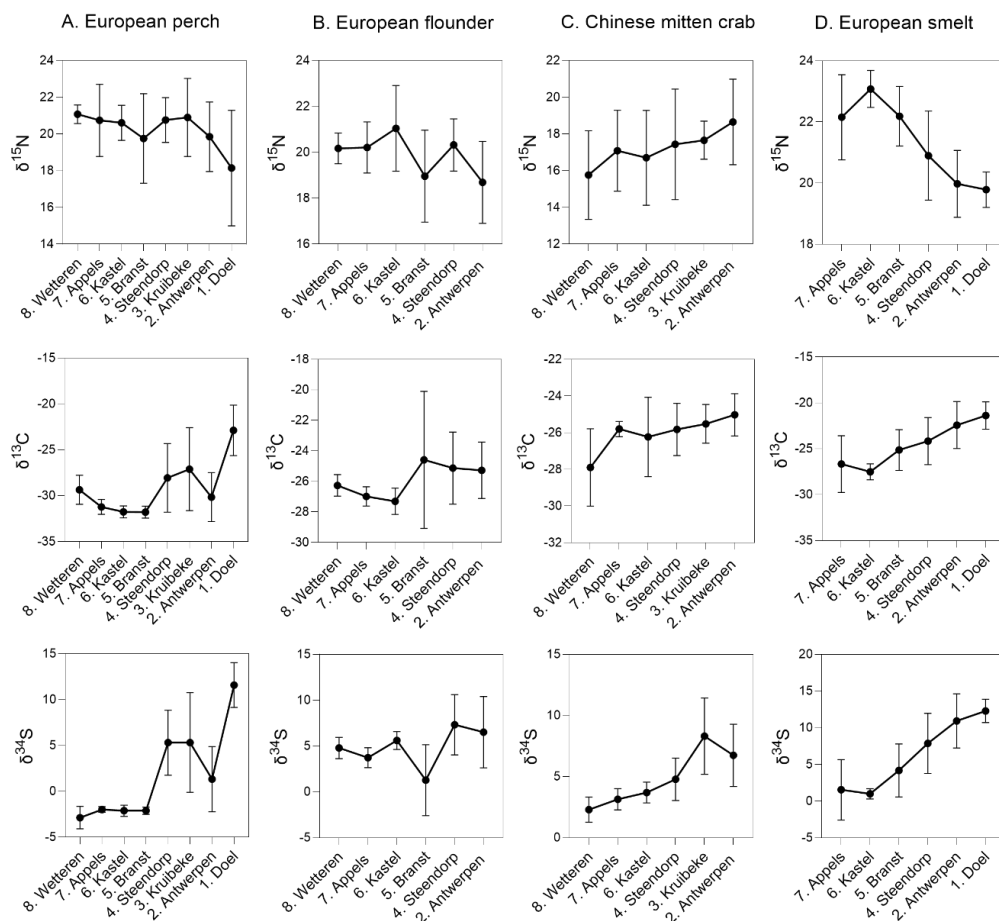


Figure 3.3: $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values (‰, average + SD) in A. European smelt, B. European flounder, C. Chinese mitten crab, D. European smelt, per sampling sites along the Scheldt river. Only sites where the specific fish species were caught were included.

The spatial patterns of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values along the Scheldt river are most pronounced in European smelt. Except for the European flounder, a gradual increase in $\delta^{34}\text{S}$ values was observed from the freshwater to the estuarine region. This increase is most pronounced in European perch and European smelt, with average minimum to maximum values ranging from -2.9‰ at Wetteren to 12‰ at Doel for perch, and from 1‰ at Kastel to 12‰ at Doel for smelt. The general patterns for carbon and nitrogen stable isotopes are less distinct, as their min-max ranges are not as pronounced. For $\delta^{13}\text{C}$, an increase in average values was noted towards the estuarine region for European perch (from -32‰ at Branst to -23‰ at Doel), European smelt (from -27‰ at Kastel to -21‰ at Doel), and Chinese mitten crab (from -28‰ at Wetteren to -

25‰ at Antwerp). No such pattern was observed for the European flounder. Conversely, $\delta^{15}\text{N}$ values showed a slight decrease towards the estuarine region for European perch (from 21‰ at Wetteren to 18‰ at Doel), European flounder (from 21‰ at Kastel to 19‰ at Antwerp), and European smelt (from 23‰ at Kastel to 20‰ at Doel). For the Chinese mitten crab, an opposite trend was observed, with a slight increase in $\delta^{15}\text{N}$ values towards the estuarine region (from 16‰ at Wetteren to 19‰ at Antwerp). This spatial variability in stable isotopes could not be linked to the spatial variability in bioaccumulated PFAS concentrations, since no significant correlation was observed per species and for all sampling sites combined.

3.3.3. Species-specific PFAS Profiles: Comparison and Tissue Distribution

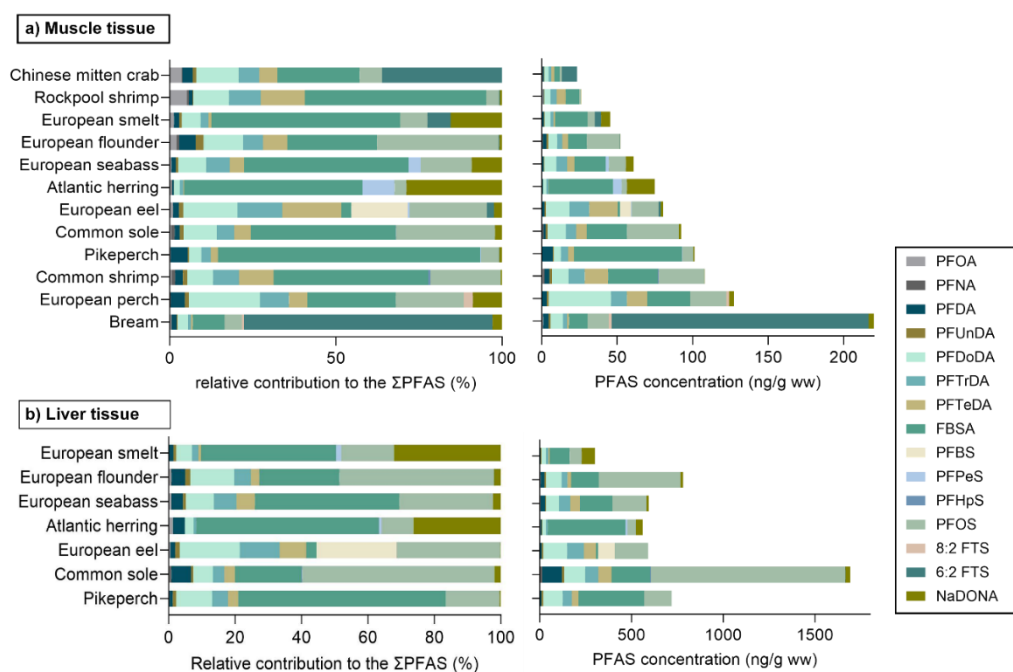


Figure 3.4: PFAS bioaccumulation profiles in a) muscle and b) liver tissue of fish and invertebrate species sampled at site 3 (Antwerp) in the Scheldt river. PFAS profiles are first expressed as the relative contribution to the Σ PFAS (corrected for the molecular weight of each compound) and secondly in PFAS concentration in ng/g ww.

A comparative analysis of PFAS profiles among species was conducted within a single sampling site to account for differences in bioaccumulation. Antwerp was chosen for this study due to its high diversity in species sampled, including eight fish species and three invertebrate species.

Detailed data on the relative contribution, mean concentration, and min-max range per species and per compound in liver and muscle tissue are presented in Tables B.4 and B.5.

The relative contribution of long-chain PFCAs (PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), PFDoDA, perfluorotridecanoic acid (PFTTrDA), perfluorotetradecanoic acid (PFTeDA)) in muscle tissue was highest in European eel (*Aguilla anguilla*) (52%), followed by European perch and rockpool shrimp (*Palaemon elegans*) (41%), European flounder (35%), Chinese mitten crab (32%), common shrimp (*Crangon crangon*) (31%), common sole (*Solea solea*) (24%), European seabass (*Dicentrarchus labrax*) (22%), pikeperch (*Sander lucioperca*) (14%), European smelt (13%), bream (*Abramis brama*) (7%), and Atlantic herring (*Clupea harengus*) (4.2%). A similar order was observed in liver tissue.

The short-chain compound FBSA was detected in all species, with a notably high relative contribution to the total PFAS in most species, ranging from 25% in Chinese mitten crabs to 79% in pikeperch. Exceptions were European eel (3%) and bream (9%). The highest mean FBSA concentration in muscle tissue was found in pikeperch (71 ng/g ww) and the lowest in European eel (1.3 ng/g ww). In liver tissue, the highest mean FBSA concentrations were detected in Atlantic herring (422 ng/g ww) and pikeperch (358 ng/g ww), with the lowest in European eel (11 ng/g ww).

European eel was the only species in which PFBS was detected in both muscle (17%, mean concentration of 7.6 ng/g ww) and liver tissue (24%, mean concentration of 91 ng/g ww). PFPeS was detected exclusively in the muscle tissue of Atlantic herring (9.8%, mean concentration: 5.9 ng/g ww) and European seabass (3.6%, mean concentration: 2.1 ng/g ww). In liver tissue, it was not detected in European seabass and was present in only four out of nine Atlantic herring samples, with a mean concentration of 10 ng/g ww.

Sodium dodecafluoro-3H-4,8-dioxanonoate (NaDONA) showed a notably high relative contribution in the muscle tissue of Atlantic herring (28%), European smelt (15%), European seabass (9.1%), and European perch (8.8%), with mean concentrations of 18 ng/g ww, 6.1 ng/g ww, 5.2 ng/g ww, and 3.2 ng/g ww, respectively. In other species, the relative contribution ranged from 0.06% (Chinese mitten crab) to 2.9% (bream). In liver tissue, the relative contribution of NaDONA was highest in herring (26%), smelt (32%), and seabass (2.4%), with mean concentrations of 38 ng/g ww, 72 ng/g ww, and 12 ng/g ww, respectively. Despite a lower

percentage detection of NaDONA in common sole (1.9%), the average concentration was relatively high (25 ng/g ww), with concentrations ranging from 0.11 to 44 ng/g ww.

The fluorotelomer 6:2 FTS exhibited a remarkably high relative contribution of 75% in bream, with an average concentration of 170 ng/g ww. Additionally, Chinese mitten crab and European smelt showed relative contributions of 36% and 6.9%, with average concentrations of 10 ng/g ww and 4.3 ng/g ww, respectively. In all other species, the relative contribution was 2.1% (European eel) or lower.

The relative contribution of PFOS to the total PFAS varied significantly among species in muscle tissue. The highest contribution was observed in European flounder (36%), followed by common sole (30%), European eel (23%), common shrimp and European perch (21%), European seabass (15%), European smelt (8.2%), Chinese mitten crab (6.8%), pikeperch (5.5%), bream (5.2%), rockpool shrimp (3.9%), and Atlantic herring (3.5%). The concentrations ranged from 1.1 ng/g ww in rockpool shrimp to 33 ng/g ww in common sole. In liver tissue, both the relative contribution of PFOS and specifically mean concentrations were generally much higher compared to muscle tissue. The highest contribution and mean concentration was found in common sole (58%, 1056 ng/g ww), followed by European flounder (47%, 449 ng/g ww), European eel (31%, 183 ng/g ww), European seabass (28%, 188 ng/g ww), pikeperch (16%, 150 ng/g ww), European smelt (16%, 66 ng/g ww), and Atlantic herring (9.7%, 47 ng/g ww).

3.3.4. Stable Isotope Ellipses: SIBER Analysis

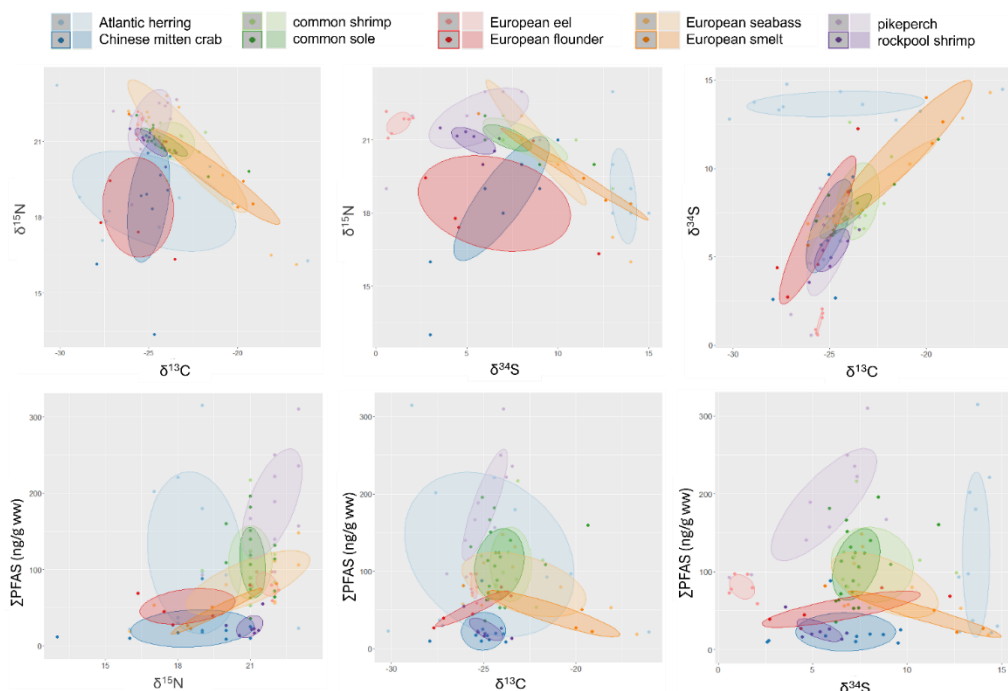


Figure 3.5: Isotopic ($\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ vs. $\delta^{34}\text{S}$, $\delta^{13}\text{C}$ vs. $\delta^{34}\text{S}$) and contamination (ΣPFAS vs. $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$) niches biplots of seven fish species and three invertebrate species sampled at site 3 (Antwerp) in the Scheldt river.

In the $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ biplot (Figure 3.5), Atlantic herring exhibited the largest isotopic niche area ($\text{SEAc: } 27\text{‰}^2$), followed by European flounder ($\text{SEAc: } 14\text{‰}^2$), European seabass ($\text{SEAc: } 11\text{‰}^2$), Chinese mitten crab ($\text{SEAc: } 8.9\text{‰}^2$), European smelt ($\text{SEAc: } 7.5\text{‰}^2$), pikeperch ($\text{SEAc: } 4.7\text{‰}^2$), common shrimp ($\text{SEAc: } 2.8\text{‰}^2$), common sole ($\text{SEAc: } 1.3\text{‰}^2$), rockpool shrimp ($\text{SEAc: } 0.99\text{‰}^2$), and European eel ($\text{SEAc: } 0.22\text{‰}^2$). However, when $\delta^{34}\text{S}$ is included, as in the $\delta^{15}\text{N}$ vs. $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$ vs. $\delta^{34}\text{S}$ biplots, the isotopic niche area for Atlantic herring is reduced, with SEAc values of 3.6‰^2 and 11‰^2 , respectively. Notably, the $\delta^{34}\text{S}$ -inclusive biplots also demonstrate a clear separation of European eel and Atlantic herring from the other species, with little to no overlap in their isotopic niches. A similar trend is observed in the contamination niches. In the ΣPFAS vs. $\delta^{34}\text{S}$ biplot, Atlantic herring, European eel, and pikeperch are distinctly separated from the other species, showing minimal overlap. This distinct separation is not observed in the ΣPFAS vs. $\delta^{15}\text{N}$ and ΣPFAS vs. $\delta^{13}\text{C}$ biplots, where the positions of the other species remain relatively

unchanged. An overview of the isotopic and contamination niche areas (SEAc) for all biplots and species is provided in Table B.6.

3.4. Discussion

3.4.1. PFAS Bioaccumulation Patterns: Spatial Distribution

Upon analysing the spatial distribution of bioaccumulated PFAS profiles along the Scheldt river, it was observed that within each species, there was a relatively uniform distribution of legacy compounds such as long-chain perfluoroalkyl carboxylic acids (PFCAs: PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA) and perfluorooctanesulfonic acid (PFOS). This consistency in distribution indicates the homogenous and consistent environmental presence and bioavailability of legacy compounds throughout the aquatic system studied, regardless of the spatial variance among sampling sites.

A distinctive pattern emerged for FBSA showing elevated concentrations in specific regions, notably at sites four (Steendorp), three (Kruibeke), and two (Antwerp) across all studied species. FBSA is a short-chain precursor whose production has increased following the phase-out and regulation of legacy long-chain chemicals such as PFOS and PFOA (Ateia et al., 2019). Furthermore, FBSA can be a degradation product and metabolite of other precursors in aqueous film-forming foams (AFFFs) and surface treatment products (Barzen-Hanson et al., 2017; Chu & Letcher, 2014). Although FBSA is still poorly monitored in biota, studies examining fish samples from diverse water bodies across Canada and the Great Lakes region indicate a widespread occurrence of FBSA in freshwater ecosystems (Chu et al., 2016; Kaboré et al., 2022). Concentrations in fish samples from more remote areas, such as Great Bear Lake, Kusawa Lake, and Lake Athabasca, ranged from below detection limits to 0.44 ng/g ww. These levels are substantially lower than those observed in the more urbanized and industrialized Great Lakes basin, where average concentrations reached 3.17 ng/g ww. In contrast, the present study recorded average FBSA concentrations up to 71 ng/g ww (in pikeperch, Antwerp), demonstrating considerably higher levels compared to those reported across Canada. Notably, the higher FBSA concentrations were more localized in a specific region rather than being uniformly distributed, which may suggest the presence of a local point source of FBSA in the Scheldt. In August 2021, multiple allegations surfaced against the 3M plant located near the

Scheldt river in Antwerp, accusing it of illegally discharging wastewater containing FBSA. These claims were extensively reported in several Belgian newspapers, leading to calls for legal action against 3M (Arnoudt, 2021). This information supports the hypothesis of the localized pollution of FBSA in the area of Antwerp and surrounding sampling sites.

In the study by Groffen et al. (2024), the bioaccumulation of PFAS in Chinese mitten crabs across Flanders in Belgium was investigated, including sites in the Scheldt river. Groffen et al. (2024) reported the highest mean FBSA concentration at Doel, measuring 9.9 ng/g ww. In contrast, the highest concentration in the present study was found slightly further upstream at Kruibeke, measuring 20 ng/g ww. However, a direct comparison is not possible as Chinese mitten crabs were not collected at Doel in the present study, nor at Kruibeke in the Groffen et al. study. Both studies included sampling at Steendorp, where Groffen et al. (2024) detected mean concentrations of 1.8 ng/g ww, while the present study found higher levels at 3.6 ng/g ww. The temporal overlap in sampling periods complicates any conclusions about changes in concentrations over time. Groffen et al. (2024)'s samples were collected between September 2020 and May to November 2021, whereas the present study's samples were collected between September and October 2020. In both studies, crabs are considered to be adults based on the carapax width.

Furthermore, in the present study significantly higher concentrations of 6:2 FTS were observed in Chinese mitten crabs in the regions of Kastel and Steendorp. Similarly, elevated concentrations were found in European perch at sites further downstream, around Kruibeke and Doel. The fluorotelomer 6:2 FTS and its derivatives are utilized as alternatives to PFOS in the production of fire-fighting foams (Yang et al., 2014). Laboratory toxicity tests on rainbow trout have demonstrated that 6:2 FTS is effectively eliminated in teleost fish and is considered unlikely to bioaccumulate or biomagnify in aquatic systems (Hoke et al., 2015; Yeung & Mabury, 2013). However, a field study by Langberg et al. (2019) detected considerable concentrations of 6:2 FTS in marine invertebrates (crabs) but not in fish. This discrepancy suggests that invertebrates may possess different detoxification pathways and enzymes compared to fish, leading to distinct accumulation patterns. However, this hypothesis is in need of further experimental confirmation. Notably, the present study also found significant levels of 6:2 FTS in certain fish species such as European perch, highlighting the potential for bioaccumulation variability among different fish species. These results underline the importance of caution when drawing conclusions from laboratory experiments conducted under controlled conditions and

based on a single test species. It is crucial to consider the complexities of field conditions and species-specific responses to assess the environmental impact of chemicals such as 6:2 FTS.

Migration is a critical factor to consider when evaluating the accumulated PFAS profiles, as it raises the question of whether these profiles accurately reflect the contamination state of specific sites. The four species examined in section 4.3.1. can be considered relatively resident during the sampling period and life stages observed. The sampled European perch specimens were all below the total length at maturity, indicating that they were juveniles (Figure B.2). Juvenile perch utilize estuarine environments for feeding and growth and primarily engage in short-range movements (Kottelat & Freyhof, 2008). As they mature, they tend to migrate to more stable freshwater environments. Similarly, European flounders, which exhibit a catadromous life cycle, spawn in oceanic waters, feed and grow in estuaries, and return to oceanic waters as adults (Summers, 1979). The flounder specimens in the present study were primarily early-settled juveniles (total length <90 mm) which are known to be highly site-restricted (Teichert et al., 2022). In contrast, the European smelt is an anadromous species that lives in schools in estuarine and coastal waters and migrates into freshwater zones to spawn in early spring (Lyle & Maitland, 1997). Based on total length measurements, specimens sampled at the upstream sites Appels and Kastel were juveniles close to adult length, while specimens from other sites were adults. As sampling was conducted outside the migration period, these specimens are not expected to migrate large distances. The migration behaviour of Chinese mitten crabs is not yet fully understood, but several studies in similar systems as the Scheldt river, such as the Thames River in Great Britain and the Elbe River in Germany, have documented downstream migration of adults during fall and winter (Dittel & Epifanio, 2009). Therefore, it is possible that the crabs sampled in our study (all in adult stage, Figure B.2) had just started their downstream migration, reflecting pollution profiles from more upstream sites. However, this does not significantly alter the hypothesis regarding the FBSA pollution source, as for all species the contamination is reflected in a region of sites, rather than being confined to a single site.

The dispersion of pollution hotspots can be attributed to the dynamic nature of the Scheldt river (Meire et al., 2005). The estuary's exceptional tidal influence (even up until the freshwater area) possibly contributes to the mixing and potential up- and downstream transport of contaminants (Vanderborght et al., 2007). Another crucial factor to consider is the salinity gradient observed during the sampling period, which ranged from 0.43‰ in Wetteren to 18‰

in Doel (Table B.2). The sorption of PFAS onto sediments increases with rising salinity, a phenomenon known as the salting-out effect (Jeon et al., 2011; You et al., 2010). This effect is particularly pronounced for longer-chain PFAS, as their hydrophobic tails interact more strongly with hydrophobic sites on sediment particles (Munoz et al., 2017a). Consequently, this partitioning process is expected to reduce water-mediated exposure pathways for PFAS. However, benthic organisms, which frequently interact with sediment, may experience an increased risk of exposure. Despite their classification as benthic feeders, no clear bioaccumulation pattern linked to this effect was observed in European flounder or Chinese mitten crabs. In contrast, European smelt, a pelagic feeder primarily consuming amphipods, mysids, and other fish (McGoran et al., 2017; Taal et al., 2014), exhibited a gradual decrease in the average concentration of long-chain perfluorinated carboxylic acids (PFCAs) from upstream to downstream locations. PFCA levels declined from 33 ng/g ww upstream to 9.8 ng/g ww downstream, suggesting that reduced water-mediated exposure may influence PFAS bioaccumulation in this species. However, further research is needed to clarify this relationship. Additionally, also other physicochemical parameters such as pH can influence PFAS bioaccumulation. For example, lower pH values enhance PFAS adsorption onto sediments through electrostatic interactions (Ohoro et al., 2024). However, in this study, pH variations across sampling stations were minor, ranging from 7.7 in Antwerp to 8.1 in Kastel, suggesting that pH is unlikely to be a major driver of PFAS bioaccumulation patterns in this system.

3.4.2. Spatial Isotope Composition Variability

Estuarine systems provide a diverse range of habitats for aquatic organisms, including marshes, subtidal and intertidal mudflats, rocky shores, and unvegetated areas (Meire et al., 2005). Consequently, individuals of the same species may exhibit different patterns of habitat utilization depending on their location within the system, potentially leading to varying levels of contaminant exposure. The observed downstream enrichment of sulfur isotope ratios in the present study might indicate a gradual transition in sulfur sources along the Scheldt river. Producers that predominantly utilize seawater sulfates, such as microalgae and phytoplankton, are isotopically enriched in ^{34}S (approximately +20‰), suggesting offshore and pelagic behaviour (Connolly et al., 2004; Peterson & Howarth, 1987). In contrast, those relying on sedimentary sulfides, such as anaerobic bacteria and marsh plants (approximately -24‰), or

sulfates from precipitation, such as emergent or intertidal plants (approximately +2‰ to +8‰), are isotopically lighter, suggesting coastal and benthic behaviour (Connolly et al., 2004; Herzka, 2005; McMahon et al., 2013). Accordingly, the slight increase in $\delta^{34}\text{S}$ values may indicate a decrease of benthic sulfur to the water column. Similarly, $\delta^{13}\text{C}$ values are more depleted in the upstream freshwater part of the Scheldt than in the downstream brackish part. This is likely due to the predominant input of riverine and terrestrial organic matter in the upper parts of the river. This pattern is supported by likewise studies that were conducted in the Scheldt estuary on suspended matter and fishes (Guelinckx et al., 2006; McMahon et al., 2013; Middelburg & Nieuwenhuize, 1998; Van Ael et al., 2013). The spatial pattern of $\delta^{15}\text{N}$ isotopic values exhibited minimal variation across the sampling stations, and variable patterns were observed among the species. This variability is likely attributable to the seasonal reversal of the $\delta^{15}\text{N}$ gradient. Previous temporal studies on fish, copepods, and particulate organic matter in the Scheldt have reported a variable $\delta^{15}\text{N}$ gradient, with depleted values observed from late autumn to early spring and enriched values from late spring to early autumn in the brackish part of the river (De Brabandere et al., 2002; Guelinckx et al., 2006; Middelburg & Nieuwenhuize, 1998). Therefore, since our samples were collected in September and October, the alternating $\delta^{15}\text{N}$ values observed across different species is likely attributed to natural variations in the isotopic composition of the inorganic nitrogen pool. Overall, the observed variations in stable isotope compositions in the present study suggest a subtle differentiation in habitat utilization and feeding ecology across the sampling stations. However, the absence of correlations between stable isotope data (analysed per species and aggregated across sampling sites) and PFAS concentrations indicates that the spatial differences in PFAS bioaccumulation patterns, such as the elevated FBSA levels observed around Antwerp, are unlikely to result from shifts in feeding ecology. Rather, these spatial variations are more plausibly attributed to heightened environmental exposure together with the environmental conditions in these specific area.

3.4.3. Species PFAS Profiles Comparison Using Stable Isotope Analysis

The isotopic niches of the fish and invertebrate species provide an estimation of their ecological niches, which can be described by two key metrics: niche area and niche overlap (Das et al., 2017; Pinzone et al., 2019). The niche area reflects the diversity of habitats and resources utilized by a species while the niche overlap, on the other hand, illustrates the extent to which

these resources are shared with other species. In the present study, niche overlap is described qualitatively rather than quantified, due to the large number of species involved. The integration of PFAS concentrations into the isotopic niches gives rise to what can be defined as the 'contamination niche' (Pinzone et al., 2019).

When examining the $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ isotopic niches (Figure 3.5), species such as pikeperch, rockpool shrimp, common shrimp, common sole, and European eel generally display higher $\delta^{15}\text{N}$ values, indicative of a higher trophic position compared to European flounder, Atlantic herring, and Chinese mitten crab. However, in the present study, it is not appropriate to directly associate $\delta^{15}\text{N}$ with trophic position due to the complexity of the Scheldt river, where multiple $\delta^{15}\text{N}$ baselines (freshwater, marine, terrestrial origins) exist. Consequently, identical $\delta^{15}\text{N}$ values can correspond to different trophic positions depending on the baseline relied upon by each species. For example, as illustrated in the contamination niche of ΣPFAS versus $\delta^{15}\text{N}$ (Figure 3.5) and the bioaccumulation profiles (Figure 3.4), the rockpool shrimp exhibited some of the lowest accumulated PFAS concentrations despite higher $\delta^{15}\text{N}$ values. In contrast, pikeperch, which shares a similar $\delta^{15}\text{N}$ range, demonstrated some of the highest accumulated ΣPFAS concentrations. These results would suggest that both species have a similar trophic position; however, it is documented in literature that pikeperch and rockpool shrimp have different feeding strategies and are likely to occupy distinct trophic positions (Bódis et al., 2007; Janas & Barańska, 2008). Therefore, it can be concluded that $\delta^{15}\text{N}$ is not an appropriate parameter for explaining bioaccumulation differences among species within the current system. In order to effectively investigate the influence of trophic position on PFAS bioaccumulation in complex systems, such as the Scheldt River, it is recommended that potential baselines are sampled, including macrophytes, benthic algae, phytoplankton, etc., in order to account for such variations.

It is notable that the niche area of Atlantic herring in the $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$, ΣPFAS versus $\delta^{15}\text{N}$ and ΣPFAS versus $\delta^{13}\text{C}$ biplots is considerable compared to the other species, indicating a high degree of variability in resource and habitat use among individuals. However, studies of feeding ecology based on the analysis of stomach contents describe smaller herrings as selective feeders that consume a limited number of zooplankton species (Casini et al., 2004). The specimens examined in the present study were all juveniles, with total lengths ranging from 9.1 to 11 cm. Temporal studies indicate that herring species can adapt their feeding behaviour in response to changes in food availability and environmental conditions (Bachiller et al., 2016;

Casini et al., 2004). This adaptability likely explains the broad range of carbon sources, as stable isotopes reflect assimilated food sources over the long term, whereas stomach content analysis provides only short-term dietary insights.

In addition to Atlantic herring, European seabass and European smelt also demonstrate a broad range of $\delta^{13}\text{C}$ values, covering a particular range where no overlap with other species is found. Markedly, these species demonstrate an elevated relative contribution of NaDONA, which is less pronounced in other species. European perch also exhibited notable NaDONA levels; however, due to an insufficient number of replicates, it was not included in the SIBER analysis. NaDONA is an alternative to PFOA that is used as an emulsifier in the polymerisation of fluoropolymers (Munoz et al., 2019). The results of experimental studies conducted on rats have indicated that NaDONA is much less toxic than legacy compounds such as PFOS and PFOA (Gordon, 2011). In addition, NaDONA has been shown to have a lower bioaccumulation potential and high solubility in water (Munoz et al., 2019). Research on the environmental occurrence of NaDONA is limited. Nevertheless, according to the review of Pan et al. (2018), numerous studies have documented the presence of NaDONA in surface waters in close proximity to fluorochemical manufacturing facilities (Pan et al., 2018). This pattern is also evident in the current study, with the proximity of the 3M fluorochemical plant in the vicinity of the sampling location in Antwerp. To the best of our knowledge, the NaDONA concentrations documented in the present study represent among the highest ever recorded in the livers and muscles of aquatic organisms (Feng et al., 2024).

The inclusion of $\delta^{34}\text{S}$ values results in a clear separation of the Atlantic herring ellipse. The ellipse representing European smelt extends into the range observed for herring, while still overlapping with those of the other species. The enrichment of $\delta^{34}\text{S}$ indicates a greater influence of water column primary production on the diet of these zooplanktonophagous fishes (Connolly et al., 2004). This may provide an explanation for the relative contribution of NaDONA being more pronounced in herring and smelt, given that exposure to NaDONA is likely to be water-mediated due to its high water solubility (Munoz et al., 2019). Furthermore, other highly water-soluble short-chain compounds, such as FBSA and PFPeS, predominantly contribute to the PFAS profile, whereas the contribution of long-chain compounds is significantly less pronounced in those species. A comparable pattern was observed in the study conducted by Teunen et al. (2021), wherein the relative contribution of short-chain PFAS in water exposed mussels was markedly higher than that in resident demersal fish, European eel and European

perch. These findings highlight the necessity for monitoring novel alternative PFAS in pelagic species, particularly in areas of potential pollution.

In contrast to Atlantic herring, European eel shows a distinct separation on the opposite side of the $\delta^{34}\text{S}$ range, indicating sulfur depletion. This indicates that the sulfur sources for the European eel are primarily derived from a demersal foraging behaviour (Connolly et al., 2004). Furthermore, the European eel has a relatively restricted isotopic niche area, suggesting small variability in sources and habitat used among individuals. However, it is often characterized as an opportunistic feeder with the capacity to readily adapt its diet (Bouchereau et al., 2009; Van Liefferinge et al., 2012). The eel exhibits a distinctive bioaccumulation profile in comparison to the other species. The highest contribution of legacy long-chain PFCAs (52%) was observed, with PFDoDA, PFTrDA, and PFTeDA the predominant compounds. Secondly, it is the only species in which a considerable amount of PFBS was detected, with average concentrations of 7.5 ng/g wet weight in muscle tissue and 91 ng/g wet weight in liver tissue. Experimental studies have demonstrated that PFBS exhibits a reduced bioaccumulation potential in comparison to FBSA (Rericha et al., 2022). In light of these findings and in accordance with the results observed in the other species, it is anticipated that FBSA would bioaccumulate more readily, as it is a precursor to PFBS as a metabolite in fish (Chu et al., 2016). However, studies have demonstrated that sulfonamide PFAS can undergo biotransformation into terminal end products, including perfluoroalkyl carboxylates and sulfonates (Buck et al., 2011). Consequently, it is plausible that the European eel employs a metabolic strategy to biotransform bioaccumulated FBSA into PFBS. However, the current understanding of these processes and strategies is generally lacking, and further experimental research is essential to enhance our knowledge in this area.

A distinct bioaccumulation pattern has been observed in both the Chinese mitten crab and bream, with a high contribution of 6:2 FTS to their PFAS profiles. Although literature characterizes both species as exhibiting typical benthic feeding behaviour, this is not supported by the stable isotope results, as there is no isotopic niche overlap between the Chinese mitten crab and the European eel (Persson & Nilsson, 2007; Rosewarne et al., 2016). Due to an insufficient number of replicates ($N = 3$), bream could not be included in the SIBER analyses. However, the stable isotope values observed for bream (mean $\delta^{15}\text{N} = 20\text{‰}$, $\delta^{13}\text{C} = -27\text{‰}$, $\delta^{34}\text{S} = 3.6\text{‰}$) fall within the range of values recorded for the Chinese mitten crab, suggesting a potential overlap in isotopic niches between these two species. Furthermore, the isotopic niche

of the Chinese mitten crab exhibits clear overlap with that of other species, such as the European flounder, indicating a shared utilization of resources and habitats. However, the bioaccumulation of 6:2 FTS is less pronounced in flounder compared to that observed in the Chinese mitten crab and bream. Therefore, the high bioaccumulation of 6:2 FTS in the Chinese mitten crab and bream cannot be readily explained by ecological factors such as benthic feeding behaviour based on the results of this study. As discussed in Section 4.4.1, this pronounced bioaccumulation is more likely attributed to species-specific metabolic differences rather than feeding ecology. It is possible that 6:2 FTS was also initially accumulated in other species but was subsequently eliminated to low levels. This pattern is consistent with findings from experimental studies on rainbow trout, where 6:2 FTS concentrations were observed to bioaccumulate during the early stages of exposure (within the first few days or weeks), followed by an increase in elimination rates, ultimately leading to low tissue concentrations (Hoke et al., 2015; Yeung & Mabury, 2013). Furthermore, none of the potential biotransformation products of 6:2 FTS, such as PFBA, PFPeA, and PFHxA, were detected in any of the species (Wang et al., 2013).

The significant overlap observed in all isotopic combinations between the common shrimp and common sole indicates a strong degree of resource and habitat sharing, a conclusion that is also supported by their similar bioaccumulation profiles.

While the concept of a 'contamination niche' has proven useful in identifying ecological factors influencing contaminant accumulation for certain substances, such as mercury (Hg) (Pinzone et al., 2019), it is less applicable when considering the sum of PFAS. Ecologically driven bioaccumulation differences are not necessarily reflected in the sum of PFAS and concentration levels; rather, they are manifested at the level of individual compounds.

3.5. Conclusions

This study provides crucial insights into the bioaccumulation patterns of PFAS in fish and invertebrates along the Scheldt river, with a particular focus on the detection of alternative PFAS in the vicinity of Antwerp. The detection of compounds such as FBSA, NaDONA, and 6:2 FTS in notable concentrations indicates the potential for local pollution sources, which are likely associated with industrial activities. These findings emphasize the necessity for the continued

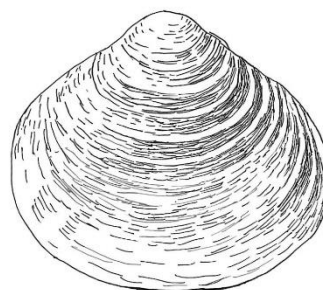
monitoring and regulation of both legacy and emerging PFAS, particularly in regions in close proximity to industrial hotspots.

By integrating toxicological data with ecological tracers, such as stable isotopes, this study provides insights into the bioaccumulation pathways and emphasises the significance of species-specific ecological dynamics in understanding PFAS contamination. In particular, the use of sulfur isotopes ($\delta^{34}\text{S}$) proved to be effective in differentiating between pelagic and benthic foraging behaviours. Pelagic species, such as Atlantic herring and European smelt, exhibited greater exposure to water-soluble short-chain PFAS, whereas benthic species like the European eel demonstrated the bioaccumulation of long-chain PFAS that are more likely to adsorb onto sediments. This distinction highlights the importance of ecological dynamics in the context of PFAS contamination, as nitrogen isotopes ($\delta^{15}\text{N}$) were less effective in explaining bioaccumulation differences within species groups.

Nevertheless, the study also suggests that not all bioaccumulation patterns can be attributed to ecological factors alone, as other variables, such as metabolic differences among species and environmental conditions, are likely to exert a significant influence. To enable a more comprehensive evaluation of the ecological and health risks associated with PFAS contamination in aquatic ecosystems, further research should focus on the long-term ecological impacts, metabolic pathways and the influence of environmental variables such as salinity and temperature on the distribution of PFAS.

Chapter IV

Active Biomonitoring of PFAS in Asian Clams: Associations With Oxidative stress, Respiration and Condition index



Manuscript under preparation

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that accumulate in aquatic ecosystems, posing a threat to wildlife. This study examines the potential of Asian clams (*Corbicula fluminea*) as an active biomonitoring species for assessing PFAS contamination in the Scheldt River, Belgium. Clams were exposed in cages at six sites along the river for a six-week exposure period, with simultaneous collection of sediment and water samples at each site. To evaluate the effects of PFAS bioaccumulation under natural conditions, three endpoints—oxidative stress, condition index, and respiration rate—were measured and analyzed in relation to bioaccumulated PFAS concentrations.

The results revealed significant bioaccumulation of both legacy and emerging PFAS in clams, exhibiting distinct spatial patterns across sampling sites. In water samples, short-chain PFAS were predominant, whereas long-chain PFAS were primarily detected in sediments. Notably, only one compound, PFOA, was detected across all three matrices. Furthermore, Asian clams bioaccumulated three PFAS compounds, PFDA, PFTeDA, and NaDONA, that were not detected in water or sediment samples, underscoring the critical role of biomonitoring in detecting contaminants that may be overlooked in abiotic assessments.

Biomarker analysis suggested oxidative stress responses to PFAS exposure, with significant positive correlations between malondialdehyde (MDA) levels and PFAS concentrations (NaDONA, PFDoDA, PFTeDA and Σ PFAS), suggesting lipid peroxidation as a potential mechanism of PFAS-induced cellular damage. Additionally, the condition index of the clams showed significant negative correlations with PFAS concentrations (NaDONA, PFOA and Σ PFAS), indicating potential physiological impairment. However, no significant relationships with respiration rates were observed.

These findings demonstrate the utility of Asian clams as effective biomonitors for PFAS contamination and suggest of physiological and molecular responses to PFAS exposure in aquatic environments.

4.1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds with fluorinated carbon chains, valued for their chemical stability and amphiphilic properties (Buck et al., 2011). Used for decades in industries such as firefighting, food packaging, cosmetics, and electronics, PFAS are highly resistant to degradation due to their strong carbon-fluorine bonds (Wang et al., 2021). Their widespread use has led to environmental contamination through industrial emissions, wastewater, and consumer products, resulting in global distribution via air and water transport (Ahrens et al., 2023; Young et al., 2007; Zhao et al., 2012).

Studies worldwide have reported the accumulation of PFAS in various aquatic species, ranging from invertebrates and fish to marine mammals and birds (Cheng et al., 2022; Houde et al., 2011; Khan et al., 2023; Miranda et al., 2021). The extent of this accumulation is influenced by several factors, including the chemical structure of PFAS, species-specific traits, biological differences such as gender and reproductive status, and dietary exposure (Lewis et al., 2022; Semerád et al., 2022). Nevertheless, despite the considerable bioaccumulation potential of PFAS, monitoring strategies often tend to focus on measurements in the abiotic environment, such as water and sediment. However, these measurements provide only single-point data and are unable to capture temporal variations in pollutant concentrations (Kördel et al., 2013). Moreover, water and sediment samples do not account for variations in bioavailability, which can be influenced by fluctuating abiotic and biotic factors (Van Ael et al., 2017). Passive biomonitoring, which entails the evaluation of pollutant bioaccumulation in indigenous species, presents an alternative methodology that can address these limitations (Lacroix et al., 2015). In river systems, benthic macroinvertebrates are considered an optimal target species due to their sedentary behavior, which allows for an accurate representation of in-situ conditions and long-term contaminant bioavailability. However, as previously observed by Byns et al. (2024) (chapter V), this approach also presents certain challenges. These include the labor-intensive nature of sampling and the difficulty of consistently collecting the same species across different sites, which in turn complicates comparative analyses. One potential solution to the limitations of passive biomonitoring is active biomonitoring, which involves exposing a selected species in cages at various sites (Wepener, 2013). This method allows for greater control over exposure conditions and enhances comparability across locations by standardizing the biomonitoring species used. The efficacy of active biomonitoring is contingent upon the selection of an

appropriate species, which should exhibit high bioaccumulation potential and tolerance to a range of pollutants (Besse et al., 2012; Smolders et al., 2002). Mussels are considered effective active biomonitoring species in both marine and freshwater systems due to their sessile nature, filter-feeding behavior, and long lifespan (Benito et al., 2024; Bervoets et al., 2005a; Roméo et al., 2003; Smolders et al., 2003; Teunen et al., 2021; Ukasha et al., 2024).

The toxicity of PFAS has been the subject of considerable research, with studies demonstrating adverse effects on the reproductive, developmental, endocrine, and immune systems of both aquatic and terrestrial organisms (Dickman & Aga, 2022; Mahoney et al., 2022). However, the majority of this research has focused on legacy compounds, such as PFOS and PFOA, with a limited understanding of the effects of alternative and emerging PFAS. Furthermore, the majority of current knowledge regarding the ecotoxicological effects of PFAS has been derived from laboratory-based toxicity experiments (Ankley et al., 2021; Gkika et al., 2023). In these studies, the test organisms are typically exposed to a single compound in simplified setups under idealized environmental conditions, often at concentrations that far exceed those found in natural environments. This controlled approach fails to capture the complexity of real-world exposure, where organisms are subject to lower, yet chronic, concentrations of pollutants within a multi-stressor environment (Sinclair et al., 2020). Moreover, organisms in natural ecosystems are often exposed to mixtures of contaminants, which may result in additive or synergistic effects (Ahrens & Bundschuh, 2014). Nevertheless, field-based studies investigating the ecological impacts of PFAS in aquatic environments remain scarce, highlighting the necessity for more comprehensive, real-world assessments (Ankley et al., 2021).

This study aims to use Asian clams (*Corbicula fluminea*) as active biomonitors for detecting PFAS contamination in estuaries, with the Scheldt river as a case study. The river presents an ideal setting for studying PFAS bioaccumulation, given the presence of a major PFAS manufacturing facility near Antwerp, a likely point source of pollution. Additionally, agricultural runoff and urban wastewater from cities such as Brussels, Ghent, and Antwerp contribute to further PFAS contamination (Stronkhorst, 1993). Asian clams were chosen as biomonitor species due to their broad salinity tolerance, making them well-suited for the Scheldt's dynamic tidal environment, which includes freshwater, brackish, and saline zones (Sousa et al., 2008). Their high filtration capacity has been previously leveraged for monitoring various pollutants, including metals, microplastics, and PFAS (Cataldo et al., 2001; Jou et al., 2009; Su et al., 2018; Teunen et al., 2021). This study will also compare PFAS profiles in clams with those in their surrounding abiotic

environment. To assess the effects of PFAS bioaccumulation under natural conditions, three key endpoints, oxidative stress, condition index, and respiration, are measured and correlated with bioaccumulated PFAS concentrations.

4.2. Materials and Methods

4.2.1. Active Biomonitoring and Sample Collection

In August 2021, Asian clams were collected from the Blaarmeersen pond in Ghent, Belgium, a site known for its low background concentrations of various pollutants, including PFAS (Bervoets et al., 2005a; Byns et al., 2024; Teunen et al., 2021). The clams were acclimatized for six weeks in a semi-natural pond system filled with dechlorinated tap water at the mesocosm facility (Mesodrome) of the University of Antwerp. Following acclimatization, the clams were deployed in Thermo Scientific Nalgene polypropylene baskets (105 x 123 x 154 mm) at six locations along the Scheldt River (Figure 5.1). The exposure sites are numbered from 1 to 6, arranged from upstream to downstream: Temse (1), Schelle (2), Hemiksem (3), Steenplein (4), Zwijndrecht (5), and Lillo (6). These sites were specifically chosen for their availability of floating platforms that adjust to changing water levels, ensuring that the clams remained submerged at a constant depth throughout the exposure period. The clams were deployed on October 13, 2021, and recollected on November 24, 2021, resulting in a total exposure duration of six weeks. Given that the Asian clam is an exotic species and typically reproduces biannually, with spawning occurring once between spring and early summer and again in late summer, the autumn exposure period was selected to minimize the likelihood of reproduction and larval release during the study (Sousa et al., 2008). At each site, 10 clams were exposed. Throughout the six-week exposure period, four sampling campaigns (every 10 days) were conducted. In each campaign, two surface water samples were collected from each site using a bucket (N=8 per site in total), along with three replicate sediment samples obtained using a Petit-Ponar grab sampler (Wildco cat. 1728; 235 cm²). Following recollection, water and sediment samples were stored in 50 mL PP tubes at -20°C until further analysis. The clams were maintained (+/- 3 hours) in site water until the assessment of their condition index and respiration

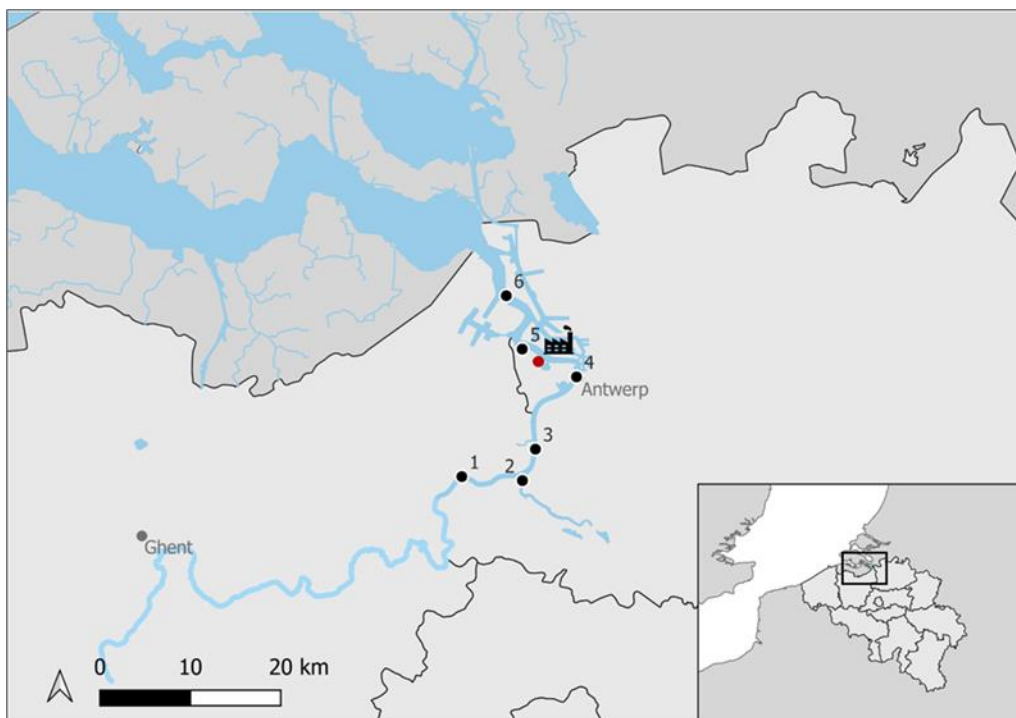


Figure 4.1: Selected exposure and sampling sites along the Scheldt river: Temse (1), Schelle (2), Hemiksem (3), Steenplein (4), Zwijndrecht (5), and Lillo (6). The fluorochemical plant is indicated with the red dot.

4.2.2. Condition Index

The condition index (CI) was calculated by dividing the total wet weight of the Asian clam (including the shell) by the shell length, following the method described by Nobles & Zhang (2015):

$$CI = \frac{\text{Total wet weight (g)}}{\text{Shell length (cm)}}$$

Upon collection from the field, the clams were immediately weighed using a precision balance (Mettler AT261 DeltaRange) and their shell length was measured with digital calipers.

4.2.3. Respiration

Following the collection of data pertaining to the condition index, an evaluation of clam respiration was conducted. Each clam was placed individually in a respiration chamber with a volume of 59 mL. Prior to introducing the clams, the chambers were filled with medium hard water (Environmental Protection Agency, 2002), and the dissolved oxygen concentration

(mg/L) was measured using a multimeter (HQ30d, HACH). A single clam was then added to each chamber, which was sealed airtight with a plastic wrap and lid. A total of seven control chambers were included to account for changes in oxygen concentration in the absence of clams. After a two-hour respiration period, the chambers were reopened, and the dissolved oxygen concentration was measured again. Respiration rates were calculated using the following formula.

$$R = \frac{(O_{2i} - O_{2f} - (O_{2ic} - O_{2fc})) * \frac{V_c - V_m}{t}}{W}$$

R represents the respiration rate, expressed as mg O₂ per hour per gram of wet weight. O_{2i} and O_{2f} denote the initial and final oxygen concentrations, respectively, while O_{2ic} and O_{2fc} correspond to the initial and final oxygen concentrations in the control chambers. V_c is the volume of the respiration chamber, adjusted for the volume of the clam (V_m). The duration of the respiration experiment is represented by t (in hours), and W refers to the wet weight of the clams (in grams). Following the respiration experiment, the clams were stored at -80°C until further analysis was conducted.

Since the clam volume was not directly measured prior to the respiration experiment, a formula was derived to estimate clam volume based on shell length. To achieve this, the length and volume of 70 individual clams across a range of sizes were measured. It is important to note that a different set of clams was utilized for this, distinct from those that were previously exposed in the field. A linear regression analysis between shell length (L, mm) and volume (V, mL) produced the following equation (Figure C.1):

$$V = 0.39 * L - 6$$

4.2.4. PFAS Analysis

Prior to the extraction process, the clams were dissected, and the soft tissues were homogenized using a mortar and pestle. To maintain the tissue in a frozen state, liquid nitrogen was added during the homogenization process. Approximately 0.3 g wet weight (ww) of soft tissue from each clam or dried (48h at 80°C) sediment sample was weighed and transferred to a 50 mL polypropylene (PP) tube. For the water samples 10 mL was transferred into the 50 mL PP tubes. The extraction procedure for biotic samples followed the method of Powley et al.

(2005), with minor modifications (detailed in the Supplementary Information). For the abiotic samples the extraction procedure as described by Groffen et al. (2019b) was followed. A total of 28 target analytes were analyzed using Ultra Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS), as detailed in the Supplementary Information in Table C.1. Additional information regarding target analytes and specific instrumental settings is provided in Groffen et al. (2021). As part of the quality control protocol, a procedural blank containing 10 mL of acetonitrile (ACN) was included for every 10 samples during the extraction process. The limit of quantification (LOQ) for each target analyte was determined in the matrix, based on the integrated peak area of a low-concentration peak with a signal-to-noise ratio of 10. To mitigate carry-over effects during the PFAS analysis, ACN was routinely injected as an instrumental blank between injections.

4.2.5. Oxidative Damage and Antioxidant Biomarker Analysis

4.2.5.1. *Frap Assay*

The ferric reducing ability of plasma (FRAP) assay measures the total antioxidant capacity (TAC) through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants present in the samples. The FRAP assay is an automated test as described by Benzie & Strain (1996). The reaction mixture was prepared by combining 70 mL of 0.3 M acetate buffer (pH 3.6), 7 mL of 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and 7 mL of 20 mM FeCl_3 , resulting in a total volume of 84 mL. In a 96-well plate, 170 μL of the reaction mixture was added to 30 μL of each sample. The plate was then kept on ice for 20 minutes before being measured using a microplate reader at an absorbance of 600 nm.

4.2.5.2. *Malondialdehyde Content*

To evaluate the malondialdehyde content (MDA), which is an end product of the lipid peroxidation response to oxidative stress, the protocol as described by Murshed et al. (2008) was followed. A 100 μL aliquot of each sample was transferred into Eppendorf tubes, and 500 μL of 0.5% (w/v) thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) was added. The samples were then incubated at 90°C for 30 minutes. Following incubation, the reaction was halted by placing the samples in an ice bath. The samples were vortexed and centrifuged for 5

minutes at 7,000 rpm. Finally, 200 μL of the supernatant was transferred to a 96-well plate, and absorbance was measured at 440, 532, and 600 nm using a microplate reader.

4.2.5.3. *Polyphenol Content*

The Folin-Ciocalteu assay as described by Zhang et al. (2006) was used to determine the total polyphenols content (TPC), which is a group of naturally occurring antioxidants. A 30 μL aliquot of each sample was added to a new 96-well plate, followed by the addition of 50 μL of Folin-Ciocalteu Phenol reagent (10 times diluted). The mixture was allowed to react for 5 minutes before 40 μL of 10% saturated Na_2CO_3 was added. The plate was then incubated in the dark for at least 1 hour. After incubation, the absorbance of the reaction mixture was measured at 725 nm using a microplate ELISA reader. A standard curve using gallic acid was prepared to calculate the phenolic content (Table C.5). The standard curve was generated from a 100 $\mu\text{g}/\text{mL}$ stock solution of gallic acid in 80% ethanol. TPC for each extract was expressed as mg gallic acid equivalents per gram of fresh sample material.

4.2.6. **Statistical Analysis**

Statistical analyses were conducted using RStudio (version 2022.12.0) and GraphPad Prism (version 10.2.2), with a significance threshold set at $p \leq 0.05$. To compare PFAS concentrations in water, sediment, and biota across different exposure sites, two-way ANOVA was performed. Post-hoc comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test, or Dunn's post-hoc test as the non-parametric alternative when the assumptions of homogeneity of variances and normality of residuals were violated. The same statistical tests were applied to assess spatial variation in biomarker responses. Additionally, Pearson correlation analysis was used to examine the relationships between bioaccumulated PFAS concentrations in clams and biomarker responses, condition index and respiration rate. When assumptions of normality, linearity, or homoscedasticity were not met, Spearman correlation was employed instead.

4.3. Results

4.3.1. Spatial Distribution of PFAS Profiles

The spatial distribution of Σ PFAS in clams, water and sediment samples is shown in Figure 4.2. An overview of the average concentrations and min – max ranges per matrix and per site is shown in the Supplementary Information in Tables C.2, C.3 and C.4. The sites are numbered from 1 to 6, arranged from upstream to downstream: Temse (1), Schelle (2), Hemiksem (3), Steenplein (4), Zwijndrecht (5), and Lillo (6). The distribution of accumulated PFAS in clams revealed significantly higher mean Σ PFAS concentrations ($p < 0.001$) at site 1 (27 ng/g ww) compared to sites 2 (3.5 ng/g ww), 4 (10 ng/g ww), 5 (17 ng/g ww), and 6 (11 ng/g ww). Additionally, mean Σ PFAS concentrations were significantly higher at site 3 (24 ng/g ww) compared to sites 4 and 6. Site 2 also exhibited significantly lower mean Σ PFAS concentrations compared to site 5. Among individual PFAS, significant differences between sites were observed only for NaDONA, with site 1 showing significantly ($p < 0.05$) higher mean concentrations (12 ng/g ww) than sites 2 (1.9 ng/g ww) and 6 (2.1 ng/g ww) and site 3 showing significantly ($p < 0.001$) higher concentrations compared to site 6. PFBA and PFHxA were detected exclusively at the most downstream site (site 6), with mean concentrations of 2.0 ng/g ww for PFBA and 0.30 ng/g ww for PFHxA. PFDA was detected only at sites 1 and 4, with mean concentrations of 1.7 ng/g ww and 0.64 ng/g ww, respectively.

In water samples, the total Σ PFAS concentrations were significantly lower at site 3 (12 ng/L) compared to site 2 (145 ng/L) and site 1 (130 ng/L). PFBA was exclusively detected at sites 5 and 6, while PFOA and PFOS were only present at sites 4, 5, and 6. Additionally, 9Cl-PF3ONS (F53B major) was detected solely at sites 1, 2, and 4.

In the sediment samples, significant differences ($p < 0.05$) in the total Σ PFAS concentrations were observed, with higher mean concentrations at site 3 (5.7 ng/g ww) compared to sites 4 (0.79 ng/g ww) and 5 (2.1 ng/g ww), and at site 1 (5.0 ng/g ww) compared to site 4. At sites 2 and 6, PFAS were below the LOQ. PFOA was detected only at sites 1, 4, and 5, while PFDoDA and PFTrDA were exclusively detected at sites 1 and 3. PFOS was detected solely at site 3.

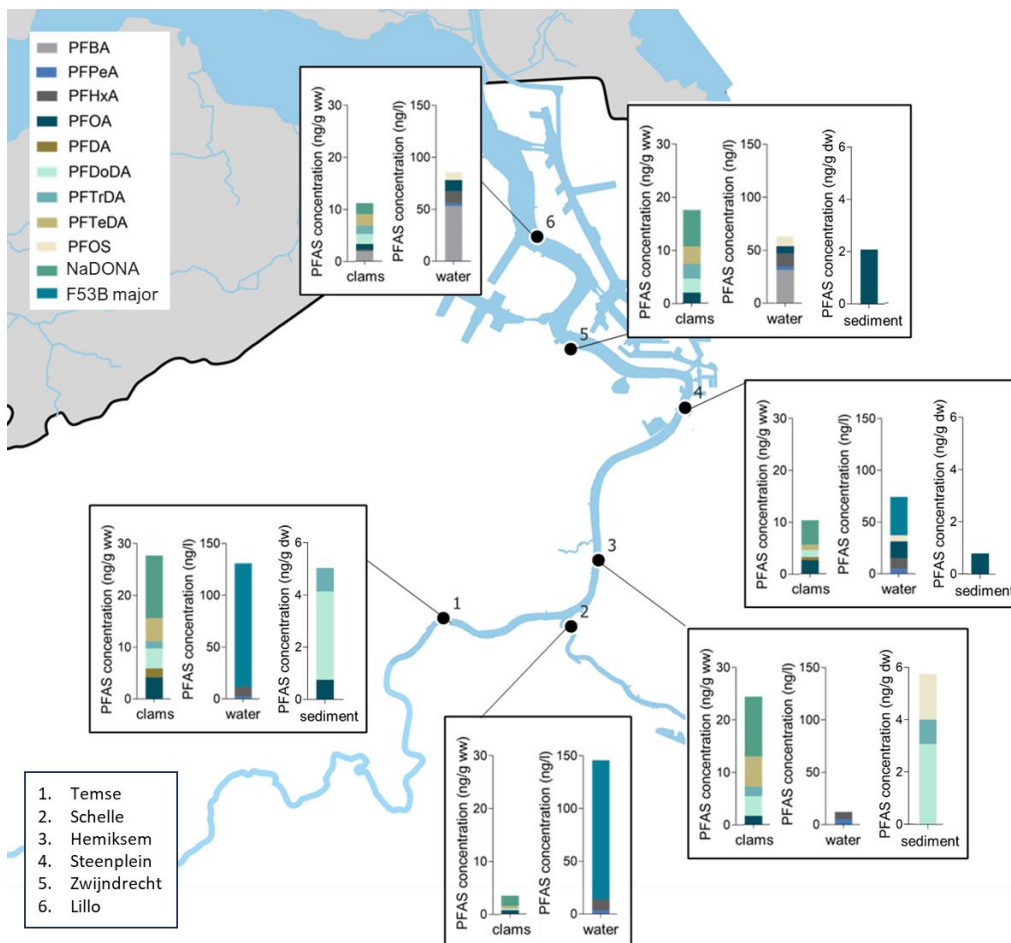


Figure 4.2: Spatial distribution of mean PFAS concentrations in Asian clams ("clams", ng/g ww), water (ng/L) and sediment (ng/g dw) samples. At site 2 and 6 no graph is available for sediment as all concentrations were below the LOQ.

The relative contribution of each compound to the total sum of PFAS detected per matrix is shown in Figure 4.3. In the sediment, PFOA is the predominant compound which accounts for 55%, followed by PFDoDA (30%), PFTrDA (8.2 %) and PFOS (6.8%). In water, both PFHxA and 9Cl-PF3ONS (F53B major) dominate with 25% followed by PFBA (21%), PFPeA (17%), PFOA (8.2%) and PFOS (3.8%). In Asian clams, NaDONA is the predominant compound with 50%, followed by PFOA (17%), PFDoDA and PFTeDA (10%), PFTrDA (5.6%), PFBA, (4.3%), PFDA (1.7%) and PFHxA (0.96%).

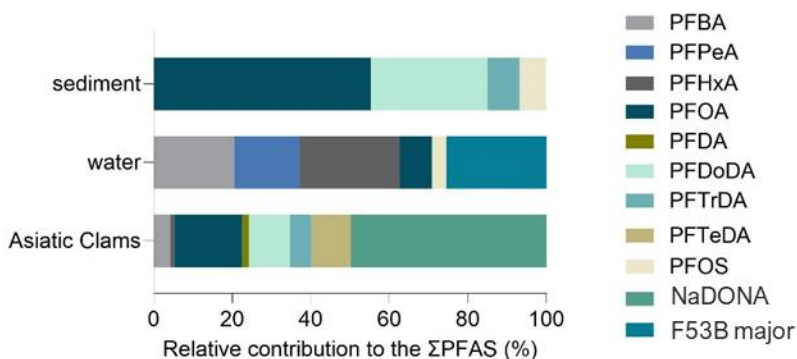


Figure 4.3: Relative contribution of PFAS detected in sediment, water and Asian clams regardless of the sampling sites. For the calculation of the relative contribution the molecular weight of each compound is taken into account.

4.3.2. Biomarker Responses

Figure 4.4 illustrates the spatial distribution of biomarker responses across the different sites. No significant differences were observed among the sites for the FRAP assay, with minimum values of 16 $\mu\text{mol Trolox/g}$ at site 2 and maximum values of 47.7 $\mu\text{mol Trolox/g}$ at site 3 (Table C.6). However, for the total polyphenol content, significantly lower values were found at site 5 compared to site 3 ($p < 0.05$) and site 6 ($p < 0.05$). The range of values for the polyphenol content spanned from 7.8 mg GA/gFW at site 2 to 17 mg GA/gFW at site 3. In the MDA assay, significantly lower values were recorded at site 2 compared to site 1 ($p < 0.05$), with values ranging from 3.6 nmol/g at site 2 to 12 nmol/g at site 3.

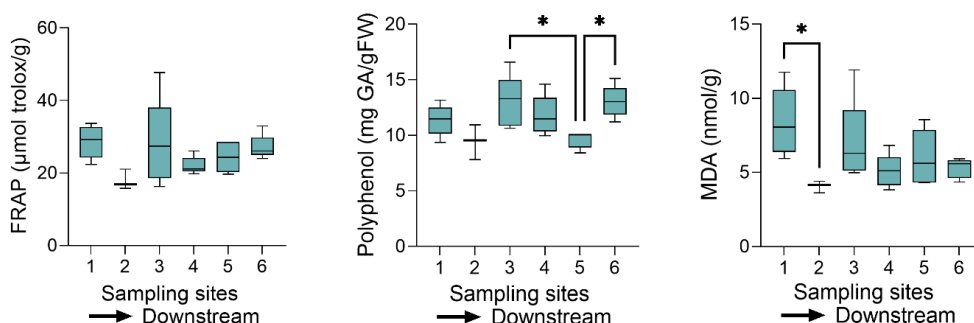


Figure 5.4: Spatial variation (mean + Standard Deviation (SD)) of biomarker responses in field exposed Asian clams: (A) FRAP ($\mu\text{mol Trolox/g}$), (B) Polyphenol content (mg GA/gFW) and (C) MDA (nmol/g). Significant differences with p -values < 0.05 are indicated with ** .

To assess the relationships between bioaccumulated PFAS in clams and biomarker responses, correlation analyses were performed. Significant positive correlations were identified between MDA and PFDODA ($p < 0.05$, $r_{\text{Spearman}} = 0.46$), PFTeDA ($p < 0.01$, $r_{\text{Spearman}} = 0.54$), NaDONA ($p < 0.05$, $r_{\text{Pearson}} = 0.41$), and ΣPFAS ($p < 0.05$, $r_{\text{Pearson}} = 0.47$), as shown in Figure 4.5. No significant correlations were observed between polyphenols or FRAP assay results with any of the PFAS (Table C.7).

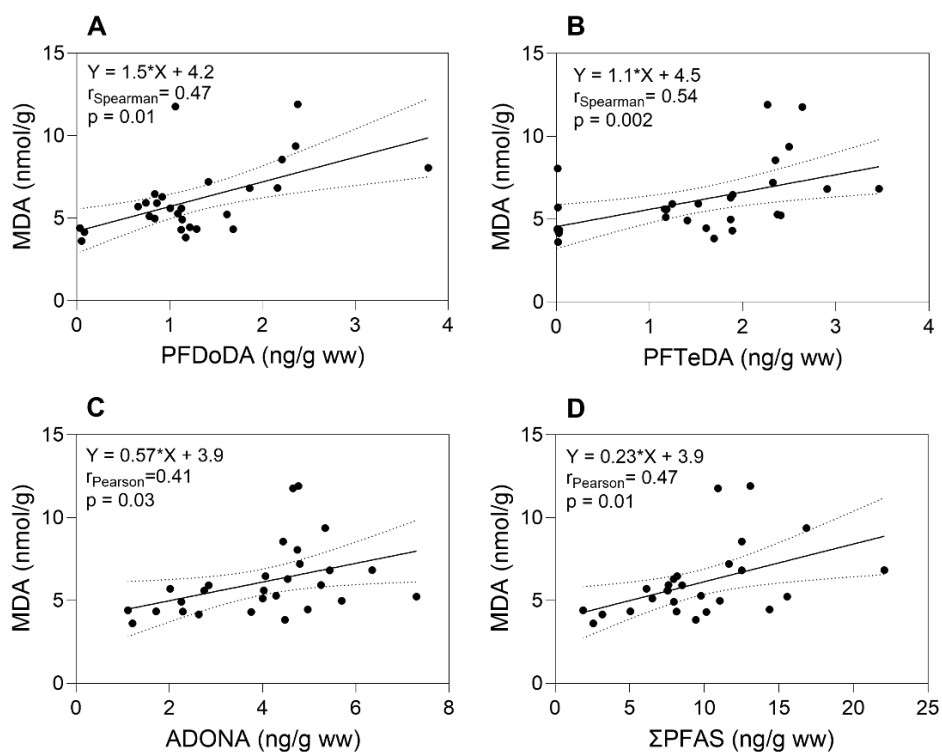


Figure 4.5: Relationship between oxidative stress response measured in field exposed Asian clams as MDA (nmol/g) versus PFDODA (A), PFTeDA (B), NaDONA (C) and ΣPFAS (D) expressed in ng/g ww. 95% confidence intervals are indicated with dotted lines.

4.3.3. Condition Index

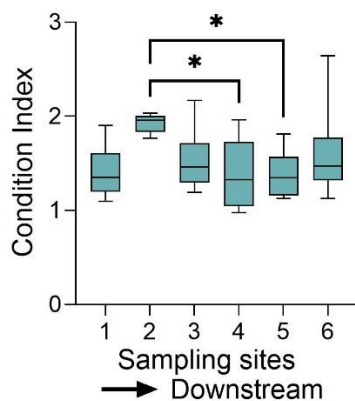


Figure 4.6: Spatial variation of the Condition Index (mean + SD) of Field exposed clams. Significant differences with p -values < 0.05 are indicated with '*'.

The condition index ranged from 0.98 at site 4 up to 2.7 at site 6 (Table C.6). A significant higher CI was observed at site 2 ($p < 0.05$) compared to site 4 and 5 (Figure 4.6). Based on the correlation analysis significant but weak negative correlations were observed between the CI and PFOA ($p < 0.05$, $r_{\text{spearman}} = -0.27$), NaDONA ($p < 0.05$), $p_{\text{Pearson}} = -0.34$) and Σ PFAS ($p < 0.05$, $p_{\text{Pearson}} = -0.33$) (Figure 4.7, Table C.7).

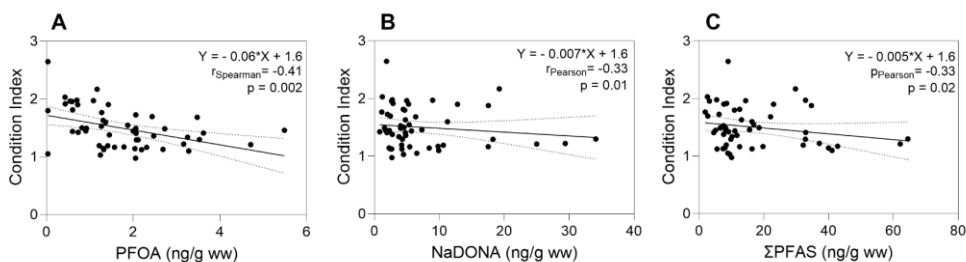


Figure 4.7: Relationship between Condition Index and accumulated PFAS concentrations in field exposed clams for (A) PFOA, (B) NaDONA, (C) Σ PFAS.

4.3.4. Respiration

Significant differences in respiration were observed between site 1 versus site 2 ($p < 0.05$, Figure 4.8) and site 4 ($p < 0.05$) (Table C.6). However, no significant correlations were found between the respiration rate and bioaccumulated PFAS concentrations (Table C.7). Furthermore, also no significant correlations were observed between any of the endpoints.

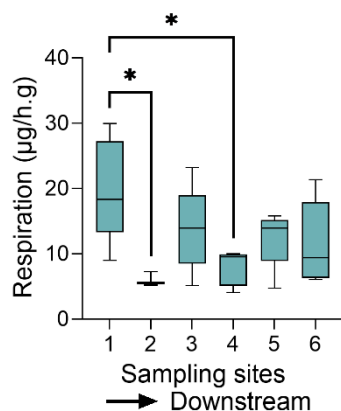


Figure 4.8: Spatial variation of the respiration rate in field exposed Asian clams ($\mu\text{g O}_2/\text{h.g}$) (mean + SD).

4.4. Discussion

4.4.1. PFAS Profiles: Spatial Distribution and comparison

4.4.1.1. Water and Sediment

The spatial distribution of PFAS in water samples exhibits significant variation across different sampling sites. At site 2 (Schelle), despite the relatively low bioaccumulation levels of PFAS in clams, surface water samples revealed notably elevated concentrations of ΣPFAS . This is largely attributed to the high levels of 9Cl-PF3ONS, commonly referred to as F53B major, which averaged 132 ng/L. F53B major was developed as an alternative to PFOS and is commonly used as a mist suppressant in electroplating industries (Du et al., 2016; Munoz et al., 2019). Elevated concentrations were also recorded at sites 1 (Temse) and 4 (Steenplein), with mean values of 119 ng/L and 37 ng/L, respectively. Globally, F53B major has been detected in surface waters, and its presence is often associated with wastewater effluents from PFAS manufacturers or chrome plating facilities (Chambers et al., 2021; Gebbink et al., 2017; Munoz et al., 2019; Pan et al., 2018). In the Scheldt catchment area, several electroplating industries are present, including sites upstream of site 1 and near the Rupel River, which may serve as potential sources of contamination to the Scheldt River through connecting waterways. However, further research is required to confirm these industries as specific sources of the compound F53B major. F53B major has raised environmental concerns due to its potential acute toxicity, which is considered comparable to PFOS (Wang et al., 2013). Studies suggest that its bioaccumulation

potential may exceed that of PFOS, classifying it as a highly bioaccumulative contaminant of emerging concern (Liu et al., 2018; Shi et al., 2015; Wang et al., 2013). However, the current study contrasts with these findings, as F53B major, despite its high concentrations in water, was not detected in the clams. This discrepancy highlights the need for ongoing monitoring and further investigation into bioaccumulation patterns in the Scheldt river.

As sampling progresses further downstream, the composition of the PFAS profiles in surface water shifts from a predominance of F53B major towards a greater prevalence of short-chain compounds, including PFBA, PFPeA, and PFHxA. The observed spatial variation in PFAS contamination may be attributed to direct inputs from multiple pollution sources. In the more downstream region, around sites 4, 5 and 6 of this study, the 3M facility in Zwijndrecht is often regarded as the primary source of PFAS pollution in the Scheldt River (Jonker, 2024). However, several other sources likely contribute to the contamination. As Antwerp is a major port and industrial hub, numerous companies in the region may be involved in PFAS-related emissions. Indaver, a waste processing company, is responsible for the destruction of PFAS waste, potentially releasing emissions in the process. Additionally, firefighter training grounds in Antwerp and Lillo represent another potential PFAS source, as Aqueous Film-Forming Foam (AFFF) historically used in training exercises contains PFAS (Kaboré et al., 2022). Furthermore, urban wastewater discharges and wastewater treatment plants (WWTPs) also play a role in PFAS pollution, as these facilities may not fully remove PFAS before releasing treated water (Jeong et al., 2022).

However, the complexity of the estuarine system itself should also be considered, as it may influence the distribution of pollutants. For example, suspended particles in surface waters have been identified as key pathway for the transport of PFAS in aquatic environments. The affinity of PFAS for these particles is largely determined by their composition, particularly the proportion of organic carbon and the presence of iron or aluminum oxides, which can fluctuate significantly across estuarine systems (Borthakur et al., 2021; Li et al., 2018). The composition of suspended matter in the Scheldt river varies between the upper and lower regions, with the upper part primarily influenced by riverine and terrestrial sources, while the lower estuary is dominated by marine inputs (Middelburg & Nieuwenhuize, 1998). However, the understanding of how these variations in suspended matter composition, along with changes in water chemistry, impact the distribution of PFAS in estuarine systems like the Scheldt requires further investigation.

In consideration of the general PFAS profile, it can be concluded that the water samples are dominated by compounds with high solubility, specifically short-chain PFAS and alternatives (F53B major), which account for 88% of the total PFAS. This finding is consistent with the results of previous studies, which have demonstrated that short-chain PFAS and alternatives contribute more significantly to the overall PFAS burden in water due to their higher solubility compared to their long-chain counterparts (Gebblink et al., 2017; Labadie & Chevreuil, 2011; Pan et al., 2018). Zhao et al. (2016) estimated that approximately 89% of the total PFAS in the water column are short-chain PFAS which is in line with the present study. This trend is likely to continue due to the increased manufacturing and use of short-chain PFAS and related substances (Land et al., 2018).

The sediment profiles are composed exclusively of long-chain PFAS, with PFTrDA predominantly found in the upper part of the river and PFOA in the lower part. This observation aligns with existing literature, which indicates that long-chain PFAS tend to partition more readily into sediments due to their lower water solubility, which decreases as the carbon chain length increases (Labadie & Chevreuil, 2011; Navarro et al., 2022). The observed variation in predominant PFAS along the river may be attributed to the frequent disturbances of the sediment bed due to dredging and disposal practices (van Dijk et al., 2021). However, since the production of PFOA was phased out in 2002, its presence in sediment can likely be attributed to historical pollution, potentially originating from 3M. Despite the expected increase in long-chain PFAS concentrations towards the lower Scheldt River due to the salting-out effect, this trend was not observed in the present study. However, due to the limited sample size, no definitive conclusions can be drawn. Additionally, the sediment samples were collected using a grab sampler, which only captures the top 10-15 cm of surface sediment, and sampling was restricted to one side of the river, failing to represent the full river width.

4.4.1.2. *Asian Clams*

Our results indicate a general decrease in bioaccumulated Σ PFAS concentrations from upstream to downstream sites. This finding contradicts our initial hypothesis, which anticipated higher PFAS concentrations downstream due to the proximity of a PFAS manufacturer located between sites 4 (Steenplein) and 5 (Zwijndrecht), potentially serving as a point source for PFAS release into the Scheldt River. It was expected that this would result in general higher concentrations downstream of the point source. However, PFAS bioaccumulation in the Scheldt

river is far more complex than can be explained by a simple downstream current. The Scheldt river is a tidal system, with tidal influence extending as far as the city of Ghent (Baeyens et al., 1997; Meire et al., 2005). Thus, both downstream and upstream currents contribute to the mixing and dispersion of pollutants, complicating the assumption of a linear downstream gradient in PFAS concentrations. Furthermore, the study investigated the bioaccumulation of PFAS along a salinity gradient, which exhibits a gradual increase from 0.15 in the freshwater zone to 30 in the saline zone (Muylaert et al., 2009). This gradual rise in salinity is likely to exert an influence on bioaccumulation patterns (Jeon et al., 2011). Munoz et al. (2017a) observed that PFAS tend to adsorb more readily onto sediments with increasing salinity, a phenomenon particularly pronounced for longer-chain PFAS, due to their hydrophobic tails, which interact more strongly with hydrophobic sites on sediment particles. Since the clams in this study were primarily exposed to the water column, this partitioning effect suggests that the water-mediated exposure pathway for PFAS may decrease as salinity increases, contributing to the observed reduction in bioaccumulation further downstream. Notably, bioaccumulation of the short-chain compounds PFBA and PFHxA was observed exclusively at the most downstream site, site 6 (Lillo). Previous studies have documented the competitive bioaccumulation potential between long- and short-chain PFAS, with longer-chain PFAS typically exhibiting higher binding affinities (Shi et al., 2018; Wen et al., 2017, 2023). The decreased water-mediated exposure of long-chain PFAS, likely due to the salting-out effect, may have reduced competition from longer-chain compounds. As a result, short-chain PFAS may have had greater opportunity to bioaccumulate at this site. Additionally, PFBA was detected in water samples only at sites 5 (Zwijndrecht) and 6 (Lillo), indicating a localized increase of this compound beginning at site 5. In contrast, PFHxA was detected in water at all sampling sites, with concentrations remaining relatively consistent across locations. Given that PFBA is currently one of the most widely used short-chain PFAS, it is possible that regional PFBA pollution in the Scheldt River can be attributed to emissions from the nearby PFAS manufacturing plant (Ateia et al., 2019). The bioaccumulation profile of NaDONA exhibited an opposite trend, with the highest concentrations detected at the upstream site (site 1, Temse). These elevated concentrations suggest potential upstream sources of NaDONA. In a study by Teunen et al. (2021), high concentrations of Σ PFAS (50-60 ng/g ww) were bioaccumulated in quagga mussels (*Dreissena bugensis*) at a site located further upstream than site 1 (Temse) in the present study. Although NaDONA was not detected in the Teunen et al. (2021) study, the elevated Σ PFAS levels suggest

the presence of other PFAS sources upstream of Temse in the Scheldt River. NaDONA, which is an alternative to PFOA, has limited documentation regarding its environmental occurrence, though several studies have reported its presence in surface waters near fluorochemical manufacturing facilities (Munoz et al., 2019; Pan et al., 2018). Notably, in the present study, NaDONA was detected in clams at all sampling locations, yet it was not detected in either water or sediment samples. Despite experimental studies indicating a low bioaccumulation potential for NaDONA, our findings highlight its significance bioaccumulation in clams (Munoz et al., 2019). This underscores the value of using biological monitoring to detect pollutants that might be overlooked by conventional water and sediment analyses. Overall, the bioaccumulation of legacy compounds, such as long-chain PFCAs, was relatively uniform across the Scheldt River, suggesting that these compounds are widespread throughout the entire system. This distribution is likely attributable to historical pollution as following the phase-out of PFOS and PFOA in 2000, there has been a notable shift in production from long-chain PFAS to short-chain PFAS and alternative compounds (Ateia et al., 2019).

Notably, low bioaccumulated PFAS concentrations were observed at site 2 (Schelle). This site is not directly located within the Scheldt river but is positioned just before the Rupel river merges with the Scheldt. The Rupel river, which carries waters from highly industrialized and densely populated regions, including Brussels, is considered a potential transport route for indirect PFAS sources into the Scheldt River. However, the low levels of bioaccumulated PFAS in clams at this site suggest that the influence of the Rupel river on PFAS contamination in the Scheldt may be limited.

The general PFAS profile indicates that short-chain PFAS and alternative substances dominate in Asian clams, accounting for 55% of the total PFAS detected. This pattern is consistent with the findings of Teunen et al. (2021), where short-chain PFAS were more prevalent in mussels compared to in fish, likely due to the differences in trophic levels and exposure dynamics. In a similar study that used Asian clams for passive biomonitoring in the Occoquan watershed in Northern Virginia, the highest bioaccumulated concentrations were also observed for short-chain perfluoroalkyl carboxylates (PFCA) and fluorotelomer sulfonic acids (FTS) (Koban et al., 2024). The total concentration of PFAS detected in clams in the study by Koban et al. (2024) ranged from 6.2 to 18 ng/g ww, with PFHxA exhibiting the highest concentrations (2.2–9.9 ng/g ww). In contrast, the present study revealed a total PFAS concentration range of 1.9 to 65 ng/g

ww, with PFHxA among the lowest concentrations (0.30–0.84 ng/g ww) and NaDONA exhibiting the highest concentrations (0.81–34 ng/g ww).

The overall comparison of PFAS profiles across different matrices suggests that measurements in water and sediment samples do not always accurately reflect the bioavailable compounds that tend to bioaccumulate in organisms. Only two compounds, PFBA and PFHxA, were consistently detected between water and clams, while in sediment, PFDoDA, and PFTrDA were the only complementary compounds. PFOA was the only compound present in all three matrices (water, sediment, and clams). Interestingly, Asian clams bioaccumulated three compounds—PFDA, PFTeDA, and NaDONA—that were not detected in water or sediment. These findings are consistent with the similar study of Koban et al. (2024), where only two compounds were detected across all three matrices, and PFPeS and PFDA were uniquely detected in Asian clams but not in water or sediment. This underscores the complexity of PFAS bioavailability and accumulation, particularly in dynamic systems such as the Scheldt River, where organisms may accumulate compounds that are not readily detectable in their surrounding environment. However, due to the small sample size, the relationship between the biotic and abiotic environments could not be thoroughly explored. Therefore, future research should focus on examining how water and sediment characteristics (e.g. salinity, pH, total organic carbon (TOC) content, clay content, ...) influence the distribution and bioaccumulation of PFAS across both biotic and abiotic systems.

4.4.2. Asian Clams as Biomonitor

The results of the present study suggest that the Asian clam may serve as an effective biomonitor species. Firstly, no mortality was observed along the exposure locations, indicating that the clams are capable of withstanding the dynamic nature and present pollution of the Scheldt river. Secondly, the bioaccumulated PFAS profiles indicate that the Asian clam has the capacity to accumulate a diverse range of PFAS, encompassing both emerging and legacy compounds. Moreover, even compounds that were not detected in water or sediment were bioaccumulated in the clams. It is important to note, however, that the clams were exposed for a limited time frame of six weeks, which may not fully reflect seasonal variations in bioavailability. Consequently, the PFAS profile of these clams may not accurately reflect the profiles of resident species that have experienced a lifetime of exposure. Accordingly, the

utilization of resident biomonitoring species may facilitate a more comprehensive understanding of the contamination profile, as previously asserted by the study of Teunen et al. (2021). However, although the study did not assess the levels of PFAS, the findings of Bervoets et al. (2004) indicate that the bioaccumulated pollutant concentrations (specifically, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hexachlorobenzene, and trace metals) in translocated zebra mussels (*Dreissena polymorpha*) were comparable to those in indigenous mussels (Bervoets et al., 2004). In addition, from an ecological standpoint, the sacrifice of an invasive species such as the Asian clam is preferable to the capture of organisms from an indigenous population. Furthermore, translocating clams is less labor-intensive compared to sampling indigenous species, and active biomonitoring ensures the availability of sufficient numbers of the same species at each site. Additionally, this approach provides the advantage of known background concentrations and controlled exposure times.

4.4.3. Oxidative Stress Biomarker Responses

Oxidative stress is defined as an imbalance between the production of oxidants, such as reactive oxygen species (ROS), and an organism's antioxidant defenses (Lushchak, 2011). This imbalance can result in damage to the DNA, alterations in enzyme activity, and a cascade of oxidative damage throughout the organism (Lee et al., 2020). The evidence from experimental studies indicates that PFAS exposure may induce oxidative stress in aquatic organisms (Liu et al., 2007; Sant et al., 2018). The oxidative status of organisms in response to pollutant exposure can be evaluated by measuring biomarkers of oxidative damage and antioxidant defenses (Beaulieu & Costantini, 2014). Nevertheless, research examining the oxidative stress responses to PFAS exposure in natural aquatic ecosystems remains scarce.

The results of the present study demonstrated a significant positive yet moderate correlation between certain PFAS and the malondialdehyde (MDA) biomarker. Additionally, the spatial distribution findings align with these observations, as the lowest MDA values were recorded at the site with the lowest bioaccumulated PFAS concentrations (site 2, Schelle). These associations suggest that the exposure to PFAS can cause oxidative stress in Asian clams. Malondialdehyde is a byproduct of lipid peroxidation, which occurs when reactive oxygen species attack lipids in cell membranes, leading to cell damage (Lee et al., 2020). Measuring the

levels of these byproducts in biological samples provides an indication of the extent of oxidative stress an organism is experiencing (Del Rio et al., 2005). However, it is important to note that the present study was conducted under field conditions, which introduces potential confounding factors in the environment (e.g., the presence of other pollutants, variations in water chemistry, fluctuations in food availability, etc.) that may further influence the production of ROS. Nevertheless, in accordance with the findings of the present study, an experimental study on the exposure of Asian clams to PFOS also reported an enhanced oxidative stress response, with MDA being identified as one of the increased markers (Liu et al., 2020). Furthermore, other experimental studies on different aquatic organisms, including freshwater tilapia (*Oreochromis niloticus*), *Gammarus insensibilis*, and freshwater mussels (*Unio ravoisieri*), have also reported increased MDA values in response to PFAS exposure (Amraoui et al., 2018; Liu et al., 2007; Touaylia et al., 2019). However, these studies do not reflect environmentally realistic conditions and fail to account for chronic exposure. To the best of our knowledge, the study by Piva et al. (2022) is the only other field-based research to assess oxidative stress responses to PFAS under environmental conditions (Piva et al., 2022). In that study, a number of oxidative stress biomarkers were evaluated in two endemic fish species (*Squalius cephalus* and *Padogobius bonelli*) from three rivers with varying PFAS concentrations. In contrast with the findings of the present study, Piva et al. (2022) observed a slight decrease in MDA levels with increasing environmental pollution, which was attributed to an increase in the activation of genes coding for antioxidant proteins. Antioxidants are formed as a protective mechanism to neutralize ROS and prevent further damage. In contrast, the present study found no correlation between antioxidants and PFAS based on polyphenol and total antioxidant capacity (TAC) assays, indicating the absence of mechanisms to counteract oxidative stress and neutralize ROS. A key distinction between this study and that of Piva et al. (2022) is that the mussels used in the present study are not endemic, but rather exposed for a certain time period. It is plausible that the exposure duration was sufficient to induce ROS formation and lipid peroxidation, yet insufficient to trigger antioxidant enzyme activation to inhibit ROS and mitigate cell membrane damage. Although this is purely speculative, given the lack of available information regarding the link between exposure time and antioxidant formation, future research should focus on extending the exposure period for Asian clams beyond six weeks, with subsampling at various time points to examine how oxidative stress biomarkers and antioxidant enzyme levels evolve over time.

4.4.4. Condition Index and Respiration

The condition index and respiration of bivalves are commonly used metrics to assess the health, nutritious and physiological status of bivalves (Andral et al., 2004; Liu et al., 2024; Nilin et al., 2012; Rao & Khan, 2000). Significant, but relatively weak, negative correlations were identified between certain PFAS and the condition index of the clams. This is consistent with the spatial distribution results, which indicate that the most beneficial average condition factor was observed at the location with the lowest bioaccumulated PFAS concentrations. These findings indicate that exposure to PFAS may have an impact on the overall health and physiological status of Asian clams or suggest that the condition index could be a determining factor in the bioaccumulation of PFAS. This is consistent with findings from Lemos et al., (2022), who reported a negative correlation between PFAS concentrations and the condition index of the American oyster (*Crassostrea virginica*) sampled from the field. In the Scheldt river, Mubiana et al. (2006) also observed a negative correlation between the condition index and environmental metal pollution in bivalve populations, particularly in the blue mussel (*Mytilus edulis*). In contrast, other studies have found no significant relationship between CI and pollutants such as PFAS and metals in various bivalve species, including the Asian clam (Islam et al., 2021; Peltier et al., 2009; Teunen et al., 2021). The physiological responses of bivalves to pollution are complex and influenced by a range of factors, making CI a challenging metric to interpret (Bervoets et al., 2005b). Although CI can provide insights into the general health of populations at various locations, it does not isolate the specific causes of stress. Therefore, while CI might be a useful starting point for understanding population health, further laboratory-based studies are necessary to determine its effectiveness as a tool for assessing PFAS toxicity in bivalves.

No significant relationship between respiration rates and bioaccumulated PFAS concentrations was observed in this study. Although, it is noteworthy that the lowest average respiration rate occurred at the site with the lowest PFAS bioaccumulation, while the highest rates were recorded at the sites with the highest bioaccumulated concentrations. Respiration rate is a widely used metric for assessing contaminant effects in marine and freshwater invertebrates, as it reflects both metabolic activity and physiological stress (Brooks et al., 2009; Sukhotin, 1992; Trigos et al., 2015; Wang et al., 2005). Although laboratory studies, such as those conducted by Fernández-Sanjuan et al. (2013), have reported increased respiration rates

following exposure to PFOS and PFOA, the results of this study indicate that respiration may not be the most reliable indicator of PFAS effects under field conditions. Due to these conditions, it is crucial to acknowledge the potential influence of various environmental factors on clam respiration. The presence of other pollutants may have obfuscated the specific effects induced by PFAS alone. Furthermore, prior to the analysis of biomarkers, the clams were exposed to EPA medium hard water instead of site water, which could have influenced the respiration rates. Other factors, such as fluctuations in water temperature during the transportation process, despite efforts to maintain consistent conditions, may also have influenced the respiration. In addition, some studies have indicated that respiration is less sensitive compared to other physiological responses, such as clearance rate and scope for growth (Nilin et al., 2012; Wang et al., 2005) suggesting that these parameters may provide more insight into the effects of PFAS exposure under field conditions.

4.5. Conclusions

This study demonstrates that Asian clams can serve as effective biomonitors for the detection of a wide range of PFAS, including both legacy and emerging compounds. The clams exhibited significant bioaccumulation of PFAS, some of which were undetectable in water and sediment samples, highlighting the importance of biological monitoring for contaminants that may be missed in abiotic matrices. The spatial variation in PFAS concentrations suggests that bioaccumulation patterns in such dynamic systems are complex and not solely determined by proximity to known pollution sources. Future research should focus on the influence of environmental parameters such as salinity to better understand the bioaccumulation patterns in such systems.

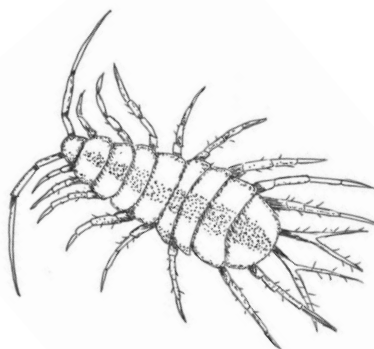
The biomarker responses indicated that PFAS exposure could induce oxidative stress in clams, as evidenced by the significant positive correlations between MDA and various PFAS. This suggests lipid peroxidation in response to PFAS exposure, although the lack of a clear antioxidant response, as indicated by polyphenol and FRAP assays, suggests that clams did not counteract oxidative damage effectively within the exposure timeframe. Further research, both in the field and under controlled laboratory conditions, is required to validate and strengthen these findings. Although significant negative correlations were observed between PFAS

concentrations and the condition index of the clams, suggesting that PFAS may affect the overall health of these organisms, no significant effects on clam respiration were detected. This suggests that while PFAS exposure may affect physiological health, the direct effects on respiration were not pronounced during the study period. These findings emphasize the importance of selecting appropriate endpoints, as antioxidants and respiration were found to be less effective in assessing the impact of PFAS contamination, particularly under field conditions.

Overall, these findings underscore the critical role of biomonitoring in assessing PFAS contamination and highlight the need for integrated approaches that combine bioaccumulation data with physiological and molecular endpoints to fully understand the ecological impacts of PFAS exposure in aquatic ecosystems.

Chapter V

Aquatic Macroinvertebrate Community Responses to PFAS Pollution: can we Define Threshold Body Burdens?



Adapted from:

Byns, C., Groffen, T., & Bervoets, L. (2024). Aquatic macroinvertebrate community responses to pollution of perfluoroalkyl substances (PFAS): Can we define threshold body burdens? *Science of the Total Environment*, 917. <https://doi.org/10.1016/j.scitotenv.2024.170611>

Abstract

The pollution of per- and polyfluorinated alkyl substances (PFAS) in aquatic environments is a worldwide concern of which the ecological impact is still not well understood. Especially field-based effect studies in aquatic ecosystems are generally lacking, creating a knowledge gap that goes along with monitoring and regulatory challenges. Therefore, this study examined if bioaccumulated PFAS concentrations could be related to ecological responses assessed by changes in the macroinvertebrate community structure. In addition, threshold body burdens that are protective of ecological damage were estimated. Aquatic macroinvertebrates were sampled in 30 streams across Flanders (Belgium) and 28 PFAS target analytes were measured in three resident taxa (*Gammarus* sp., *Asellus* sp. and *Chironomus* sp.) and translocated zebra mussels (*Dreissena polymorpha*). The macroinvertebrate community structure was assessed by calculating the Multimetric Macroinvertebrate Index Flanders (MMIF). Primarily long-chain perfluorinated carboxylic acids (PFCAs) were detected in both resident taxa (passive biomonitoring) and zebra mussels (active biomonitoring). Based on a 90th quantile regression model, safe threshold body burdens could be calculated for PFTeDA (7.1 ng/g ww) and Σ PFAS (2264 ng/g ww) in *Gammarus* sp. and for PFOA (5.5 ng/g ww), PFDoDA (1.7 ng/g ww), PFTrDA (0.51 ng/g ww), PFTeDA (2.4 ng/g ww), PFOS (644 ng/g ww) and Σ PFAS (133 ng/g ww) in zebra mussel. An additional threshold value was calculated for most compounds and species using the 95th percentile method. However, although these estimated thresholds are pertinent and indicative, regulatory applicability requires further lines of evidence and validation. Nevertheless, this study offers first-time evidence of associations between accumulated PFAS concentrations in invertebrates and a reduced ecological water quality in terms of macroinvertebrate community structure and highlights the potential of *Gammarus* sp. and zebra mussels to serve as reliable PFAS biomonitoring species.

5.1. Introduction

The pollution of per- and polyfluorinated alkyl substances (PFAS) in aquatic environments is a significant global environmental concern with complex regulatory challenges (Ankley et al., 2021). PFAS, characterized by strong carbon-fluorine bonds and an amphiphilic nature, are water and oil repellent and exhibit resistance to heat, making them highly useful in various industrial and commercial applications since the 1940s (Buck et al., 2011). However, the widespread application of PFAS has resulted in both direct and indirect releases into the environment. Once in the environment, PFAS are persistent and tend to bioaccumulate in aquatic organisms (Houde et al., 2011). While most scientific studies have primarily focused on the distribution and bioaccumulation of PFAS, the ecological impact of PFAS contamination on aquatic communities has received comparatively less attention (Ankley et al., 2021).

This limited understanding is coupled with a lack of regulatory measures, particularly the derivation and implementation of appropriate quality standards (QS). So far, in Europe, biota and environmental quality standards have only been established for a very limited subset of PFAS, despite the production of over 7 million compounds (Schymanski et al., 2023). Additionally, the Biota Quality Standard (BQS) for perfluorooctane sulfonic acid (PFOS; 9.1 ng/g ww) primarily aims to protect human health and to prevent secondary poisoning of top predators, but does not consider the protection of aquatic communities (European Union, 2013). In October 2022, the European Commission proposed new thresholds for PFAS in biota ($QS_{\text{biota,sec pois}}$), in particular for fish (22.3 $\mu\text{g/kg ww}$) and bivalves (6.2 $\mu\text{g/kg ww}$) expressed as PFOA equivalents (SCHEER, 2022). However, these BQs were also derived based on a secondary poisoning assessment. For the protection of human health, a more stringent BQS of 0.077 $\mu\text{g/kg ww}$ has been derived, expressed in PFOA equivalents (SCHEER, 2022). So far, no BQS has been derived for the protection of aquatic organisms at community or ecosystem level.

Furthermore, the monitoring of PFAS contamination predominantly relies on measurements taken from the abiotic environment, such as water and sediment (Kördel et al., 2013). However, these measurements only provide a momentary snapshot of the pollution status and fail to account for variations in bioavailability caused by fluctuating abiotic (e.g. water hardness, temperature, ...) and biotic factors (e.g. feeding mode, physiological status,...) (Van Ael et al., 2017). As a result, derived water and sediment quality criteria may not always be adequate for

the protection of aquatic communities. The monitoring of PFAS in biota can address the limitations of monitoring solely in the abiotic environment by considering bioaccumulated levels that integrate over time and reflect the bioavailability of PFAS. Two different biomonitoring approaches can be distinguished, namely passive and active biomonitoring. Passive biomonitoring (PBM) includes the collection of indigenous organisms as representatives of the in-situ conditions (Lacroix et al., 2015). Benthic macroinvertebrates have been considered to be a good indicator group to conduct passive biomonitoring as they are sedentary, easy to collect and identify, they can integrate fluctuations of environmental conditions, and have a wide geographical abundance (Resh et al., 1995; Basset et al., 2004; Li et al., 2010; Lin et al., 2020). They comprise a diverse group of organisms that play a crucial role in freshwater ecosystems by facilitating essential ecological processes, including nutrient cycling through organic matter decomposition, sediment mixing, and serving as a pivotal link in the aquatic food web (Nieto et al., 2017). Given that certain invertebrate taxa are more sensitive to pollution than others, the composition and structure of macroinvertebrate communities can serve as an indicator of the cumulative impact of both short-term and long-term pollution events (López-López & Sedeño-Díaz, 2015). Several studies on metal pollution demonstrated that bioaccumulated metal concentrations in aquatic macroinvertebrates can be associated with the macroinvertebrate community structure, offering potential for deriving biota quality standards that provide community-level protection (De Jonge et al., 2015; Rainbow et al., 2012; Méndez-Fernández et al., 2017). Active biomonitoring (ABM), on the other hand, refers to translocating caged organisms collected from a reference population to a specific site of interest (Wepener, 2013). After a fixed period of exposure, the biota will accumulate a contaminant profile that reflects the state of the local environment. Several animal groups can be used for ABM in aquatic environment including bivalves, crustaceans and fish (Moschino et al., 2016; Bertrand et al., 2018; Santana et al., 2018). One of the most commonly used species in Europe is the zebra mussel (*Dreissena polymorpha*), which has been shown to have a high uptake efficiency for metals and Persistent Organic Pollutants (POPs) and is tolerant to a wide range of contaminants (Smolders et al., 2002; Riva et al., 2008; Besse et al., 2012; Evariste et al., 2018). Both PBM and ABM have their advantages and disadvantages. Therefore, it is suggested to integrate both methods in order to achieve a comprehensive picture.

To date, most knowledge on the ecological effects of PFAS is based on laboratory toxicity data, while field-based effect studies are generally lacking (Ankley et al., 2021). In order to understand the potential effects of PFAS on wildlife, it is necessary to integrate more field studies that examine the effects of PFAS at a community and population level. Therefore, the objective of the present study was first to study the relationship between bioaccumulated PFAS concentrations (in macroinvertebrates and mussels) and the ecological responses assessed as the macroinvertebrate community structure. Secondly, we aim to evaluate the applicability of zebra mussels as ABM for PFAS pollution. Thirdly, we aimed to determine if this approach is suitable to derive threshold body burdens that are protective of ecological damage. To the best of our knowledge this is the third field-based effect study that has ever been conducted on the macroinvertebrate community responses to PFAS pollution (Bakke et al., 2010; Rusconi et al., 2015).

5.2. Materials and Methods

5.2.1. Passive and Active Biomonitoring

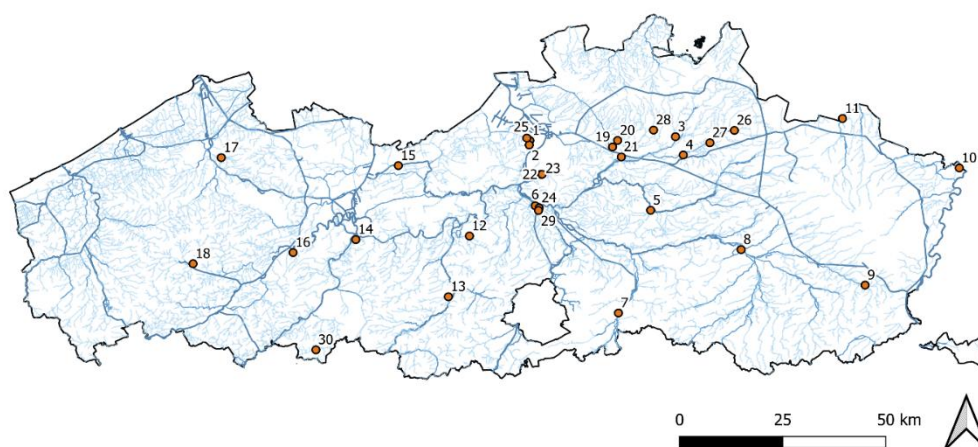


Figure 5.1: Overview of sampling locations in Flanders, Belgium.

Between September 2020 and December 2020, as well as from September 2021 to November 2021, resident macroinvertebrates were collected from 30 sites located in small rivers and streams across Flanders, Belgium (Figure 5.1; Table D.1 in Supplementary Information). The recommended sampling period for macroinvertebrates by Gabriels et al. (2010) is spring,

summer and autumn to avoid extreme regimes and temperatures (Gabriels et al., 2010). However, weather in Belgium is shifting towards milder winters and warmer summers, culminating in unprecedented temperature records during the summer months (De Ridder et al., 2020). Therefore, the sampling period of the present study was selected to avoid extreme weather conditions during the summer months. Sampling was conducted by collecting a 10-meter stretch at each site using a standard hand net with a mesh size of 0.5 μm . The kick sampling technique, as outlined by De Pauw and Vanhooren (1983) was employed to ensure the capture of a diverse range of macroinvertebrates. In the laboratory, all samples were carefully sieved (0.5 μm) and sorted, and the three most abundant invertebrate species, namely larvae of the non-biting midge *Chironomus* sp., the isopod *Asellus* sp., and the amphipod *Gammarus* sp., were selected and stored in a freezer at -20°C for subsequent PFAS analysis. All other invertebrate taxa were preserved in 70% ethanol to facilitate further identification.

To ensure an appropriate representation of individuals from the same species across all locations, active biomonitoring was also employed. Zebra mussels (*Dreissena polymorpha*) were exposed at 23 sites, which coincided with the locations where resident taxa were sampled. In order to minimise potential differences in bioavailability and to allow for comparison, mussels were exposed during the same macroinvertebrate sampling period. The mussels were sourced from a reference lake, the Blaarmeersen in Gent, known for its low background concentrations of pollutants (Teunen et al., 2021). Upon collection, the mussels underwent a two-week acclimatization period in artificial ponds containing dechlorinated tap water at the mesocosm facility of the University of Antwerp. Subsequently, the mussels were exposed for six weeks in cages constructed from polyethylene pond baskets (11 x 11 x 22 cm) with a mesh size of 2 x 4 mm, facilitating water circulation within the cages (Smolders et al., 2002). In total, 15 individual mussels were exposed per site. The cages were positioned approximately 20-30 cm above the bottom to prevent sediment accumulation. As previously described by Bervoets et al. (2004), a six-week exposure period is found optimal for zebra mussels, allowing for the accumulation of comparable pollutant levels to those found in indigenous mussels (Bervoets et al., 2004). Unfortunately, recollection of the mussels was only possible for 15 out of 23 sites, as many cages got destroyed or lost during the exposure time. Following collection, the mussels underwent a depuration phase in particle-free water obtained from the corresponding sampling sites, with a duration of 24 hours at a temperature of 15°C, before subsequent dissection. Given that zebra mussels are non-native species, and on top of

the existing evidence of the widespread distribution of *Dreissena* species in Flanders (Bervoets et al., 2004), the exposure was intentionally carried out during autumn and winter when water temperatures below 12°C act as a threshold to mussel reproduction (Mackie & Schloesser, 1996).

5.2.2. Ecological Quality Assessment

For each sampling site, all macroinvertebrate taxa preserved in ethanol were identified and calculated using a stereo microscope (Leica S8AP0) and the identification guide by De Pauw & Vannevel (1991). In order to evaluate the macroinvertebrate community responses to PFAS pollution, the Multimetric Macroinvertebrate Index of Flanders (MMIF) was calculated per site, following the methodology outlined by Gabriels et al. (2010). Specifically developed for surface waters in Flanders, this index incorporates five equally weighted metrics along with considerations for the specific water body type. The metrics used in the MMIF calculation include taxonomic richness (TAX), the number of EPT-taxa (Ephemeroptera, Plecoptera, Trichoptera), the number of other sensitive taxa (NST), the Shannon-Wiener Diversity index (SWD), and the mean tolerance score (MTS). These metrics are recognized as indicators sensitive to pollution in general, with particular emphasis on the diversity of EPT-taxa as markers for micropollutants (Berger et al., 2018). The resulting MMIF scores range from 0 to 1, where a score of 0 indicates poor ecological quality and a score of 1 indicates high ecological quality (Table D.2).

5.2.3. PFAS analyses

Before the extraction process, the mussels were dissected, and the soft tissue was homogenized with a bead homogeniser (TissueLyser LT Qiagen GmbH, Germany) with stainless steel beads (5 mm; Qiagen GmbH, Germany). Approximately 0.3 g ww of each individual mussel was weighed and placed in a 50 ml polypropylene (PP) tube. To ensure an adequate amount of tissue for the extraction of the other, resident, macroinvertebrates, five to ten individuals per species and per location were pooled together, homogenised with a bead homogeniser, and weighed in separate 50 ml PP tubes. The extraction procedure for biotic samples, as described by Powley et al. (2005), was carried out with minor modifications (Supplementary Information in appendix B). In total, 28 target analytes were analysed using Ultra Performance Liquid

Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) (Table D.3). Further information regarding the target analytes and specific instrumental settings can be found in Groffen et al. (2021). As a quality control measure, a procedural blank containing 10 mL of acetonitrile (ACN) per every 10 samples was included during the extraction process. The limit of quantification (LOQ) for each target analyte was determined per matrix, based on the integrated peak area of a low concentration peak with a corresponding signal-to-noise ratio of 10 (Table D.4). To prevent carry-over effects to the next sample, ACN was regularly injected during PFAS analysis as an instrumental blank.

5.2.4. Statistical Analyses

In addition to PFAS bioaccumulation, the macroinvertebrate community structure can also be influenced by various factors, including other pollutants, water characteristics, habitat quality, and food availability. To specifically examine the relationship between accumulated PFAS levels and the response of the invertebrate community, a 90th quantile regression analyses was employed. Hereby, only the maximum ecological response is considered, in order to correct for these unmodelled factors. All quantile regression models were performed using the software package R (version 4.1.3) and a significance level of p-value < 0.05 was considered. To define threshold body burdens above which a good ecological water quality is not achieved, two methods were used. The first and more robust approach is based on the 90th quantile regression line. In case no significant relationship was identified, the 95th percentile of accumulated PFAS concentrations measured at locations exhibiting good ecological quality (MMIF ≥ 0.7) was calculated (Gabriels et al., 2010). Two-way ANOVA analyses were performed to compare PFAS concentrations between species. Post-hoc comparisons were performed using Tukey's honestly significant difference (HSD) adjusted post-hoc test or the non-parametric equivalent Dunn's post-hoc test when model assumptions (homogeneity of variances and normality of residuals) were not met.

5.3. Results

5.3.1. PFAS Profiles

From the resident taxa, *Asellus* sp. could be sampled at 22 out of 30 sampling sites and *Gammarus* sp. and *Chironomus* sp. were each collected at 12 sites. To have enough datapoints, regression analyses were only done for compounds with a detection frequency $\geq 50\%$ as shown in Figure 5.3. Based on the detection frequency profiles, similar profiles were found for *Asellus* sp., *Gammarus* sp. and zebra mussels with mainly long-chain perfluoroalkyl carboxylic acids (PFCAs) such as perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) with a frequency $\geq 50\%$. Remarkable is the detection of perfluoro-3,6-dioxaheptanoic acid (3,6-OPFHpA) in only *Asellus* sp. and not in any other species, even not at the same locations. Despite the detection of 3,6-OPFHpA throughout the whole sampling area, elevated concentrations were mainly detected in rivers restricted to the Nete catchment area and the lower Scheldt catchment area. For chironomids, the detection frequency is less pronounced with only PFOA, PFDA and PFOS being $\geq 50\%$. This is also reflected in the concentration profiles, where PFAS concentrations are generally lower in *Chironomus* sp. compared to the other species. The lowest average concentration of Σ PFAS was found in *Chironomus* sp. (17 ng/g ww, min. 0.15 – max. 105 ng/g ww), followed by *D. polymorpha* (89 ng/g ww, min. 1.1 – max. 1388 ng/g ww), *Asellus* sp. (360 ng/g ww, min. 17 – 6324 ng/g ww) and *Gammarus* sp. (1409 ng/g ww, min. 18 – max. 5405 ng/g ww). However, the comparison of these concentration profiles must be made with some caution, as they are highly dependent on the different sites and therefore different conditions to which a given species is exposed. For example, *Asellus* sp. and zebra mussels were collected and exposed at a specific hotspot location, which explains the accumulation of compounds such as perfluorobutane sulfonamide (FBSA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonic acid (PFHxS) and perfluoroheptanesulfonic acid (PFHpS), which were not detected at other locations and in the other species. Comparisons between all four species within the same site could not be made because they were never sampled or exposed all together. For three species, *Gammarus* sp., *Asellus* sp. and zebra mussel comparisons could be made based on five sites (Figure 5.2). Significant higher concentrations were found for PFDoDA

in *Asellus* sp. ($p < 0.01$) and *Gammarus* sp. ($p < 0.001$) compared to zebra mussel with mean concentrations being 7.4_{*Asellus* sp.}, 13_{*Gammarus* sp.} and 1.4_{zebra mussel} ng/g ww. For all other compounds no significant difference was found for this comparison. However, generally higher PFTrDA and PFTeDA concentrations were detected in *Asellus* sp. and *Gammarus* sp. compared to zebra mussels with mean concentrations being 3.1_{*Asellus* sp.}, 3.1_{*Gammarus* sp.} and 0.38_{zebra mussel} ng/g ww for PFTrDA and 5.6_{*Asellus* sp.}, 5.7_{*Gammarus* sp.} and 1.7_{zebra mussel} ng/g ww for PFTeDA. Significantly higher PFDoDA concentrations were also found when comparing only *Gammarus* sp. to zebra mussels based on 11 sites ($p = 0.017$). For the other comparisons such as *Gammarus* sp. vs. *Asellus* sp. (based on eight sites) and *Asellus* sp. vs. zebra mussel (based on five sites), no significant difference was found for any of the compounds. For *Chironomus* sp. it was only possible to statistically compare with *Asellus* sp. for PFOA (based on five sites) and no significant difference was found. All other compounds could not be compared as they were often not detected in *Chironomus* sp. at these specific sites, resulting in insufficient data for statistical comparison. The same was true for *Chironomus* sp. vs. zebra mussels, which overlapped at 4 sites. *Gammarus* sp. and *Chironomus* sp. were never sampled at the same sites. An overview of all concentrations detected per species and per location is shown in Table D.5 – D.8.

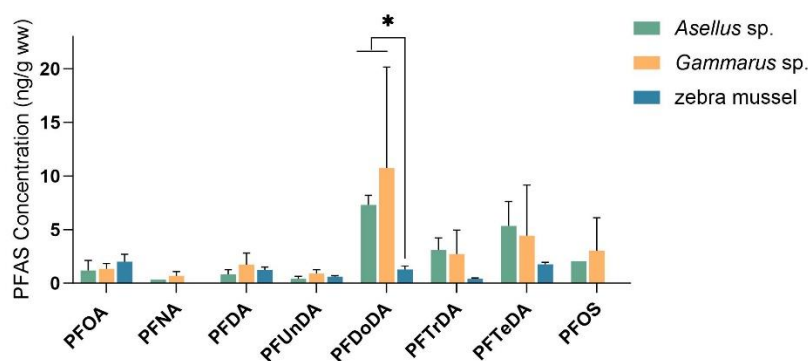


Figure 5.2: Comparison of PFAS concentrations and standard deviation (ng/g ww) among *Asellus* sp. *Gammarus* sp. and zebra mussels for five sampling sites where all three species were sampled or exposed.

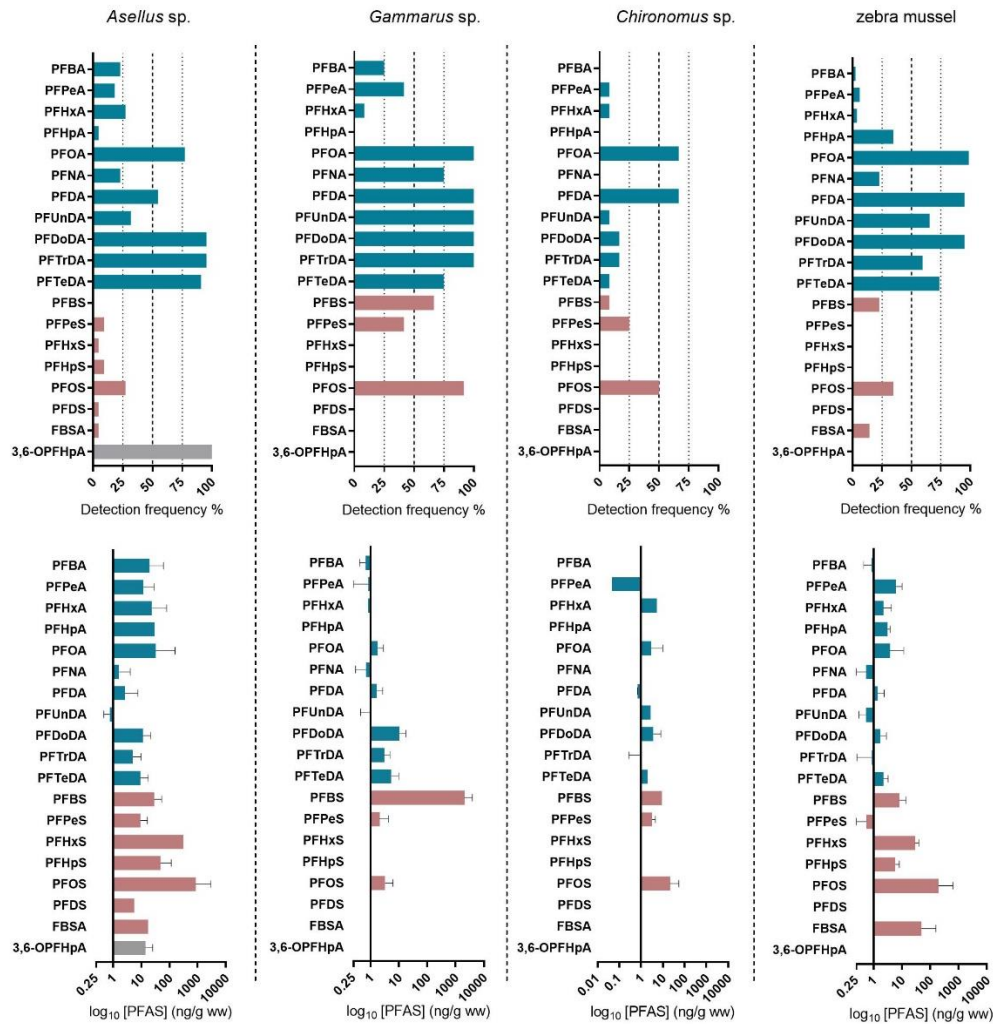


Figure 5.3: Detection frequencies (%) and mean PFAS concentrations with Standard Deviation (SD) (ng/g ww) detected over all sites per species: *Asellus sp.*, *Gammarus sp.*, *Chironomus sp.* and zebra mussel. Perfluoroalkyl carboxylic acids (PFCAs) are indicated in green, perfluoroalkyl sulfonic acids (PFSAs) and sulfonamides in pink and perfluoropolyether carboxylic acids (PFECAs) in grey. Note that the x-axis showing the PFAS concentrations is on a log scale.

5.3.2. Passive Biomonitoring and Ecological Responses

Figure 5.4 displays scatterplots illustrating the relationship between the MMIF and the accumulated PFAS concentrations in resident taxa, specifically *Asellus* sp., *Gammarus* sp., and *Chironomus* sp. However, based on the 90th quantile regression analyses, no significant relationship was observed between the ecological water quality and accumulated PFAS concentrations, except for PFTeDA ($p=0.03$) and Σ PFAS ($p<0.01$) in *Gammarus* sp. Consequently, threshold body burdens for PFTeDA and Σ PFAS in *Gammarus* sp. were calculated using the quantile regression model, resulting in a value of 7.1 ng/g ww and 2264 ng/g ww respectively. Despite the absence of significant relationships, threshold body burdens could still be derived for PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFOS, and Σ PFAS in both *Asellus* sp. and *Gammarus* sp. using the 95th percentile method (Table 5.1). Additionally, a threshold value of 35 ng/g ww was calculated in *Asellus* sp. for 3,6-OPFHpA. For *Chironomus* sp., no threshold burden could be derived due to the absence of a significant relationship between accumulated PFAS concentrations and the ecological water quality. Additionally, it is important to note that the sites where *Chironomus* sp. were sampled consistently demonstrated MMIF values below the threshold of 0.7. As a result, the 95th percentile method could not be applied to derive threshold burdens for *Chironomus* sp. in relation to the accumulated PFAS concentrations.

Hence, besides the MMIF, the relationship between each individual metric and accumulated PFAS concentrations was also examined. This was done to investigate whether specific metrics might have distinct associations that are overshadowed by other metrics within the composite MMIF. Hereby, significant negative relationships were found between PFTeDA concentrations in *Gammarus* sp. and the number of EPT taxa ($p=0.04$), number of other sensitive taxa ($p=0.04$) and the mean tolerance score ($p=0.03$) (Figure D.1). For *Asellus* sp. a significant negative relationship was found between PFTeDA and taxonomic richness ($p<0.01$) (Figure D.2).

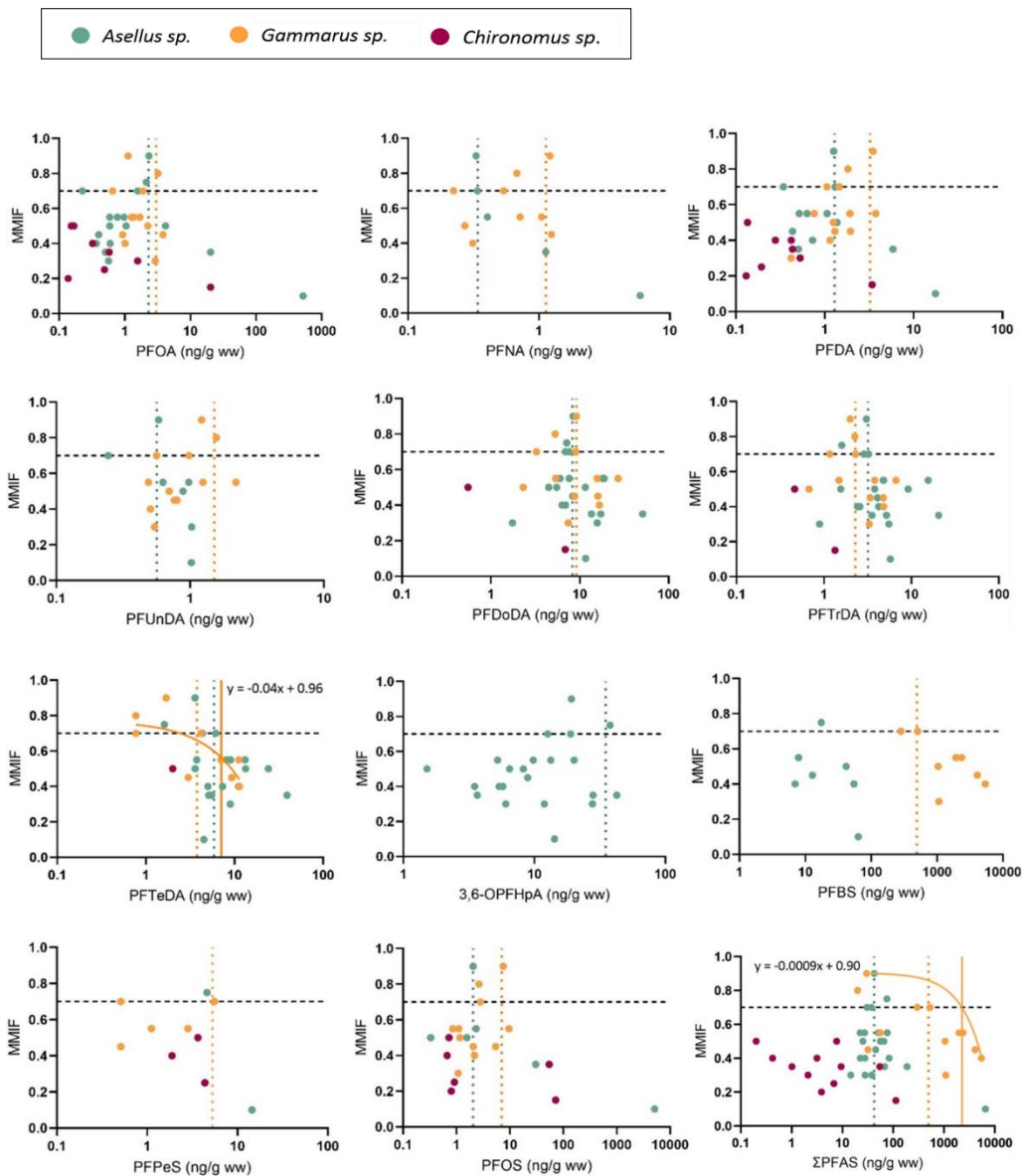


Figure 5.4: Scatterplots of the relationship between bioaccumulated PFAS concentrations in resident taxa versus the ecological water quality index (indicated as the MMIF). The horizontal dashed line indicates the threshold for a good ecological water quality (MMIF > 0.7). The vertical dashed lines indicate the threshold body burdens per species (green = *Asellus sp.*, yellow = *Gammarus sp.* and red = *Chironomus sp.*) calculated based on the 95th percentile method. Vertical solid lines indicate the threshold body burdens calculated based on the 90th quantile regression model.

5.3.3. Active Biomonitoring and Ecological Responses

Figure 5.5 depicts the scatterplots illustrating the relationship between the MMIF and the accumulated PFAS concentrations in translocated zebra mussels. Significantly observed quantile regression models ($p < 0.01$) were found for the compounds PFOA, PFDoDA, PFTrDA, PFTeDA, PFOS, and Σ PFAS.

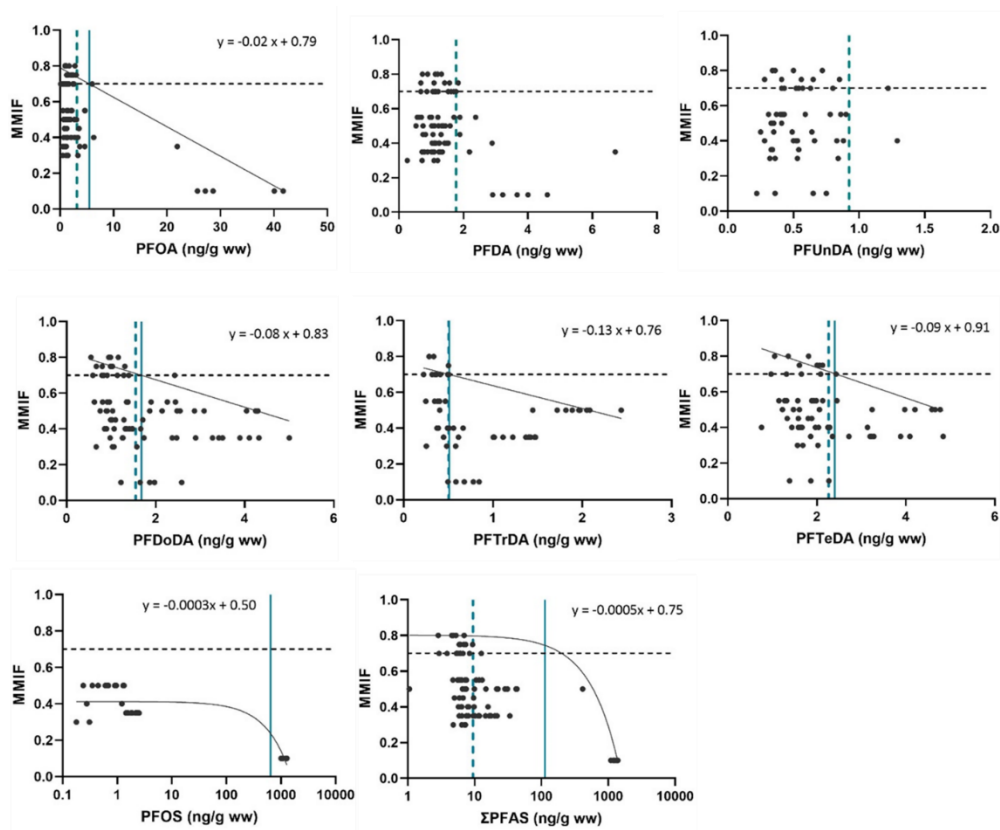


Figure 5.5: Significant 90th quantile regressions between the accumulated PFAS concentrations (ng/g ww) in zebra mussels (*D. polymorpha*) and the ecological water quality index (MMIF). The horizontal dashed line indicates the threshold for a good ecological water quality (MMIF > 0.7). The vertical dashed lines indicate the threshold body burdens based on the 95th percentile method and the vertical solid lines indicate the threshold body burdens calculated based on the 90th quantile regression model.

Consequently, threshold body burdens for these compounds in zebra mussels were calculated using both methods and are presented in Table 5.1. Since PFOS was not detected in the mussels at sites exhibiting a good ecological quality (MMIF > 0.7), the threshold value could not be calculated using the 95th percentile method. However, this method was applicable for deriving the threshold values of PFDA and PFUnDA only (Table 5.1).

Based on the quantile regression analysis with the individual metrics significant relationships ($p < 0.01$) were found for PFOA, PFDoDA, PFTrDA, PFTeDA and Σ PFAS versus all individual metrics (TAX, EPT, SWD and MST) except for NST. For PFOS a significant relationship was found with EPT, TAX, MTS and NST but not with SWD ($p < 0.01$). For NST, a significant relationship was only found for PFOA, PFOS and Σ PFAS ($p < 0.01$). As a result, EPT is the only index that showed significant models for all the same compounds as for the MMIF. The scatterplots of these significant relationships can be found in the appendix (Figure D.3 – D.7).

Table 5.1: Derived threshold body burdens (ng/g ww) for PFAS in zebra mussels and resident taxa based on the 95th percentile and the 90th quantile regression model. 'ns' means that no significant relationship was found. '-' indicates that the threshold body burden could not be calculated because this compound was not detected at any location with an MMIF ≥ 7 or the compound was not detected in this species.

ng/g ww	95 th Percentile			90 th Quantile regression model		
	<i>Asellus</i> sp.	<i>Gammarus</i> sp.	zebra mussel	<i>Asellus</i> sp.	<i>Gammarus</i> sp.	zebra mussel
PFOA	2.3	3.0	3.2	ns	ns	5.5
PFNA	0.34	1.1	-	ns	ns	ns
PFDA	1.3	3.2	1.8	ns	ns	ns
PFUnDA	0.56	1.5	0.92	ns	ns	ns
PFDoDA	8.3	9.1	1.6	ns	ns	1.7
PFTrDA	3.2	2.3	0.51	ns	ns	0.51
PFTeDA	5.9	3.8	2.3	ns	7.1	2.4
PFBS	-	493	-	ns	ns	-
PFPeS	-	5.3	-	-	-	-
PFOS	2.0	7.0	-	ns	ns	644
3,6-OPFHpA	35	-	-	-	-	-
Σ PFAS	42	493	9.4	ns	2264	113

5.4. Discussion

5.4.1. PFAS Profiles

The PFAS profiles observed in the present study exhibit similarity among the different invertebrate species, with long-chain PFCAs being the most dominant compounds. This finding aligns with previous research conducted by Lasier et al. (2011), who exposed aquatic oligochaete worms (*Lumbriculus variegatus*) to PFAS-contaminated field sediments and observed that PFOS and long-chain PFCAs exhibited the highest bioaccumulation potential (Lasier et al., 2011). A similar trend was also observed in the study conducted by Bertin et al. (2016), where Biota Sediment Accumulation Factors (BSAFs) for Gammarids increased with increasing chain length of PFAS (Bertin et al., 2016). A study involving the comparative analysis of PFAS accumulation kinetics in *Chironomus riparius* and *Gammarus* sp. revealed notable distinctions (Bertin et al., 2014, 2016). Specifically, the elimination rate of PFAS within chironomids was observed to be higher than within gammarids, and correspondingly, the BSAFs were found to be higher in gammarids compared to chironomids. This is in line with the present study, where the overall patterns show that the detection frequencies and the accumulated concentrations of PFAS are generally lower in chironomids. In a study on PFAS bioaccumulation in the Rhone River, invertebrate samples were pooled and Σ PFAS concentrations ranged from 6.56 ng/g ww in a chironomid sample to 356 ng/g ww in a gammarid sample (Babut et al., 2017). In the present study mean Σ PFAS concentrations range from 17 ng/g ww in *Chironomus* sp. to 1409 ng/g ww in *Gammarus* sp. It should be noted, however, that our study covers multiple water bodies, while the study by Babut et al. (2017), focuses on one river system.

Furthermore, it is important to consider that the different foraging behaviour of the studied species may result in different PFAS exposure routes and profiles. Zebra mussels, being filter feeders, are primarily exposed to PFAS through their diet and water, while the studied resident taxa, which dwell in sediments, experience additional exposure through sediment contact (Lewis et al., 2022). In contrast with our findings, it was initially anticipated that the relative contribution of short-chain PFAS would be higher in mussels than in bottom-dwelling resident taxa, given the greater water solubility of short-chain PFAS (Li et al., 2020). The dominance of long-chain PFAS in the profiles could be attributed to their higher bioaccumulation potential compared to short-chain PFAS (Goodrow et al., 2020). Additionally, it should be noted that the

placement of mussel cages in close proximity to the sediment in relatively shallow streams may also have influenced the bioaccumulation pattern. A similar pattern in mussels was observed in a recent study by Teunen et al. (2022), where quagga (*Dreissena rostriformis bugensis*) and Asian clams (*Corbicula fluminea*) were exposed in larger rivers across Flanders. Consistent with our findings, the profile was mainly characterized by PFOA (approximately 40%), followed by other long-chain PFCA's such as PFUnDA, PFDoDA and PFTrDA. Although accumulated concentrations in zebra mussels were lower than in resident taxa for some compounds such as PFDoDA, PFTrDA and PFTeDA, zebra mussels and resident taxa have similar detection profiles, suggesting that zebra mussels may provide an overview of compounds to which resident taxa are exposed. Additionally, the present study shows some of the highest concentrations of PFOS and PFOA that have ever been reported in mussels with mean PFOS concentrations of 1137 ng/g ww and mean PFOA concentrations of 37 ng/g ww measured at a specific hotspot location, the palingbeek in Zwijndrecht. PFOS concentrations in zebra mussels from the Great Lakes ranged from 2.4 to 3.1 ng/g ww (Kannan et al., 2005). However, most data on PFAS accumulation in mussels is on marine species and often restricted to PFOS and PFOA. In brown mussels from Guanabara Bay, Brazil, PFOS and PFOA concentrations ranged from 3.5 to 4.5 and 2.1 to 6.0 ng/g ww (Quinete et al., 2009); PFOS concentrations in blue mussels from Danish coastal waters ranged from 0.11 to 0.58 ng/g ww (Bossi et al., 2008); South China and Japanese coast concentrations of PFOS and PFOSA in mussels ranged from 0.11 to 0.59 and from 0.04 to 2.9 ng/g ww (So et al., 2006). High PFOS concentrations were also reported in Mediterranean mussel (36.8–126 ng/g ww) collected in Portugal (Cunha et al., 2005). The mean Σ PFAS concentration reported in dreissenid mussels from Lake Huron was 4.1 ± 0.53 ng/g ww, while the mean Σ PFAS in the present study in zebra mussels was 89 ng/g ww (range 1.1 – 1388 ng/g ww) (Ren et al., 2022). Again, it should be noted that the present study does not focus on one specific water body, but covers several streams and small rivers throughout Flanders. In the study by Teunen et al. (2021), which also covers several river systems in Flanders, Σ PFAS in bivalves (quagga mussels and Asian clams) ranged from 8.56 to 157 ng/g ww. In Manila clams (*Venerupis philippinarum*) from the Po River Delta in Italy, concentrations of PFOA and PFOS dominated, ranging from 1.6 to 5.8 ng/g ww (PFOA) and from 0.3 to 1.6 ng/g ww (PFOS) (Mazzoni et al., 2016). Conversely, in blue mussels (*Mytilus galloprovincialis*) from the same region, the highest concentrations were observed for PFOA, PFDoDA and PFUnDA, ranging from 0.2 to 1.1 ng/g ww, 0.2 to 0.7 ng/g ww and 0.1 to 0.2 ng/g ww, respectively (Mazzoni et al.,

2016). Notably, the concentrations in blue mussels were significantly lower compared to those in Manila clams. A separate study by Corsolini et al. (2014) also found that the Σ PFAS concentrations in Manila clams were markedly higher than those in blue mussels sampled at the same locations. This underscores the importance of selecting the right organisms and species for a successful active biomonitoring campaign. In general, the above-mentioned PFAS concentrations are relatively low compared to the concentrations measured in the present study, suggesting that zebra mussels have the potential to serve as an effective biomonitoring species for PFAS.

5.4.2. Macroinvertebrate Community Responses to PFAS Pollution

Studying the relationship between accumulated micropollutant levels in resident taxa and macroinvertebrate based biotic indices has been found to be a good method to predict ecological effects and derive critical threshold values that are protective of ecological damage (Luoma et al., 2010; Rainbow et al., 2012; De Jonge et al., 2013; Bervoets et al., 2016). However, to our knowledge this type of research has not been done for PFAS up until now. Overall, research on the effects of PFAS on aquatic wildlife is lacking compared to controlled laboratory studies, especially for macroinvertebrates. Based on the review study of Ankley et al. (2021), 39 field-based effect studies were reported with only two studies focussing on aquatic invertebrates. One of these studies investigated the macro-benthic community structure up- and downstream of a PFOA point source in Northern Italy (Rusconi et al., 2015). Although more sensitive taxa were found upstream compared to downstream no significant differences were observed based on a multimetric index. Therefore, the relationship between each individual metric and accumulated PFAS concentrations was also examined in the current study. As such, individual metrics showed inconsistent results, making the MMIF the preferred ecological endpoint for assessing community responses as it also takes into account watercourse type, which mitigates the effect of external factors on the index (Gabriels et al., 2010).

Because *Chironomus* species are well known for their tolerance to pollutants, they were only found in sufficient numbers at 12 out of 35 sites and never at the sites with good ecological quality (Saether, 1979; Lindegaard, 1995; Mousavi et al., 2003). Among aquatic invertebrates, chironomids are one of the most studied taxa regarding PFAS toxicity. However, findings from laboratory studies present conflicting outcomes. For example, Ankley et al. (2021) report that

Chironomus riparius is one of the most sensitive species to PFOS, with adverse reproductive effects occurring at a concentration of 4 µg PFOS/L. Conversely, the multi-generational study conducted by Marziali et al. (2019), examining the effects of PFOS, PFOA, and PFBS on *Chironomus* sp. for 10 generations to 10 µg/L nominal concentrations, did not identify any reproductive or population-level impacts, suggesting a limited influence under field conditions (Marziali et al., 2019). Crustaceans have also been identified as one of the more sensitive groups. However, most toxicity studies focus primarily on Cladocerans (particularly *Daphnia* sp.), leaving a gap in toxicity data for other crustaceans such as *Asellus* sp. and *Gammarus* sp. (Ankley et al., 2021).

Based on the scatter plots (Figure 5.4) it can be observed that certain locations exhibit a low ecological quality, despite low accumulated PFAS concentrations. This is likely due to the influence of other factors such as environmental stressors (e.g., low oxygen, high nutrient loads, habitat degradation, ...), as well as the presence of other contaminants. This observation lends further support to the notion that accumulated pollutants and the ecological water quality do not necessarily reflect a causal relationship. Therefore, the use of 90th quantile regression models appear to be a good strategy to relate internal PFAS concentrations to the maximum ecological response to mitigate the effects of unmodeled factors. For the resident taxa, a significant negative relationship was only found for PFTTrDA and ΣPFAS in *Gammarus* sp. The lack of significant relationships for the other compounds may be because the accumulated concentrations in lower trophic levels, such as invertebrates, are too low to cause changes in macroinvertebrate community structure. However, sites exhibiting higher levels of accumulated PFAS concentrations consistently remain below the threshold value for a good ecological quality (MMIF=0.7). Furthermore, in sites characterized by an MMIF>0.7 the accumulated concentration of 9.0 ng/g ww was never exceeded for any of the compounds except for PFBS in *Gammarus* sp.. It is important to note that the nature of this fieldwork introduces challenges to consistently sample the same species across all sites. Consequently, the available dataset per compound and species is often constrained, representing a pivotal limitation in this study.

Translocated zebra mussels were exposed to overcome the limitations of passive biomonitoring with resident taxa. This active biomonitoring technique has been used for decades to assess the bioavailability of pollutants in aquatic environments (Mersch et al., 1996; Bervoets et al., 2004; Minier et al., 2006). Although zebra mussels have been found to be a good bioindicator

for predicting the impact of contaminants such as metals and pesticides on aquatic ecosystems, literature on the use of this approach for PFAS is limited (Bervoets et al., 2004; Mersch et al., 1996). In the present study it was found that increasing PFOA, PFDoDA, PFTrDA, PFTeDA, PFOS and Σ PFAS concentrations in zebra mussels can be related to a decreasing ecological water quality, as assessed by the MMIF. Overall, as was also found for resident taxa, the congruity between outcomes derived from individual metrics and the multimetric index (MMIF) suggests that the MMIF is a good approach. This suggests that translocated zebra mussels can be a suitable biomonitoring tool to study the bioavailability of PFAS in aquatic environments and to predict the ecological responses of macroinvertebrate communities. However, the experimental study of Fernández-Sanjuan et al. (2013) indicated that the bioaccumulation potential of PFOS and PFOA in zebra mussels is low compared to other aquatic organisms (Fernández-Sanjuan et al., 2013). It was found that above an accumulated concentration of 9 ng/g ww, the bioaccumulation kinetics of PFOS and PFOA are inversely related to the activity of efflux transporters. It is therefore possible that some of the accumulated compounds in mussel tissues are being actively excreted. However, in the present study, no major differences in accumulated PFAS concentrations were observed compared to resident species, supporting our statement that zebra mussels are suitable biomonitoring species. In addition, as described above, the present study shows some of the highest concentrations of PFOS and PFOA that have ever been reported in mussels, suggesting that they are good bioaccumulators of PFAS. Although ABM offers great potential for biomonitoring PFAS, we also experienced some drawbacks, including losing 8 out of 23 cages during our exposure period. Some cages were simply taken out of the water, while other cages had ropes cut through, probably as a result of riverbank maintenance practices such as mowing. For this reason, we recommend to place the cages on small bridges over the stream, rather than along the banks.

5.4.3. Derivation of Threshold Body Burdens

This type of field-based effect study allows to provide evidence of pollutant bioavailability and has been used to estimate critical body levels to protect aquatic invertebrate communities for a range of pollutants (Meador et al., 2014; Bervoets et al., 2016). For PFAS, to the best of our knowledge only the study of Teunen et al. (2022) has applied this approach to estimate threshold body burdens for PFOS in two fish species, eel (*Anguilla anguilla*) and perch (*Perca fluviatilis*). In perch the threshold values were 9.5 ng/g ww (95th percentile) and 12 ng/g ww

(90th quantile) and in eel a threshold of 49 ng/g ww (95th percentile) was derived. In general, invertebrates have been found to be more sensitive to contaminants than fish (Buckler et al., 2005). Therefore, a lower threshold body burden was expected for invertebrates compared to fish. This was the case for *Gammarus* sp. and *Asellus* sp. with PFOS thresholds of 7.0 and 2.0 ng/g ww respectively, but not for zebra mussels (644 ng/g ww). This is likely because the body burden of PFOS (90th quantile) in zebra mussels was only based on four sites including one hotspot. When the hotspot site is excluded from the dataset, the quantile regression is no longer significant. In general, the quantile regression method can be considered as the most robust method for the derivation of threshold values as it is based on a significant quantile regression model. However, compared to the 95th percentile method, this method is more likely to be influenced by hotspots as shown in the present study. The 95th percentile on the other hand is solely based on concentrations measured at sites with a good ecological quality. This method is often applied as no relationship was observed which might indicate that the threshold or turning point is not even reached yet in the dataset. Therefore, it is possible that the effective threshold is higher. The advantages and disadvantages of both methods must be considered for further validation of the threshold body burdens.

Despite that Σ PFAS concentrations are often reported in literature and monitoring studies, the present study indicates that this approach is less relevant for the estimation of thresholds. For example, remarkable high threshold values of 492 ng/g ww (95th percentile) and 2264 ng/g ww (Quantile) were estimated for Σ PFAS in *Gammarus* sp.. This is mainly due to the high PFBS concentrations, ranging from 281 – 5367 ng/g ww, detected in *Gammarus* sp. at eight out of 12 sites. When PFBS was excluded from the Σ PFAS, the quantile regression was not significant anymore and the 95th percentile threshold resulted in 29 ng/g ww. Therefore, the threshold for Σ PFAS is likely to be biased, given that all compounds are accorded equal weight, thereby failing to account for variations in the toxicity of distinct compounds.

An additional crucial aspect requiring further investigation to validate these threshold values is the impact of season on critical body levels. The seasonal fluctuation in water temperatures can alter the metabolic activity of aquatic organisms, consequently affecting the bioaccumulation kinetics (Vidal et al., 2019). For example, in mussels, cooler water temperatures often lead to reduced respiration and feeding rates in response to lower metabolic demands, potentially resulting in diminished contaminant bioaccumulation (Maulvault et al., 2018; Rao & Khan, 2000; Wang et al., 2005). Conversely, a contrasting trend

has been noted for the bioaccumulation of Hg in zebra mussels, where reduced metabolism is assumed to weaken excretion processes, leading to higher bioaccumulated concentrations (Wilman et al., 2023). Therefore, it is imperative to gain a clearer understanding of the impact of seasonal variations in water temperature on the bioaccumulation of PFAS in the considered species. Besides temperature, also other factors can contribute to seasonal variations in PFAS bioavailability. For example, PFAS tend to adsorb onto suspended particles entering the aquatic environment through surface runoff, becoming a significant pathway for sediment contamination through deposition (Borthakur et al., 2021). Hence, external elements such as heavy rainfall and increased river flow velocity can profoundly influence PFAS bioavailability in aquatic environments due to increased particle loading and resuspension of river sediments (Borthakur et al., 2021).

Moreover, it is crucial to recognize that due to variations in bioaccumulation kinetics among different species, estimates of critical body burdens are applicable exclusively to the specific species for which they have been established (Bertin et al., 2014, 2016).

Despite some limitations in the estimation approach and the need for further validation through additional research, the precautionary thresholds identified in the present study remain pertinent and indicative. This is particularly significant given the pivotal role played by macroinvertebrates in aquatic ecosystems, serving as a significant biomagnification pathway for PFAS within the aquatic food web (Wallace & Webster, 2003). Therefore, the relevance of these thresholds extends beyond the protection of macroinvertebrate communities to encompass the broader safeguarding of the entire aquatic ecosystem.

5.5. Conclusions

First, predominantly long-chain perfluorinated carboxylic acids (PFCAs) were consistently detected in both resident taxa and zebra mussels across rivers and streams in Flanders, Belgium. Consequently, we strongly recommend prioritizing these compounds in future environmental monitoring programs. Given that the present study focused on a limited number of PFAS, further testing of PFAS is advised to encompass the influence of precursor compounds and provide a comprehensive understanding of PFAS present in aquatic ecosystems.

Secondly, given the observed relationship between increasing PFAS concentrations in *Gammarus* sp. and zebra mussels and a declining ecological water quality, it can be inferred that both species have the potential to serve as reliable biomonitoring tools. They can fulfil two primary purposes: (I) monitoring the bioavailability of PFAS and (II) predicting the ecological responses of macroinvertebrate communities to PFAS pollution. In contrast, due to the absence of significant relationships, *Asellus* sp. and *Chironomus* sp. appear to be less suitable for predicting the ecological effects of PFAS on aquatic ecosystems. Nevertheless, our results provide first-time evidence of associations between accumulated PFAS concentrations in benthic macro-invertebrates and a reduced ecological water quality in terms of the macroinvertebrate community structure.

Additionally, this study marks the first reporting of threshold body burdens for a set of PFAS in aquatic invertebrates. However, for regulatory applicability, it is imperative to gather additional lines of evidence to validate the estimated threshold values. Specifically, assessing the consistency of findings between laboratory, mesocosm studies (involving invertebrates, fish, etc.) and observations from field-based effect studies involving similar species would be of significant value.

Chapter VI

General Discussion

6.1. Biomonitor or Bioindicator?

Throughout the chapters of this thesis different biomonitor species and techniques have been applied. However, a good biomonitor is not necessarily also a good bioindicator, although, these terms are often used within the wrong context and misunderstood. Bioindicators are organisms or biological responses used to assess the health of an ecosystem and the presence of environmental changes or stressors (Holt & Miller, 2010). They provide qualitative data, indicating whether or not a certain level of pollution or ecological disturbance is present. Bioindicators help signal the impact of environmental changes through observable biological responses, such as changes in behavior, physiology, or population structure. Biomonitor, on the other hand, are organisms used to measure the magnitude of environmental contamination over time, providing quantitative data (Holt & Miller, 2010). They accumulate pollutants in their tissues and reflect the concentrations present in their surroundings, allowing researchers to assess and monitor pollutant levels in a specific ecosystem. Biomonitor can indicate the extent and type of pollution and offer insights into how contaminant levels fluctuate over time or between locations.

When comparing the PFAS profiles accumulated in the exposed Asian clams (Chapter IV) to those in resident fish species from the Scheldt river (Chapter III), distinct differences in the profiles are observed. Overall, 15 PFAS were detected in the fish samples, whereas only eight compounds were detected in the Asian clams. This indicates that the PFAS profiles identified in clams may not accurately reflect the extent of contamination experienced by resident species within the same system. Certain compounds, such as FBSA and PFOS, were detected in the fish but not in the clams, and vice versa. These differences can be attributed to the distinct ecological and behavioral characteristics of the organisms. Clams, being sessile organisms, remain fixed in a single location and are continuously exposed to contaminants present in the surrounding water column. In contrast, fish exhibit greater mobility, allowing them to forage across multiple contaminated areas and interact with sediments, which may contribute to variations in exposure and bioaccumulation patterns. The same reasoning was also described by Teunen et al. (2021) and Miranda et al. (2022). It is important to note that the sampling for the two studies was not conducted within the same timeframe. Consequently, the FBSA concentrations detected in fish, which were attributed to a local release, may no longer have been present at comparable levels during the exposure of the clams. This is likely due to the

mobile nature of FBSA, which could have reduced its availability for bioaccumulation in clams by the time their sampling occurred. While Asian clams may not be the most reliable indicator of PFAS bioaccumulation profiles in other resident species, they remain a valuable tool for active biomonitoring. They can provide time-integrated data on the bioavailability of contaminants in the water column at specific sites, which makes them particularly useful for assessing spatial variations in contaminant levels. From an ecological perspective, it is more appropriate to prioritize the collection and sacrifice of a non-native species such as Asian clams, over the sampling of indigenous species, which play a pivotal role in maintaining the local ecosystem balance. Moreover, their resilience to diverse environmental conditions, ease of collection and handling, and cost-effectiveness render them a practical choice for long-term monitoring programs (Sousa et al., 2008).

The aquatic macroinvertebrate communities examined in Chapter V serve as exemplary bioindicators due to their limited mobility and the capacity of their community structure to reflect and integrate the cumulative effects of water quality over time (Holt & Miller, 2010; López-López & Sedeño-Díaz, 2015). The zebra mussels exposed in the present study exhibit PFAS profiles comparable to those observed in the resident macroinvertebrates, indicating their potential as effective indicators for the PFAS profiles of these specific resident species. This finding supports the implementation of active biomonitoring over passive biomonitoring in freshwater systems, as exposing zebra mussels is less disruptive and time-efficient while ensuring the consistent use of the same target species, with the same background, across all sampling sites. In addition, the exact exposure time is known and equal over all study sites. One limitation of active biomonitoring is that organisms are exposed for a fixed time period, which does not account for seasonal variations, factors that are inherently captured through passive biomonitoring. Therefore, to study seasonal variation, active biomonitoring must be conducted multiple times throughout the year. In the context of long-term monitoring programs, this limitation can be addressed by standardizing exposure periods to the same time each year, ensuring consistency and comparability in temporal data collection.

6.2. Spatial Dynamics of PFAS Bioaccumulation Across Aquatic Systems

Throughout this thesis, the bioaccumulation of PFAS was studied across a gradient of aquatic systems, ranging from the smallest streams to larger rivers such as the Scheldt, and ultimately to the North Sea. Although different species were analyzed in these studies, necessitating caution in directly comparing bioaccumulation dynamics, an observable trend emerged: the smaller the system studied, the more diverse the PFAS profile detected. In smaller streams, as described in Chapter V, a total of 19 different PFAS were detected in macroinvertebrates and mussels. Moving downstream to the Scheldt River, the diversity decreased, with 8 to 15 different compounds detected in clams and fish (Chapter III and IV), respectively. Finally, in the North Sea, only 7 compounds were detected in fish (Chapter II).

This trend can be explained by several interrelated factors. Smaller systems often receive direct inputs from localized sources of PFAS contamination, such as industrial discharges, urban runoff, and wastewater treatment plants, which can introduce a wide variety of PFAS compounds depending on the specific sources and activities in the area. In contrast, larger systems experience significant dilution due to their sheer water volume, potentially masking the presence of less abundant or less persistent compounds. Consequently, it is possible that organisms in these larger systems predominantly bioaccumulate the most persistent PFAS compounds, which are detectable in higher quantities. For instance, the compounds detected in North Sea fish are predominantly long-chain PFAS, known for their high bioaccumulation potential and environmental persistence (Buck et al., 2011). Additionally, PFAS can undergo degradation or transformation during downstream transport (Butt et al., 2014). Precursor compounds in smaller systems may break down into more stable end-products, resulting in fewer and more uniform PFAS compounds in larger systems (Eriksson et al., 2017). Changes in physicochemical properties across these systems might also influence the partitioning behavior and bioavailability of PFAS, further contributing to differences in detected profiles.

In conclusion, the observed reduction in PFAS diversity from smaller streams to larger water bodies reflects a complex interplay of localized inputs, dilution effects, degradation processes, and physicochemical dynamics, underscoring the importance of system size and context in understanding PFAS bioaccumulation patterns.

It is important to note that in Chapter II, only 15 target analytes were analyzed, whereas the analytical method used in the other chapters was optimized to include a total of 28 target analytes. Notably, recent alternative compounds such as NaDONA, fluorotelomers, GenX, and precursor compounds like FBSA were not analyzed in the North Sea study. This exclusion potentially contributed to the lower number of detected compounds in Chapter II. An overview of the target analytes analyzed in each chapter is provided in Table E.2 of the Supplementary Information.

These findings emphasize the critical importance of continuously optimizing analytical techniques to ensure comprehensive detection and an accurate monitoring of PFAS. Although this work focused solely on the detection of PFAS through target analysis, the integration of advanced analytical techniques, such as TOP Assay, suspect screening, and non-target screening, in future research holds significant potential for identifying previously unrecognized PFAS. The TOP Assay is particularly valuable for evaluating the contribution of biotransformation of PFAS precursors to the persistence of PFAS in the environment (Ateia et al., 2023). While the assay does not directly measure biotransformation, it simulates oxidative conditions, converting precursors into stable PFAS that can then be detected. By comparing samples before and after oxidation, the hidden burden of PFAS contamination originating from precursors, that would otherwise remain undetected, can be estimated (Ateia et al., 2023). In addition to targeted approaches, suspect screening serves as a semi-targeted analytical method for identifying known or suspected PFAS in environmental and biological samples. This approach relies on high-resolution mass spectrometry to compare detected chemical signatures against existing PFAS reference databases (Jeong et al., 2022). Non-target screening, by contrast, is an untargeted approach that detects and identifies unknown PFAS without prior knowledge or a predefined chemical list (Wang et al., 2022). These methods are particularly valuable for identifying emerging PFAS contaminants in hotspot locations near industrial manufacturing sites, such as the Scheldt River or Palingbeek (Chapter V) (Joerss et al., 2022). By leveraging these complementary analytical techniques, future research can significantly enhance the detection, characterization, and regulation of PFAS contamination.

6.3. Biota Quality Standards: PFAS Compliance and Challenges

Over the course of writing this thesis, new regulations concerning PFAS standards in biota have been introduced, resulting in some chapters not reflecting the most current regulatory framework. For instance, while the maximum contaminant levels (MLs) established for certain food categories, including fish, are described in the introduction, they were not addressed in Chapter II, as these standards had not been determined at the time of its writing. Additionally, comparisons with quality standards are not consistently made or discussed across all chapters, as they did not always align with the scope of the study. Therefore, Tables 6.1 and 6.2 provide a detailed overview of current biota quality standards and the percentage of samples exceeding these thresholds, organized by chapter and species. In 2020, the European Food Safety Authority (EFSA) recommended a new Tolerable Weekly Intake (TWI) of 4.4 ng/kg body weight for the combined total of four PFAS: PFOA, PFOS, PFNA, and PFHxS. The TWI represents the estimated amount of a contaminant, unintentionally present in food or drinking water, that can be safely consumed weekly over a lifetime without causing significant health risks (EFSA, 2020). Based on this EFSA guideline, maximum contaminant levels (MLs) have been established to protect human health for these four compounds individually and their cumulative sum. Separate MLs were derived for fish and shellfish, which are presented in Tables 6.1 and 6.2, respectively. For fish, the MLs are further subdivided into three categories based on species. Category 1 includes all fish species, except those listed in category 2 and 3. An overview of the species enlisted in Category 2 and 3 is given in the Supplementary Information Table E.1.. It is noteworthy that PFHxS ($ML_{cat.1}=0.20 \mu\text{g/kg}$, $ML_{cat.2}=0.20 \mu\text{g/kg}$, $ML_{cat.3}=1.5 \mu\text{g/kg}$) is excluded from Table 6.1, as this compound was not detected in any fish samples analyzed.

In accordance with regulatory guidelines, food items exceeding the specified MLs are prohibited from entering the commercial market (EU, 2022). The findings presented in Tables 6.1 and 6.2 indicate that a considerable proportion of the fish and shellfish analyzed in this thesis exceed the established standards, rendering them unsuitable for commercial sale. Predictably, fish species classified in category 3, which are subject to the highest MLs, demonstrate the lowest percentage of exceedance. However, the rationale for the specific categorization of these fish species and the criteria upon which this classification is based are not explicitly detailed in the regulatory frameworks. It is important to recognize that not all fish and shellfish included in this thesis are species of commercial interest or commonly found in

markets. Additionally, as discussed in Chapter II, many of the sampled specimens were still juveniles, which may not reflect contaminant concentrations in adult specimens typically available for consumption. Moreover, the practice of fishing for consumption in the Scheldt river is prohibited, which significantly reduces the likelihood of fish caught in this river being sold as food. However, special attention should be given to the common shrimp, which consistently exhibits a high percentage of exceedance across all quality standards. Although common shrimp for human consumption are not harvested from the specific sampling region, they are fished downstream in the Dutch part of the Scheldt estuary. These findings underscore the importance of continued monitoring of shrimp populations to ensure the safety of human health. In addition, commercial fish species such as sole, European plaice, European flounder and Atlantic herring also often exceed the standards. Therefore, enhanced monitoring and rigorous quality control of specimens harvested from the Belgian North Sea is strongly recommended.

When examining the Environmental Quality Standard derived for the protection of human health for PFOS ($EQS_{biota,hh} = 9.1 \mu\text{g PFOS/kg ww}$) which is implemented under the Water Framework Directive, a clear disparity in exceedance levels emerges between specimens sampled in the Scheldt river (Chapter III) and those from the North Sea (Chapter II). Exceedance rates are significantly higher in the Scheldt river, likely attributable to the greater dilution of PFAS concentrations in the North Sea compared to the localized, elevated concentrations observed in the Scheldt river. It is important to note that the $EQS_{biota,hh}$ standard is still based on the TWI from 2008 (15 ng/kg/day), which is nearly 240 times higher than the updated TWI set in 2020 (EFSA, 2008).

The quality standards discussed thus far have primarily focused on protecting human health. Currently, only one biota quality standard considers the protection of aquatic ecosystems, specifically addressing the risk of secondary poisoning by PFOS in top predators which is the $EQS_{biota,secpois}$ of 33 $\mu\text{g PFOS/kg ww}$. To evaluate exceedance of this standard in fish specimens, the combined concentrations in muscle and liver tissues were analyzed. This approach was adopted based on the assumption that top predators consume whole fish, targeting multiple tissues rather than muscle tissue alone. However, the guidance documents do not explicitly specify how this standard should be applied or interpreted, potentially leading to confusion.

Table 6.1: Percentage of samples exceeding the current biota quality standards determined for fish. PFHxS is excluded as it was not detected in any of the fish samples analyzed.

	Maximum Contaminant Levels for Fish												EQS _{biota, hh}	EQS _{biota, secpois}
compound:	PFOS			PFOA			PFNA			Σ4PFAS			PFOS	PFOS
	cat. 1	cat.2	cat.3	cat. 1	cat.2	cat.3	cat. 1	cat.2	cat.3	cat. 1	cat.2	cat.3		
standard (µg/kg ww):	2.0	7.0	35	0.2	1.0	8.0	0.5	2.5	8.0	2.0	8.0	45	9.1	33
Chapter II: North Sea														
Mediterranean scaldfish	78%			67%			11%			89%			0%	0%
Atlantic herring	30%			80%			0%			30%			0%	0%
three-bearded rockling	0%			46%			9.0%			0%			0%	0%
whiting	3.8%			58%			0%			12%			0%	0%
surmullet	33%			100%			22%			55%			0%	11%
common sole	32%			73%			18%			41%			4.5%	28%
European plaice	6.9%			6.9%			0%			6.9%			6.9%	43%
Chapter III: Scheldt river														
Atlantic herring	60%			70%			0%			80%			0%	78%
common sole	95%			73%			86%			95%			90%	100%
European flounder	95%			18%			0%			95%			90%	100%
European seabass	80%			0%			0%			80%			80%	89%
bream	0%			0%			0%			0%			67%	-
European eel	8.3%			0%			0%			0%			92%	100%
European perch	15%			0%			0%			9.0%			55%	-
European smelt	2.1%			0%			0%			2.1%			35%	100%
pikeperch	0%			0%			0%			0%			10%	100%

Table 6.2: Percentage of samples exceeding the current biota quality standards determined for shellfish.

	Maximum Contaminant Levels for Shellfish					EQS _{biota, hh}	EQS _{biota, secpois}
compound:	PFOS	PFOA	PFNA	PFHxS	Σ4PFAS	PFOS	PFOS
standard (µg/kg ww)	3	0.7	1	1.5	5	9.1	33
Chapter II: North Sea							
flying crab	31%	62%	17%	-	31%	0%	0%
common shrimp	30%	50%	0%	-	10%	0%	0%
Chapter III: Scheldt river							
Chinese mitten crab	19%	25%	0%	-	10%	4.1%	0%
common shrimp	100%	63%	47%	-	94%	84%	16%
rockpool shrimp	0%	57%	0%	-	2.9%	0%	0%
Chapter IV: Scheldt river							
Asian clam	-	84%	-	-	5.5%	-	-
Chapter V: freshwater streams							
zebra mussel	6.0%	86%	2.4%	4.8%	3.6%	6.0%	6.0%

For the shrimp and crab species in chapter III, only muscle tissue was analyzed; thus, comparisons for these organisms were based solely on the concentrations detected in muscle tissue. However, the study by Groffen et al. (2024) revealed significant differences in PFAS bioaccumulation across various tissues of Chinese mitten crabs, including muscle tissue, hepatopancreas, and carapace. These variations could result in differing exceedance levels depending on whether all tissues are considered collectively or individually. This highlights the need for clearer guidance on the interpretation and application of biota quality standards, particularly in relation to tissue-specific analyses. The exceedance results indicate that fish sampled from the Scheldt river present a particularly high risk for secondary poisoning of top predators, with exceedance percentages ranging from 78% to 100%. Keeping in mind that this estimated risk is solely based on the risk exposed by PFOS.

The detection of numerous compounds throughout the chapters of this thesis is noteworthy, particularly given the limited availability of EQS for other compounds except for PFOS. This abundance of distinct PFAS poses a significant challenge for regulators in developing and implementing environmental or biota quality standards for such a diverse group of chemicals. To address this issue, the draft EQS dossier, prepared by the Joint Research Centre (JRC) and reviewed by the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER), introduced a novel approach involving the Relative Potency Factor (RPF) and PFOA equivalents (SCHEER, 2022). The PFOA equivalents approach provides a framework for evaluating the combined toxicity of various PFAS by expressing their effects relative to PFOA, a well-researched PFAS (Bil et al., 2023). This method calculates the total PFAS concentration in a

sample as an equivalent amount of PFOA, enabling a standardized assessment of PFAS mixtures. In parallel, the RPF approach offers a method for quantifying the toxicity of individual PFAS relative to a reference substance, typically PFOA. Each PFAS is assigned a RPF value based on its relative toxicity compared to PFOA. For example, a PFAS with half the toxicity of PFOA would have an RPF value of 0.5. By multiplying the concentration of each PFAS by its respective RPF and summing the results, the overall toxicity of a PFAS mixture can be estimated (Bil et al., 2021). This approach enables a more comprehensive and refined risk assessment by accounting for the varying toxicities of individual PFAS within complex mixtures. Based on this methodology, a new quality standard for the protection of human health ($QS_{\text{biota, hh}}$) was proposed for the sum of PFAS concentrations (expressed as PFOA equivalents) measured in fish, with a value of $0.077 \mu\text{g/kg}$. Additionally, using the PFOA-equivalent approach, the draft EQS dossier also discussed new quality standards for secondary poisoning, with a specific focus on fish and mollusks. A distinction was made between the marine environment ($QS_{\text{biota, secpois, sw}}$) and freshwater environments ($QS_{\text{biota, secpois, fw}}$) as detailed in Table 6.3. However, a notable limitation of this approach is that the RPF values are derived primarily from toxicity experiments conducted in rats, specifically focusing on liver toxicity (Bil et al., 2021). When considering other endpoints, such as immune toxicity, the RPF values, and consequently the relative potency of certain compounds compared to PFOA, may differ significantly. This variability highlights the potential for inconsistencies in the assessment of toxicity across different biological effects and underscores the need for further research to refine and expand the applicability of RPF values. Although these standards have not yet been officially implemented, the percentage of exceedance for the organisms studied in this thesis relative to these proposed standards has been calculated and is presented in Table 6.3. Since fish from chapter III were not only sampled in freshwater environment but also in brackish waters, a comparison with the QS for marine environment was also conducted. For the assessment of secondary poisoning, the sum of PFAS concentrations in muscle and liver tissues was used.

Notably, the results in Table 6.3 demonstrate a high level of exceedance across all standards and organisms studied, underscoring the significant risks associated with both human consumption and the secondary poisoning of top predators. The establishment of standards at such low thresholds, which are likely to be exceeded on a large scale, carries the risk of being disregarded by governments and environmental institutions. This is due to the potential for the extent of non-compliance to appear overwhelming and unmanageable, leading to regulatory

inaction. However, it is important to recognize that these newly derived standards are based on the latest toxicity research and represent the first comprehensive regulatory framework encompassing a broad range of PFAS compounds. This raises a critical question: are these proposed quality standards excessively stringent, or does the extent of PFAS pollution in aquatic ecosystems across Flanders, Belgium indicate a severe and large-scale environmental crisis?

Table 6.3: Percentage of samples exceeding the newly proposed Quality Standards (QS) for fish and mollusks.

	QS _{biota,hh}	QS _{biota, secpois, sw}		QS _{biota, secpois, fw}	
compound:	ΣPFOA eq.	ΣPFOA eq.		ΣPFOA eq.	
Target organism:	fish	fish	mollusks	fish	mollusks
standard (µg/kg ww):	0.077	6.99	2.0	22.3	6.2
Chapter II: North sea					
Mediterranean scaldfish	100%	100%			
Atlantic herring	100%	56%			
three-bearded rockling	100%	64%			
Whiting	100%	100%			
Surmullet	100%	100%			
common sole	100%	95%			
European plaice	100%	100%			
Chapter III: Scheldt river					
Atlantic herring	100%	100%		100%	
common sole	100%	100%		100%	
European flounder	100%	100%		100%	
European seabass	100%	100%		100%	
breem	100%				
European eel	100%	100%		100%	
European perch	100%				
European smelt	100%	100%		100%	
Pikeperch	100%	100%		100%	
Chapter IV: Scheldt river					
Asian clam		91%		58%	
Chapter V: freshwater streams					
zebra mussel				96%	

Overall, the regulation of PFAS within the European Union is currently fragmented and lacks coherence across food safety, water quality, and environmental standards. This regulatory misalignment necessitates urgent harmonization to ensure consistency, scientific credibility, and effective risk management. A key example of this inconsistency is the proposed

environmental quality standard for biota ($EQS_{\text{biota,hh}} = 9.1 \mu\text{g/kg ww}$), which establishes a limit for PFOS in fish intended to protect human health. However, this threshold is incongruent with the Maximum Contaminant Levels (MLs) for PFOS in fish under food safety regulations, which allow concentrations ranging from 2 to 35 $\mu\text{g/kg ww}$ (depending on the fish category). This discrepancy means that fish from the same waterbody could be deemed unsafe under one regulation while remaining legally marketable for human consumption under another, highlighting a fundamental regulatory contradiction. Furthermore, regulatory thresholds differ in how they classify and group PFAS, making cross-comparisons difficult. Some standards apply to individual PFAS such as PFOS (e.g., EQS_{biota} , $EQS_{\text{surface water}}$), while others regulate the sum of specific PFAS groups—such as the sum of four PFAS (PFOS, PFOA, PFNA, PFHxS) under the EFSA TWI or the sum of 20 selected PFAS (ΣPFAS_{20}) under the drinking water quality standard. These varying grouping methodologies result in inconsistent exposure assessments, where contamination flagged under one regulation may not be recognized under another, even in identical exposure scenarios.

Additionally, the scientific robustness of key PFAS guidelines, such as the EFSA TWI, remains questionable, despite its widespread use as a reference point for implementing regulatory limits across Europe. The EFSA TWI is derived from a single epidemiological study that identified an association between PFOA exposure in breastfed infants and reduced vaccine antibody response (Abraham et al., 2020). However, no such association was observed for PFOS, PFNA, or PFHxS, yet these substances were included in the TWI calculation without sufficient scientific justification. Moreover, other well-documented toxicological endpoints, such as liver toxicity, lipid metabolism disruption, and cholesterol dysregulation, were excluded from the TWI derivation, despite having stronger and more consistent toxicological evidence (Reinikainen et al., 2024). Given these limitations, the EFSA TWI lacks a sufficiently rigorous scientific foundation and may not serve as an appropriate reference value for establishing PFAS regulatory limits.

Despite the extensive focus on human health and top predators, current EU PFAS regulations fail to explicitly address the protection of entire ecosystems or the preservation of ecological integrity. The methodology outlined in Chapter V, which proposes deriving threshold body burdens in aquatic invertebrates to prevent ecological damage, presents a promising approach for addressing this regulatory gap. A regulatory focus solely on human health and higher trophic

levels ignores the interconnected nature of ecosystems, where contamination at lower levels of the food chain can cascade into widespread ecological disruption.

The current inconsistencies in PFAS regulatory thresholds create regulatory confusion and hinder enforcement across sectors. A harmonized, science-based approach is imperative to ensure that PFAS limits across different exposure pathways are consistent, transparent, and grounded in robust scientific evidence.

In my opinion, a critical starting point for achieving harmonization is revising the approach to setting EQS. At present, EQS are primarily based on human health concerns, despite the fact that these risks are already regulated under drinking water standards and maximum contaminant levels in food. A more effective approach would be to base all EQS limits on ecological risks rather than human health risks, aligning with the original intent of EQS in protecting ecosystems. Moreover, the interconnection between human health and environmental health requires greater recognition in regulatory frameworks. For instance, quality standards for surface and groundwater should be consistent with drinking water standards, given that drinking water is sourced directly from these water bodies. Aligning these thresholds would eliminate contradictions, improve regulatory coherence, and ensure a comprehensive, science-based approach to managing PFAS contamination.

6.4. PFAS Pollution, a Multifaceted Issue

Throughout the course of this thesis, it has become increasingly evident that PFAS pollution is not just an isolated environmental problem, but a highly complex, multidimensional challenge involving a wide range of stakeholders with diverse and often conflicting interests. These stakeholders encompass governmental institutions, PFAS-producing and utilizing industries, environmental agencies, scientists, media and the general public, all of whom play a crucial role in addressing PFAS contamination. From the perspective and experience of a scientist working on PFAS, Figure 6.1 illustrates the complex interplay between researchers and key stakeholders involved in PFAS management.

Researchers play a crucial role in advancing knowledge to better understand the extent of the problem. This is achieved through studying the effects and distribution of PFAS, as well as exploring potential solutions by developing and evaluating mitigation technologies.

Consequently, researchers must adopt a collaborative role with environmental agencies to provide a robust scientific foundation for the development of monitoring programs and the enforcement of environmental standards. From this perspective, the current work offers valuable insights into the various monitoring strategies applied in this thesis. Environmental agencies, in turn, could contribute by providing large monitoring datasets on the ecological water quality and PFAS concentrations in water bodies across Flanders. Such an approach could strengthen the estimation of threshold values and serve as a significant step toward implementing thresholds that effectively protect aquatic systems at community level.

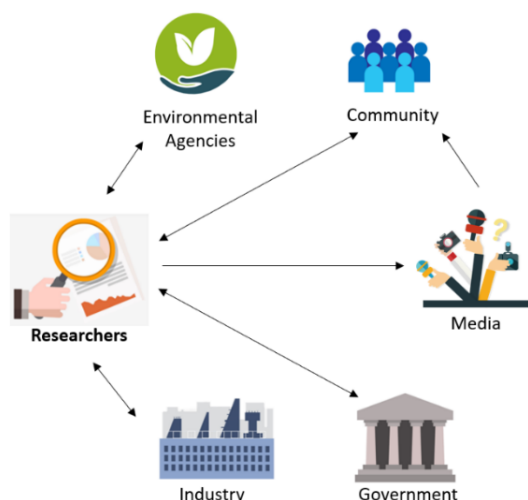


Figure 6.1: Map of interrelationships between researchers and the various stakeholders involved in the PFAS pollution problem (icons sourced from www.pngtree.com).

Furthermore, the collaboration between researchers and industry plays a crucial role in bridging the gap between fundamental science and practical application. Academic research can drive the optimization of existing technologies or the development of innovative processes, with a focus on promoting environmental sustainability and ensuring compliance with regulatory standards. In turn, industries can contribute valuable data essential for conducting such research. This relationship fosters the translation of scientific advancements into practical solutions, benefiting both environmental outcomes and industrial practices. An example of this collaboration can be observed in PFAS research conducted in Belgium, where the fluorochemical company 3M, located in Antwerp, has allowed research on PFAS contamination in songbirds within its industrial site (Groffen et al., 2017 and 2019a).

Researchers primarily rely on government funding, which supports research projects through grants and fellowships. In return, researchers provide governments with critical scientific evidence on PFAS toxicity, persistence, and bioaccumulation, as well as identifying sources, pathways, and contamination hotspots. This knowledge enables governments to prioritize interventions effectively. The scientific foundation provided by researchers is essential for the development and implementation of science-based regulations, comprehensive risk assessments, and informed policy decisions.

Lastly, researchers maintain a relationship with the community, either directly or indirectly through the media. Researchers play a crucial role in helping communities understand and address PFAS-related risks by translating complex scientific concepts into accessible language. The media serves as a key intermediary, disseminating this knowledge to minimize public concerns while ensuring transparency about existing uncertainties. A psychological study conducted in the Veneto region of Italy, a heavily PFAS-contaminated area, reported that PFAS pollution has significant psychosocial impacts on affected communities (Menegatto et al., 2022). In particular, uncertainty about potential health effects emerged as a key contributor to stress. These findings underscore the importance of effective science communication to build trust and address community concerns. Communities, in turn, can provide researchers with valuable local knowledge, which can inform the design and prioritization of studies that align with their concerns. Additionally, citizen science initiatives engage communities by involving them in the monitoring of PFAS contamination, such as collecting water or soil samples for analysis. This participation empowers communities and fosters a sense of ownership in tackling the issue. Furthermore, community members can directly contribute to scientific studies, for instance, by donating blood samples to assess PFAS exposure levels. A notable example of such collaboration is a monitoring study in which PFAS concentrations were analyzed in blood samples from adolescents living near the contaminated region around 3M (UAntwerpen et al., 2023). The findings of this study were shared through detailed reports and explained in several informational events, showcasing effective collaboration between researchers and the community. These efforts highlight the importance of transparent communication and active community involvement in addressing PFAS pollution.

The relationship between researchers and stakeholders involved in the PFAS problem is a complex but essential collaboration that drives progress in addressing this environmental and

public health issue. Sustained collaboration among all parties is critical to addressing the multifaceted challenges posed by PFAS contamination.

6.5. Conclusive Remarks and Future Perspectives

Environmental pollution with micropollutants such as PFAS is a challenging environmental issue to tackle, partly because their presence is invisible in the environment. Unlike macroplastic pollution, which is easily seen piling up in waterways, PFAS contamination remains undetectable to the naked eye, making it much harder to grasp the scale of the problem. These persistent chemicals can accumulate silently in ecosystems, with their effects unfolding gradually over time. Furthermore, the ecological consequences of PFAS pollution are far more difficult to visualize compared to immediate and distressing impacts such as marine organisms entangled in debris. From my own experience, this invisibility makes raising public awareness significantly more challenging, and as a result, the urgency to take action is often diminished.

Therefore, following the motto "to measure is to know", this research provides crucial scientific evidence of the widespread presence and bioaccumulation of PFAS in aquatic ecosystems. By uncovering this contamination, it highlights both human health risks and ecological effects while also exposing shortcomings in the current regulatory framework. As a result, this work serves as an important scientific baseline for improving future monitoring strategies and addressing regulatory challenges to safeguard both environmental and public health for generations to come. However, this research also acknowledges certain limitations and identifies critical knowledge gaps that require further investigation. Addressing these gaps will be essential to developing more effective regulations and mitigation strategies in the future.

In Chapter II, the trophic range of the studied species was found to be too limited to draw solid conclusions about the trophic transfer of PFAS in the North Sea food web. As a result, three key recommendations were outlined to guide future research on PFAS transfer in aquatic food webs. First, we recommend expanding the trophic sampling range to encompass at least two trophic levels to achieve a more comprehensive understanding of predator-prey dynamics and PFAS transfer within the food web (Borgå et al., 2012). Second, it is crucial to evaluate the confounding effects of biotransformation, particularly in higher trophic levels. This can be achieved through the implementation of advanced analytical techniques, such as the Total

Oxidizable Precursor (TOP) assay, as discussed in Section 6.2. Third, environmental characteristics of the sampled area must be considered, as abiotic factors (e.g., pH, conductivity, dissolved organic carbon, and oxygen levels) can significantly influence PFAS distribution and bioavailability (Ohoro et al., 2024). In addition to these recommendations, it is essential to emphasize that the choice of analyzed body tissues plays a critical role in data interpretation, as PFAS accumulate differentially in various tissues, which may affect the assessment of trophic transfer (Miranda et al., 2022). Therefore, the use of whole-body concentrations is strongly encouraged to minimize discrepancies between predator and prey PFAS burdens and to enhance consistency across studies.

Stable isotope analysis has proven to be a particularly valuable tool, not only for studying the trophic transfer of PFAS, but also for assessing the influence of ecological characteristics such as habitat use and feeding preferences. This approach, which was particularly insightful in Chapter III, provides an additional dimension to understanding the ecological dynamics that influence PFAS bioaccumulation. Notably, the inclusion of sulfur stable isotopes in chapter III yielded additional and significant insights, complementing the more commonly analyzed carbon and nitrogen stable isotopes. Given these findings, future studies are strongly recommended to incorporate all three stable isotopes (C, N, and S) rather than solely focusing on C and N, as has been the predominant approach in research to date. This expanded isotopic framework could improve interpretations of PFAS bioaccumulation patterns in aquatic food webs.

Furthermore, the results presented in Chapters II and III demonstrated the extensive bioaccumulation of PFAS across a wide range of fish and invertebrate species, raising a critical question: How will this exposure impact the long-term population dynamics of these organisms? To address these concerns, it would be valuable to integrate PFAS monitoring with population dynamics assessments in affected species. Additionally, investigating the population genetics of species from heavily polluted sites and comparing them with those from reference (less polluted) sites could provide crucial insights into the long-term ecological and evolutionary effects of PFAS exposure. Such an approach could help determine whether chronic contamination leads to genetic adaptations, population declines, or shifts in species resilience, ultimately shaping the structure and stability of aquatic ecosystems.

The scope of the studies conducted in Chapter III and Chapter IV did not account for physicochemical parameters, despite their likely influence on PFAS bioaccumulation. The Scheldt River is an interesting system for such research, as parameters are expected to vary significantly along its course. In Chapter III, parameters such as temperature, pH, salinity, oxygen, and turbidity were measured only twice at the sampling sites and were used in a descriptive manner to support and explain the observed trends. For future studies aiming to quantify the impact of these parameters on PFAS bioaccumulation, obtaining a more comprehensive dataset is recommended to enable robust statistical analyses, such as multivariate statistics. To achieve this, it would be interesting to incorporate continuous monitoring data from environmental sensors that are placed at multiple locations along the Scheldt River (www.scheldemonitor.be). These sensors would allow for high-frequency measurements of parameters such as temperature, oxygen levels, conductivity, and salinity, providing a detailed and temporally resolved dataset. Thereby, translocating Asian clams in proximity to the sensors would provide valuable insights into PFAS bioaccumulation under real-time environmental conditions.

In Chapter IV, the discrepancies between PFAS profiles in biotic and abiotic environments became evident, emphasizing the continued need for biomonitoring. Across the chapters, the variations in PFAS bioaccumulation among species were clearly demonstrated, highlighting the critical importance of standardizing biota monitoring practices. A key challenge of passive biomonitoring is the difficulty of sampling the same target species across different sites. When different monitoring species are used, variations in PFAS profiles can arise due to e.g. differences in feeding ecology and associated exposure routes, complicating cross-site comparisons. As discussed in Section 6.1, active biomonitoring has demonstrated considerable potential in overcoming existing limitations and offers significant promise for future PFAS monitoring in aquatic environments. In this context, zebra mussels (Chapter V) have proven particularly suitable for freshwater exposure studies, while Asian clams (Chapter IV) have shown applicability in dynamic systems with salinity gradients, such as the Scheldt River. To effectively integrate these species into long-term PFAS monitoring programs, further research is needed to determine the optimal exposure duration for PFAS bioaccumulation in both zebra mussels and Asian clams. The current exposure period of six weeks was based on the study by Bervoets et al. (2004), which found that bioaccumulation of certain contaminants reaches equilibrium after this period. However, PFAS were not included in that study, and given their

unique bioaccumulation behavior, which differs significantly from many other pollutants, it remains uncertain whether this exposure duration is appropriate for PFAS biomonitoring. Further investigation is therefore required to ensure accurate and representative PFAS accumulation assessments in these biomonitoring species.

Chapter V provides novel insights into the effects of PFAS bioaccumulation on macroinvertebrate community dynamics and represents the first study to establish threshold body burdens in aquatic invertebrates that are protective against ecological damage. To validate these threshold values and strengthen their applicability, expanding the dataset is essential. Additionally, mesocosm studies offer a promising approach to bridge the gap between highly controlled laboratory experiments and the complexities of field studies. Mesocosms simulate natural environmental conditions while allowing for precise control over PFAS concentrations and key environmental variables (e.g., pH, temperature, etc.) across treatments. These systems enable researchers to assess macroinvertebrate community composition, population dynamics, and even potential transgenerational effects of PFAS exposure (Barmantlo et al., 2018; Grantham et al., 2012).

Furthermore, during the field campaign described in Chapter V, also water and sediment samples were collected, and PFAS concentrations were analyzed alongside physicochemical water parameters at each sampling location. The future objective is to integrate these additional datasets with the data from Chapter V to address the following key research goals: (1) examine the relationship between PFAS concentrations in biotic and abiotic compartments, (2) estimate sediment threshold values that are protective against ecological damage and (3) investigate the influence of physicochemical parameters on the distribution and bioaccumulation of PFAS in water, sediment, and biota, as well as their relationship with the MMIF.

To conclude this work, I would like to reflect on the first time I conducted PFAS extractions on samples from the North Sea. At that moment, I questioned whether I would even detect any PFAS, and if I didn't, where would my research lead? Now, after five years of research, that uncertainty is long gone. Detecting PFAS is no longer a question, instead, my focus has shifted to a far more pressing question: which PFAS will be detected, and in what concentrations? The real challenge is no longer if PFAS are there, but rather what we do with the knowledge of their pervasive presence. The widespread bioaccumulation of PFAS across the diverse aquatic

ecosystems and organisms studied in this work is deeply concerning, presenting a challenge that may seem overwhelming and beyond control. However, I firmly believe that advancing scientific knowledge and maintaining rigorous, long-term monitoring are critical imperatives for driving the implementation of science-based policies. These efforts are essential to establishing a comprehensive, sustainable, and enforceable regulatory framework capable of effectively mitigating and ultimately controlling PFAS pollution at a global scale.

Bibliography

A

- Abercrombie, S. A., de Perre, C., Iacchetta, M., Flynn, R. W., Sepúlveda, M. S., Lee, L. S., & Hoverman, J. T. (2021). Sublethal effects of eermal exposure to poly- and perfluoroalkyl substances on postmetamorphic amphibians. *Environmental Toxicology and Chemistry*, 40(3), 717–726. <https://doi.org/10.1002/ETC.4711>
- Abraham, K., Mielke, H., Fromme, H., Völkel, W., Menzel, J., Peiser, M., Zepp, F., Willich, S. N., & Weikert, C. (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. *Archives of Toxicology*, 94(6), 2131–2147. <https://doi.org/10.1007/s00204-020-02715-4>
- Ahrens, L., & Bundschuh, M. (2014). Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. *Environmental Toxicology and Chemistry*, 33(9), 1921–1929. <https://doi.org/10.1002/etc.2663>
- Ahrens, L., Gashaw, H., Sjöholm, M., Gebrehiwot, S. G., Getahun, A., Derbe, E., Bishop, K., & Åkerblom, S. (2016). Poly- and perfluoroalkylated substances (PFASs) in water, sediment and fish muscle tissue from Lake Tana, Ethiopia and implications for human exposure. *Chemosphere*, 165, 352–357. <https://doi.org/10.1016/j.chemosphere.2016.09.007>
- Ahrens, L., Rakovic, J., Ekdahl, S., & Kallenborn, R. (2023). Environmental distribution of per- and polyfluoroalkyl substances (PFAS) on Svalbard: Local sources and long-range transport to the Arctic. *Chemosphere*, 345. <https://doi.org/10.1016/j.chemosphere.2023.140463>
- Ahrens, L., Siebert, U., & Ebinghaus, R., (2009). Temporal trends of polyfluoroalkyl compounds in harbor seals (*Phoca vitulina*) from the German Bight, 1999–2008. *Chemosphere* 76, 151–158. <https://doi.org/10.1016/j.chemosphere.2009.03.053>
- Alves, A. V., Tsianou, M., & Alexandridis, P. (2020). Fluorinated Surfactant Adsorption on Mineral Surfaces: Implications for PFAS Fate and Transport in the Environment. *Surfaces*, 3 (4),516–566. <https://doi.org/10.3390/SURFACES3040037>
- Amin, M.A., Luo, y., Nolan, A., Robinson, F., Niu, J., Warner, S., Liu, Y., Dharmarajan, R., Mallaarapu, M., Naidu, R., & Fang, C. (2021). Total oxidisable precursor assay towards selective detection of PFAS in AFFF. *Journal of Cleaner Production*, 328, 129568. <https://doi.org/10.1016/j.jclepro.2021.129568>
- Amraoui, I., Khalloufi, N., & Touaylia, S. (2018). Effects to perfluorooctane sulfonate (PFOS) on the mollusk *Unio ravoisieri* under laboratory exposure. *Chemistry and Ecology*, 34(4), 324–339. <https://doi.org/10.1080/02757540.2018.1433168>
- Anderson, R. H., Long, G. C., Porter, R. C., & Anderson, J. K. (2018). Occurrence of select perfluoroalkyl substances at US Air Force aqueous film-forming foam release sites other than fire training areas: Field validation of critical fate and transport properties. In S. C. Halogen & C. J. Belcher (Eds.), *Perfluoroalkyl substances in the environment* (pp. 353–372). CRC Press. <https://doi.org/10.1201/9780429487125-15>
- Anderson, R., Adamson, D. T., & Stroo, H. F. (2019). Partitioning of poly- and perfluoroalkyl substances from soil to groundwater within aqueous film-forming foam source zones. *Journal of Contaminant Hydrology*, 220, 59–65. <https://doi.org/10.1016/J.JCONHYD.2018.11.011>
- Andral, B., Stanisiere, J. Y., Sauzade, D., Damier, E., Thebault, H., Galgani, F., & Boissery, P. (2004). Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging.

Marine Pollution Bulletin, 49(9), 704–712.
<https://doi.org/https://doi.org/10.1016/j.marpolbul.2004.05.008>

Ankley, G. T., Cureton P., Hoke R. A., Houde M., Kumar A., Kurias J., Lanno R., McCarthy C., Newsted J., Salice C. J., Sample B. E., Sepúlveda M. S., Steevens J., & Valsecchi S. (2021). Assessing the ecological risks of per- and polyfluoroalkyl substances: Current state-of-the Science and a proposed path forward. *Environmental Toxicology and Chemistry*, 40(3), 564-605. <https://doi.org/10.1002/etc.4869>

Ankley, G. T., Kuehl, D. W., Kahl, M. D., Jensen, K. M., Linnum, A., Leino, R. L., & Villeneuve, D. A. (2005). Reproductive and developmental toxicity and bioconcentration of perfluorooctanesulfonate in a partial life-cycle test with the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry*, 24(9), 2316–2324. <https://doi.org/10.1897/04-634R.1>

Armitage, J. M., Macleod, M., & Cousins, I. T. (2009). Comparative assessment of the global fate and transport pathways of long-chain perfluorocarboxylic acids (PFCAs) and perfluorocarboxylates (PFCs) emitted from direct sources. *Environmental Science & Technology*, 43(15), 5830–5836. <https://doi.org/10.1021/ES900753Y>

Arnoudt, R. (2021). Chemiebedrijf 3M geeft nu zelf aan dat het de mogelijk giftige stof FBSA in de Schelde loost. <https://vrtnws.be/p.3BkxWNZVE>

Ateia, M., Chiang, D., Cashman, M., & Acheson, C. (2023). Total Oxidizable Precursor (TOP) Assay–Best Practices, Capabilities and Limitations for PFAS Site Investigation and Remediation. *Environmental Science & Technology Letters*, 10(4), 292–301. <https://doi.org/10.1021/acs.estlett.3c00061>

Ateia, M., Maroli, A., Tharayil, N., & Karanfil, T. (2019). The overlooked short- and ultrashort-chain poly- and perfluorinated substances: A review. *Chemosphere*, 220, 866–882. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2018.12.186>

ATSDR (2021). Toxicological Profile for Perfluoroalkyls. <https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf> (accessed 24 Januari 2022)

Augustsson, A., Lennqvist, T., Osbeck, C. M. G., Tibblin, P., Glynn, A., Nguyen, M. A., Westberg, E., & Vestergren, R. (2021). Consumption of freshwater fish: A variable but significant risk factor for PFOS exposure. *Environmental Research*, 192, 110284. <https://doi.org/10.1016/j.envres.2020.110284>

B

Baabish, A., Sobhane, S., & Fiedler, H. (2021). Priority perfluoroalkyl substances in surface waters - A snapshot survey from 22 developing countries. *Chemosphere*, 273, 129612. <https://doi.org/10.1016/j.chemosphere.2021.129612>

Babut, M., Labadie, P., Simonnet-Laprade, C., Munoz, G., Roger, M. C., Ferrari, B. J. D., Budzinski, H., & Sivade, E. (2017). Per- and poly-fluoroalkyl compounds in freshwater fish from the Rhône River: Influence of fish size, diet, prey contamination and biotransformation. *Science of The Total Environment*, 605–606, 38–47. <https://doi.org/10.1016/J.SCITOTENV.2017.06.111>

Bachiller, E., Skaret, G., Nøttestad, L., & Slotte, A. (2016). Feeding ecology of Northeast Atlantic Mackerel, Norwegian spring-spawning herring and blue whiting in the Norwegian Sea. *PLOS ONE*, 11(2). <https://doi.org/10.1371/journal.pone.0149238>

- Baeyens, W., Van Eck, B., Lambert, C., Wollast, R., & Goeyens, L. (1997). General description of the Scheldt estuary. *Hydrobiologia*, 366(1–3), 1–14. https://doi.org/10.1007/978-94-017-3573-5_1
- Bai, X., & Son, Y. (2021). Perfluoroalkyl substances (PFAS) in surface water and sediments from two urban watersheds in Nevada, USA. *Science of The Total Environment*, 751, 141622. <https://doi.org/10.1016/J.SCITOTENV.2020.141622>
- Bakke, T., Källqvist, T., Ruus, A., Breedveld, G. D., & Hylland, K. (2010). Development of sediment quality criteria in Norway. *Journal of Soils and Sediments*, 10(2), 172–178. <https://doi.org/10.1007/S11368-009-0173-Y/TABLES/3>
- Bangma, J., Guillelte, T. C., Bommarito, P. A., Ng, C., Reiner, J. L., Lindstrom, A. B., & Strynar, M. J. (2022). Understanding the dynamics of physiological changes, protein expression, and PFAS in wildlife. *Environment International*, 159, 107037. <https://doi.org/https://doi.org/10.1016/j.envint.2021.107037>
- Bangma, J., Reiner, J. L., Lowers, R. H., Cantu, T. M., Scott, J., Korte, J. E., Scheidt, D. M., McDonough, C., Tucker, J., Back, B., Adams, D. H., & Bowden, J. A. (2018). Perfluorinated alkyl acids and fecundity assessment in striped mullet (*Mugil cephalus*) at Merritt Island national wildlife refuge. *Science of The Total Environment*, 619–620, 740–747. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.11.141>
- Barisci, S., & Suri, R. (2021). Occurrence and removal of poly/perfluoroalkyl substances (PFAS) in municipal and industrial wastewater treatment plants. *Water Science & Technology*, 84, 3442. <https://doi.org/10.2166/wst.2021.484>
- Barmantlo, S. H., Schrama, M., Hunting, E. R., Heutink, R., van Bodegom, P. M., de Snoo, G. R., & Vijver, M. G. (2018). Assessing combined impacts of agrochemicals: Aquatic macroinvertebrate population responses in outdoor mesocosms. *Science of The Total Environment*, 631–632, 341–347. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.03.021>
- Barzen-Hanson, K. A., Roberts, S. C., Choyke, S., Oetjen, K., McAlees, A., Riddell, N., McCrindle, R., Ferguson, P. L., Higgins, C. P., & Field, J. A. (2017). Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater. *Environmental Science and Technology*, 51(4), 2047–2057. https://doi.org/10.1021/ACS.EST.6B05843/SUPPL_FILE/ES6B05843_SI_002.XLSX
- Basset, A., Sangiorgio, F., & Pinna, M. (2004). Monitoring with benthic macroinvertebrates: advantages and disadvantages of body size descriptors. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 14(S1), S43–S58. <https://doi.org/10.1002/AQC.649>
- Beaulieu, M., & Costantini, D. (2014). Biomarkers of oxidative status: Missing tools in conservation physiology. *Conservation Physiology*, 2(1), cou014. <https://doi.org/10.1093/conphys/cou014>
- Benito, D., Briand, M., Herlory, O., Izagirre, U., Bouchoucha, M., & Briaudeau, T. (2024). Active mussel biomonitoring for the health status assessment of the Western Mediterranean Sea. *Marine Pollution Bulletin*, 207, 116898. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2024.116898>
- Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/https://doi.org/10.1006/abio.1996.0292>
- Berger, E., Haase, P., Schäfer, R. B., & Sundermann, A. (2018). Towards stressor-specific macroinvertebrate indices: Which traits and taxonomic groups are associated with vulnerable and

tolerant taxa? Science of The Total Environment, 619–620, 144–154. <https://doi.org/10.1016/J.SCITOTENV.2017.11.022>

Berger, U., Glynn, A., Holmström, K. E., Berglund, M., Ankarberg, E. H., & Törnkvist, A. (2009). Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden - Analysis of edible fish from Lake Vättern and the Baltic Sea. Chemosphere, 76(6), 799–804. <https://doi.org/10.1016/j.chemosphere.2009.04.044>

Bertin, D., Ferrari, B. J. D., Labadie, P., Sapin, A., Garric, J., Budzinski, H., Houde, M., & Babut, M. (2014). Bioaccumulation of perfluoroalkyl compounds in midge (*Chironomus riparius*) larvae exposed to sediment. Environmental Pollution, 189, 27–34. <https://doi.org/10.1016/J.ENVPOL.2014.02.018>

Bertin, D., Labadie, P., Ferrari, B. J. D., Sapin, A., Garric, J., Geffard, O., Budzinski, H., & Babut, M. (2016). Potential exposure routes and accumulation kinetics for poly- and perfluorinated alkyl compounds for a freshwater amphipod: *Gammarus* spp. (Crustacea). Chemosphere, 155, 380–387. <https://doi.org/10.1016/J.CHEMOSPHERE.2016.04.006>

Bertrand, L., Monferrán, M. V., Mouneyrac, C., & Amé, M. V. (2018). Native crustacean species as a bioindicator of freshwater ecosystem pollution: A multivariate and integrative study of multi-biomarker response in active river monitoring. Chemosphere, 206, 265–277. <https://doi.org/10.1016/J.CHEMOSPHERE.2018.05.002>

Bervoets, L., De Jonge, M., & Blust, R. (2016). Identification of threshold body burdens of metals for the protection of the aquatic ecological status using two benthic invertebrates. Environmental Pollution, 210, 76–84. <https://doi.org/10.1016/J.ENVPOL.2015.12.005>

Bervoets, L., Voets, J., Chu, S., Covaci, A., Schepens, P., & Blust, R. (2004). Comparison of accumulation of micropollutants between indigenous and transplanted zebra mussels (*Dreissena polymorpha*). Environmental Toxicology and Chemistry, 23(8), 1973–1983. <https://doi.org/10.1897/03-365>

Bervoets, L., Voets, J., Covaci, A., Chu, S., Qadah, D., Smolders, R., Schepens, P., & Blust, R. (2005a). Use of transplanted zebra mussels (*Dreissena polymorpha*) to assess the bioavailability of microcontaminants in Flemish surface waters. Environmental Science and Technology, 39(6), 1492–1505. <https://doi.org/10.1021/ES049048T/ASSET/IMAGES/LARGE/ES049048TF00003.JPEG>

Bervoets, L., Voets, J., Smolders, R., & Blust, R. (2005b). Metal accumulation and condition of transplanted zebra mussel (*Dreissena polymorpha*) in metal polluted rivers. Aquatic Ecosystem Health & Management, 8(4), 451–460. <https://doi.org/10.1080/14634980500360100>

Besse, J. P., Geffard, O., & Coquery, M. (2012). Relevance and applicability of active biomonitoring in continental waters under the Water Framework Directive. Trends in Analytical Chemistry, 36, 113–127. <https://doi.org/10.1016/J.TRAC.2012.04.004>

Bil, W., Govarts, E., Zeilmaker, M. J., Woutersen, M., Bessems, J., Ma, Y., Thomsen, C., Haug, L. S., Lignell, S., Gyllenhammar, I., Palkovicova Murinova, L., Fabelova, L., Tratnik, J. S., Kosjek, T., Gabriel, C., Sarigiannis, D., Pedraza-Díaz, S., Esteban-López, M., Castaño, A., ...& Vinggaard, A. M. (2023). Approaches to mixture risk assessment of PFASs in the European population based on human hazard and biomonitoring data. International Journal of Hygiene and Environmental Health, 247, 114071. <https://doi.org/https://doi.org/10.1016/j.ijheh.2022.114071>

Bil, W., Zeilmaker, M., Fragki, S., Lijzen, J., Verbruggen, E., & Bokkers, B. (2021). Risk Assessment of Per- and Polyfluoroalkyl Substance Mixtures: A Relative Potency Factor Approach. Environmental Toxicology and Chemistry, 40(3), 859–870. <https://doi.org/https://doi.org/10.1002/etc.4835>

- Blasco, J., Chapman, P.M., Campana, O., Hampel, M. (2016). *Marine Ecotoxicology: Current Knowledge and Future Issues*, Elsevier.
- Blaxter, J.H.S., & Parrish, B.B. (1965). The importance of light in shoaling, avoidance of nets and vertical migration by herring. *J. Cons. Int. Explor. Mer.* 30, 40–57. <https://doi.org/10.1093/icesjms/30.1.40>
- Bódis, M., Kucska, B., & Bercsényi, M. (2007). The effect of different diets on the growth and mortality of juvenile pikeperch (*Sander lucioperca*) in the transition from live food to formulated feed. *Aquaculture International*, 15(1), 83–90. <https://doi.org/10.1007/s10499-006-9063-0>
- Borgå, K., Kidd, K. A., Muir, D. C. G., Berglund, O., Conder, J. M., Gobas, F. A. P. C., Kucklick, J., Malm, O., & Powell, D. E. (2012). Trophic magnification factors: Considerations of ecology, ecosystems, and study design. *Integrated Environmental Assessment and Management*, 8(1), 64–84. <https://doi.org/10.1002/ieam.244>
- Borthakur, A., Wang, M., He, M., Ascencio, K., Blotevogel, J., Adamson, D. T., Mahendra, S., & Mohanty, S. K. (2021). Perfluoroalkyl acids on suspended particles: Significant transport pathways in surface runoff, surface waters, and subsurface soils. *Journal of Hazardous Materials*, 417, 126159. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2021.126159>
- Bossi, R., Strand, J., Sortkjær, O., & Larsen, M. M. (2008). Perfluoroalkyl compounds in Danish wastewater treatment plants and aquatic environments. *Environment International*, 34(4), 443–450. <https://doi.org/10.1016/J.ENVINT.2007.10.002>
- Boucher, J. M., Cousins, I. T., Scheringer, M., Hungerbühler, K., & Wang, Z. (2019). Toward a Comprehensive Global Emission Inventory of C4-C10 Perfluoroalkanesulfonic Acids (PFSA)s and Related Precursors: Focus on the Life Cycle of C6- and C10-Based Products. *Environmental Science and Technology Letters*, 6(1), 1–7. https://doi.org/10.1021/ACS.ESTLETT.8B00531/ASSET/IMAGES/LARGE/EZ-2018-00531V_0001.JPEG
- Bouchereau, J.-L., Marques, C. R., Pereira, P., Guélorget, O., & Lourie, S. M. (2009). Feeding behaviour of *Anguilla anguilla* and trophic resources in the Ingril Lagoon (Mediterranean, France). *Cahiers de Biologie Marine*, 50(4), 319–332. <https://www.researchgate.net/publication/234037838>
- Bowman, J. S. (2015). Fluorotechnology is critical to modern life: The fluorocouncil counterpoint to the madrid statement. *Environmental Health Perspectives*, 123(5), 112–113. <https://doi.org/10.1289/ehp.1509910>
- Brandsma, S.H., Koekkoek, J.C., van Velzen, M.J.M., & de Boer, J. (2019). The PFOA substitute GenX detected in the environment near a fluoropolymer manufacturing plant in the Netherlands. *Chemosphere* 220, 493–500. <https://doi.org/10.1016/j.chemosphere.2018.12.135>
- Breine J., De Bruyn A., Galle L., Lambeens I., Maes Y., Terrie T., Van Thuyne G. & Mertens W. (2021). Opvolgen van het visbestand in het Zeeschelde-estuarium. Viscampagnes met schietfuisen 2020. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2021 (3). Instituut voor Natuur- en Bosonderzoek, Brussel. doi.org/10.21436/inbor.29281830
- Brendel, S., Fetter, É., Staude, C., Vierke, L., & Biegel-Engler, A. (2018). Short-chain perfluoroalkyl acids: environmental concerns and a regulatory strategy under REACH. *Environmental Sciences Europe*, 30(1). <https://doi.org/10.1186/s12302-018-0134-4>

Brooks, S., Lyons, B., Goodsir, F., Bignell, J., & Thain, J. (2009). Biomarker Responses in Mussels, an Integrated Approach to Biological Effects Measurements. *Journal of Toxicology and Environmental Health, Part A*, 72(3–4), 196–208. <https://doi.org/10.1080/15287390802539038>

Buck, R. C., Franklin, J., Berger, U., Conder, J. M., Cousins, I. T., Voogt, P. De, Jensen, A. A., Kannan, K., Mabury, S. A., & van Leeuwen, S. P. J. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment and Management*, 7(4), 513–541. <https://doi.org/10.1002/ieam.258>

Buckler, D. R., Mayer, F. L., Ellersieck, M. R., & Asfaw, A. (2005). Acute toxicity value extrapolation with fish and aquatic invertebrates. *Archives of Environmental Contamination and Toxicology*, 49(4), 546–558. <https://doi.org/10.1007/S00244-004-0151-8/TABLES/21>

Burkhard, L. P. (2021). Evaluation of Published Bioconcentration Factor (BCF) and Bioaccumulation Factor (BAF) Data for Per- and Polyfluoroalkyl Substances Across Aquatic Species. *Environmental Toxicology and Chemistry*, 40(6), 1530–1543. <https://doi.org/10.1002/ETC.5010>

Butt, C. M., Muir, D. C. G., & Mabury, S. A. (2014). Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: A review. *Environmental Toxicology and Chemistry*, 33(2), 243–267. <https://doi.org/10.1002/ETC.2407>

Byns, C., Groffen, T., & Bervoets, L. (2024). Aquatic macroinvertebrate community responses to pollution of perfluoroalkyl substances (PFAS): Can we define threshold body burdens? *Science of the Total Environment*, 917. <https://doi.org/10.1016/j.scitotenv.2024.170611>

Byns, C., Teunen L., Groffen, T., Lasters, R., & Bervoets, L. (2022). Bioaccumulation and trophic transfer of perfluorinated alkyl substances (PFAS) in marine biota from the Belgian North Sea: Distribution and human health risk implications. *Environmental Pollution*, 311. <https://doi.org/10.1016/j.envpol.2022.119907>

C

Cai, D., Li, Q.-Q., Chu, C., Wang, S.-Z., Tang, Y.-T., Appleton, A. A., Qiu, R.-L., Yang, B.-Y., Hu, L.-W., Dong, G.-H., & Zeng, X.-W. (2020). High trans-placental transfer of perfluoroalkyl substances alternatives in the matched maternal-cord blood serum: Evidence from a birth cohort study. *Science of The Total Environment*, 705, 135885. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.135885>

Carlioni, D., (2009). Perfluorooctane Sulfonate (PFOS) Production and Use: Past and Current Evidence. United Nations Industrial Development Organization (UNIDO). 38 pages. <https://www.unido.org/researchers/publications> (assessed 10 July 2019).

Casas, G., Martínez-Varela, A., Roscales, J. L., Vila-Costa, M., Dachs, J., & Jiménez, B. (2020). Enrichment of perfluoroalkyl substances in the sea-surface microlayer and sea-spray aerosols in the Southern Ocean. *Environmental Pollution*, 267, 115512. <https://doi.org/https://doi.org/10.1016/j.envpol.2020.115512>

Casini, M., Cardinale, M., & Arrhenius, F. (2004). Feeding preferences of herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) in the southern Baltic Sea. *ICES Journal of Marine Science*, 61(8), 1267–1277. <https://doi.org/10.1016/j.icesjms.2003.12.011>

Cataldo, D. H., Boltovskoy, D., Stripeikis, J., & Pose, M. (2001). Condition index and growth rates of field caged *Corbicula fluminea* (Bivalvia) as biomarkers of pollution gradients in the Paraná river delta

- (Argentina). *Aquatic Ecosystem Health & Management*, 4(2), 187–201. <https://doi.org/10.1080/14634980127712>
- Chambers, W. S., Hopkins, J. G., & Richards, S. M. (2021). A Review of Per- and Polyfluorinated Alkyl Substance Impairment of Reproduction. *Frontiers in Toxicology*, 3, 2673–3080. <https://doi.org/10.3389/ftox.2021.732436>
- Chen, H., Zhang, C., Yu, Y., & Han, J. (2012). Sorption of perfluorooctane sulfonate (PFOS) on marine sediments. *Marine Pollution Bulletin*, 64, 902–906. <https://doi.org/10.1016/j.marpolbul.2012.03.012>
- Chen, M., Zhu, L., Wang, Q., & Shan, G. (2021). Tissue distribution and bioaccumulation of legacy and emerging per-and polyfluoroalkyl substances (PFASs) in edible fishes from Taihu Lake, China. *Environmental Pollution*, 268, 115887. <https://doi.org/10.1016/j.envpol.2020.115887>
- Cheng, H., Lv, C., Li, J., Wu, D., Zhan, X., Song, Y., Zhao, N., & Jin, H. (2022). Bioaccumulation and biomagnification of emerging poly- and perfluoroalkyl substances in marine organisms. *Science of The Total Environment*, 851, 158117. <https://doi.org/10.1016/j.scitotenv.2022.158117>
- Christensen, K.Y., Raymond, M., Blackowicz, M., Liu, Y., Thompson, B.A., Anderson, H.A., & Turyk, M., 2017. Perfluoroalkyl substances and fish consumption. *Environmental Research*, 154, 145–151. <https://doi.org/10.1016/j.envres.2016.12.032>
- Chu, S., & Letcher, R. J. (2014). In vitro metabolic formation of perfluoroalkyl sulfonamides from copolymer surfactants of pre- and post-2002 scotchgard fabric protector products. *Environmental Science and Technology*, 48(11), 6184–6191. https://doi.org/10.1021/ES500169X/SUPPL_FILE/ES500169X_SI_001.PDF
- Chu, S., Letcher, R. J., McGoldrick, D. J., & Backus, S. M. (2016). A New Fluorinated Surfactant Contaminant in Biota: Perfluorobutane Sulfonamide in Several Fish Species. *Environmental Science & Technology*, 50(2), 669–675. <https://doi.org/10.1021/acs.est.5b05058>
- Clara, M., Scheffknecht, C., Scharf, S., Weiss, S., & Gans, O. (2008). Emissions of perfluorinated alkylated substances (PFAS) from point sources - Identification of relevant branches. *Water Science and Technology*, 58(1), 59–66. <https://doi.org/10.2166/wst.2008.641>
- Cnudde, C., Moens, T., Werbrouck, E., Lepoint, G., Van Gansbeke, D., & De Troch, M. (2015). Trophodynamics of estuarine intertidal harpacticoid copepods based on stable isotope composition and fatty acid profiles. *Marine Ecology Progress Series*, 524, 225–239. <https://doi.org/10.3354/meps11161>
- Coat, S., Monti, D., Legendre, P., Bouchon, C., Massat, F., & Lepoint, G. (2011). Organochlorine pollution in tropical rivers (Guadeloupe): Role of ecological factors in food web bioaccumulation. *Environmental Pollution*, 159(6), 1692–1701. <https://doi.org/10.1016/j.envpol.2011.02.036>
- Conder, J.M., Hoke, R.A., De Wolf, W., Russell, M.H., & Buck, R.C. (2008). Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. *Environmental Science and Technology* 42(4), 995–1003. <https://doi.org/10.1021/es070895g>
- Connolly, R. M., Guest, M. A., Melville, A. J., & Oakes, J. M. (2004). Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia*, 138(2), 161–167. <https://doi.org/10.1007/s00442-003-1415-0>
- Corsolini, S., Mazzoni, M., Ng, C., Polesello, S., Rusconi, M., & Valsecchi, S. (2014). Perfluorinated alkyl acids in bivalves, water, and sediments of the Po river delta (Adriatic Sea). *Organohalogen Compounds*, 76(February 2015), 684–687. <http://www.dioxin20xx.org/pdfs/2014/882.pdf>

Cousins, I. T., Dewitt, J. C., Glüge, J., Goldenman, G., Herzke, D., Lohmann, R., Ng, C. A., Scheringer, M., & Wang, Z. (2020). The high persistence of PFAS is sufficient for their management as a chemical class. In *Environmental Science: Processes and Impacts*, 22(12), 2307–2312. <https://doi.org/10.1039/d0em00355g>

Crawford, K., McDonald, R. A., & Bearhop, S. (2008). Applications of stable isotope techniques to the ecology of mammals. *Mammal Review*, 38(1), 87–107. <https://doi.org/10.1111/j.1365-2907.2008.00120.x>

Cunha, I., Hoff, P., Van De Vijver, K., Guilhermino, L., Esmans, E., & De Coen, W. (2005). Baseline study of perfluorooctane sulfonate occurrence in mussels, *Mytilus galloprovincialis*, from north-central portuguese estuaries. *Marine Pollution Bulletin*, 50(10), 1128–1132. <https://doi.org/10.1016/J.MARPOLBUL.2005.06.046>

Custer, T.W., Kannan, K., Tao, L., Yun, S.H., & Trowbridge, A. (2010). Perfluorinated Compounds and Polybrominated Diphenyl Ethers in Great Blue Heron Eggs from Three Colonies on the Mississippi River, Minnesota. *Waterbirds* 33, 89–95. <https://doi.org/10.1675/063.033.0110>

D

Dams, R., & Hintzer, K. (2016). Industrial Aspects of Fluorinated Oligomers and Polymers. *RSC Polymer Chemistry Series*, 24, 3–31. <https://doi.org/10.1039/9781782629368-00001>

Das, K., Holleville, O., Ryan, C., Berrow, S., Gilles, A., Ody, D., & Michel, L. N. (2017). Isotopic niches of fin whales from the Mediterranean Sea and the Celtic Sea (North Atlantic). *Marine Environmental Research*, 127, 75–83. <https://doi.org/10.1016/j.marenvres.2017.03.009>

Dasu, K., Xia, X., Siriwardena, D., Klupinski, T. P., & Seay, B. (2022). Concentration profiles of per- and polyfluoroalkyl substances in major sources to the environment. *Journal of Environmental Management*, 301, 113879. <https://doi.org/10.1016/J.JENVMAN.2021.113879>

Dauchy, X., Boiteux, V., Rosin, C., & Munoz, J. F. (2012). Relationship between industrial discharges and contamination of raw water resources by perfluorinated compounds. Part I: Case study of a fluoropolymer manufacturing plant. *Bulletin of Environmental Contamination and Toxicology*, 89(3), 525–530. <https://doi.org/10.1007/S00128-012-0704-X/TABLES/2>

Dauwe, T., Van de Vijver, K., De Coen, W., & Eens, M. (2007). PFOS levels in the blood and liver of a small insectivorous songbird near a fluorochemical plant. *Environment International*, 33(3), 357–361. <https://doi.org/https://doi.org/10.1016/j.envint.2006.11.014>

de Brabandere, L., Dehairs, F., van Damme, S., Brion, N., Meire, P., & Daro, N. (2002). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ dynamics of suspended organic matter in freshwater and brackish waters of the Scheldt estuary. *Journal of Sea Research*, 48, 1–15. [https://doi.org/10.1016/S1385-1101\(02\)00132-6](https://doi.org/10.1016/S1385-1101(02)00132-6)

De Jonge, M., Belpaire, C., Van Thuyne, G., Breine, J., & Bervoets, L. (2015). Temporal distribution of accumulated metal mixtures in two feral fish species and the relation with condition metrics and community structure. *Environmental Pollution*, 197, 43–54. <https://doi.org/10.1016/J.ENVPOL.2014.11.024>

de Jonge, M., Tipping, E., Loft, S., Bervoets, L., & Blust, R. (2013). The use of invertebrate body burdens to predict ecological effects of metal mixtures in mining-impacted waters. *Aquatic Toxicology*, 142–143, 294–302. <https://doi.org/10.1016/J.AQUATOX.2013.08.018>

- D'eon, J. C., Simpson, A. J., Kumar, R., Baer, A. J., & Mabury, S. A. (2010). Determining the molecular interactions of perfluorinated carboxylic acids with human sera and isolated human serum albumin using nuclear magnetic resonance spectroscopy. *Environmental Toxicology and Chemistry*, 29(8), 1678–1688. <https://doi.org/10.1002/etc.204>
- De Pauw, N., & Vanhooren, G. (1983). Method for biological quality assessment of watercourses in Belgium. *Hydrobiologia*, 100(1), 153–168. <https://doi.org/10.1007/BF00027428/METRICS>
- De Pauw, N., & VANNEVEL, R. (n.d.). Macroinvertebraten en waterkwaliteit. Determineersleutels van macroin-vertebraten en beoordelingsmethoden van de waterkwaliteit. Stichting Leefmilieu, Antwerpen, 1991, Dossier nr. 11, 316 p.
- De Ridder, K. De, Couderé, K., Depoorter, M., Liekens, I., Pourria, X., Steinmetz, D., Vanuytrecht, E., Verhaegen, K., & Wouters, H. (2020). Evaluation of the Socio-Economic Impact of Climate Change in Belgium. Study commissioned by the National Climate Commission (Vol. 2020, Issue July).
- De Silva, A. O., Armitage, J. M., Bruton, T. A., Dassuncao, C., Heiger-Bernays, W., Hu, X. C., Kärrman, A., Kelly, B., Ng, C., Robuck, A., Sun, M., Webster, T. F., & Sunderland, E. M. (2021). PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. *Environmental Toxicology and Chemistry*, 40(3), 631–657. <https://doi.org/10.1002/ETC.4935>
- De Standaard. (2021, June 14). Geen eieren van eigen kippen meer: deze maatregelen neemt Vlaamse regering rond 3M-site. https://www.standaard.be/cnt/dmf20210614_95881839.
- De Veen, J.F., 1978. On selective tidal transport in the migration of North Sea Plaice (*Pleuronectes platessa*) and other flatfish species. *Journal of Sea Research*, 12(2), 115–147. [https://doi.org/10.1016/0077-7579\(78\)90001-7](https://doi.org/10.1016/0077-7579(78)90001-7)
- de Vos, M. G., Huijbregts, M. A. J., van den Heuvel-Greve, M. J., Vethaak, A. D., Van de Vijver, K. I., Leonards, P. E. G., van Leeuwen, S. P. J., de Voogt, P., & Hendriks, A. J. (2008). Accumulation of perfluorooctane sulfonate (PFOS) in the food chain of the Western Scheldt estuary: Comparing field measurements with kinetic modeling. *Chemosphere*, 70(10), 1766–1773. <https://doi.org/10.1016/j.chemosphere.2007.08.038>
- Decré, H., & Baert, D. (2021, June 16). Ophef over deal PFOS-vervuiling bij Oosterweel: bouwheer Lantis benadrukt dat alle instanties op de hoogte waren. <https://www.vrt.be/vrtnws/nl/2021/06/15/dading-pfos/>.
- Del Rio, D., Stewart, A. J., & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, Metabolism and Cardiovascular Diseases*, 15(4), 316–328. <https://doi.org/https://doi.org/10.1016/j.numecd.2005.05.003>
- Delhez, E.J.M., & Carabin, G., 2001. Integrated modelling of the Belgian Coastal Zone. *Estuarine, Coastal and Shelf Science*, 53(4), 477–491. <https://doi.org/10.1006/ecss.1999.0628>
- Deng, M., Wu, Y., Xu, C., Jin, Y., He, X., Wan, J., Yu, X., Rao, H., & Tu, W. (2018). Multiple approaches to assess the effects of F-53B, a Chinese PFOS alternative, on thyroid endocrine disruption at environmentally relevant concentrations. *Science of The Total Environment*, 624, 215–224. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.12.101>
- Deniro, M. J., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, 45(3), 341–351. [https://doi.org/https://doi.org/10.1016/0016-7037\(81\)90244-1](https://doi.org/https://doi.org/10.1016/0016-7037(81)90244-1)

Dickman, R. A., & Aga, D. S. (2022). A review of recent studies on toxicity, sequestration, and degradation of per- and polyfluoroalkyl substances (PFAS). *Journal of Hazardous Materials*, 436, 129120. <https://doi.org/10.1016/j.jhazmat.2022.129120>

Dittel, A. I., & Epifanio, C. E. (2009). Invasion biology of the Chinese mitten crab *Eriocheir sinensis*: A brief review. *Journal of Experimental Marine Biology and Ecology*, 374(2), 79–92. <https://doi.org/https://doi.org/10.1016/j.jembe.2009.04.012>

Dorjbayar, A., Merckx, V., Bontinck, H., Pauwels, L., & De Bie, A. (2021, June 23). Tot 110 keer te veel PFOS in Vlaamse waterlopen, “erin zwemmen was sowieso al geen goed idee.” <https://www.vrt.be/vrtnws/nl/2021/06/22/Tot-110-keer-te-veel-Pfos-in-Vlaamse-Meren-En-Rivieren/>.

Dreyer, A., Weinberg, I., Temme, C., & Ebinghaus, R. (2009). Polyfluorinated compounds in the atmosphere of the Atlantic and Southern Oceans: Evidence for a global distribution. *Environmental Science and Technology*, 43, 6507–6514. <https://doi.org/10.1021/es9010465>

Du, Z., Deng, S., Liu, D., Yao, X., Wang, Y., Lu, X., Wang, B., Huang, J., Wang, Y., Xing, B., & Yu, G. (2016). Efficient adsorption of PFOS and F53B from chrome plating wastewater and their subsequent degradation in the regeneration process. *Chemical Engineering Journal*, 290, 405–413. <https://doi.org/https://doi.org/10.1016/j.cej.2016.01.077>

D’Hollander, W., De Bruyn, L., Hagenars, A., de Voogt, P., & Bervoets, L. (2014). Characterisation of perfluorooctane sulfonate (PFOS) in a terrestrial ecosystem near a fluorochemical plant in Flanders, Belgium. *Environmental Science and Pollution Research*, 21(20), 11856–11866. <https://doi.org/10.1007/s11356-013-2449-4>

D’Hollander, W., De Voogt, P., De Coen, W., & Bervoets, L., 2010. Perfluorinated substances in human food and other sources of human exposure. *Reviews of Environmental Contamination and Toxicology*. 208, 179–215. https://doi.org/10.1007/978-1-4419-6880-7_4

E

Ebnesajjad, S. (2013). *Introduction to Fluoropolymers: Materials, Technology, and Applications*. William Andrew Publishing, 1–325. <https://doi.org/10.1016/C2012-0-06184-4>

ECHA, (2023). PFAS restriction proposal. <https://echa.europa.eu/-/echa-publishes-pfas-restriction-proposal> (assessed 10 September 2024).

EFSA, (2020). Risk to human health related to the presence of perfluoroalkyl substances in food. <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6223> (assessed 28 Januari 2022).

EFSA, (2008). Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts - Scientific Opinion of the Panel on Contaminants in the Food chain. *The EFSA Journal*, 653, 1–131. <https://doi.org/10.2903/j.efsa.2008.653>

Environmental Protection Agency. (2002). *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms* (5th ed.). https://www.epa.gov/sites/default/files/2015-08/documents/acute-freshwater-and-marine-wet-manual_2002.pdf

- Ericson, I., Martí-Cid, R., Nadal, M., Van Bavel, B., Lindström, G., & Domingo, J.L., (2008). Human exposure to perfluorinated chemicals through the diet: Intake of perfluorinated compounds in foods from the Catalan (Spain) market. *Journal of Agricultural and Food Chemistry*, 56, 1787–1794. <https://doi.org/10.1021/jf0732408>
- Eriksson, U., Haglund, P., & Kärrman, A. (2017). Contribution of precursor compounds to the release of per- and polyfluoroalkyl substances (PFASs) from waste water treatment plants (WWTPs). *Journal of Environmental Sciences (China)*, 61, 80–90. <https://doi.org/10.1016/J.JES.2017.05.004>
- Espartero, J. L. L., Yamada, M., Ford, J., Owens, G., Prow, T., & Juhasz, A. (2022). Health-related toxicity of emerging per- and polyfluoroalkyl substances: Comparison to legacy PFOS and PFOA. *Environmental Research*, 212, 113431. <https://doi.org/https://doi.org/10.1016/j.envres.2022.113431>
- EUMOFA, 2017. Country Profile: Belgium in the world and in the EU. <https://www.eumofa.eu/belgium> (assessed 2 Februari 2022).
- European Union. (2022). Commission Regulation 2022/2388 of 7 December 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of perfluoroalkyl substances in certain foodstuffs (Text with EEA relevance). *Official Journal of the European Union*, L 316/38.
- European Union. (2013). Directives of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Official Journal of the European Union*, 1–17.
- European Union. (2014). Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 32 on Biota Monitoring. <https://circabc.europa.eu/sd/a/62343f10-5759-4e7c-ae2b-12677aa57605/Guidance%20No%2032%20-%20Biota%20Monitoring.pdf> (assessed 28 Januari 2022).
- Evariste, L., David, E., Cloutier, P. L., Brousseau, P., Auffret, M., Desrosiers, M., Groleau, P. E., Fournier, M., & Betoulle, S. (2018). Field biomonitoring using the zebra mussel *Dreissena polymorpha* and the quagga mussel *Dreissena bugensis* following immunotoxic responses. Is there a need to separate the two species? *Environmental Pollution*, 238, 706–716. <https://doi.org/10.1016/J.ENVPOL.2018.03.098>

F

- Faxneld, S., Danielsson, S., Nyberg, E., Faxneld, S., & Danielsson, S., (2014). Distribution of PFAS in liver and muscle of herring, perch, cod, eelpout, arctic char, and pike from limnic and marine environments in Sweden. *Swedish Museum of Natural History*.
- Feng, S., Lu, X., Ouyang, K., Su, G., Li, Q., Shi, B., & Meng, J. (2024). Environmental occurrence, bioaccumulation and human risks of emerging fluoroalkylether substances: Insight into security of alternatives. *Science of the Total Environment*, 922, 171151. <https://doi.org/10.1016/j.scitotenv.2024.171151>
- Fernández-Sanjuan, M., Faria, M., Lacorte, S., & Barata, C. (2013). Bioaccumulation and effects of perfluorinated compounds (PFCs) in zebra mussels (*Dreissena polymorpha*). *Environmental Science and Pollution Research*, 20(4), 2661–2669. <https://doi.org/10.1007/s11356-012-1158-8>
- Filipovic, M., Woldegiorgis, A., Norström, K., Bibi, M., Lindberg, M., & Österås, A. H. (2015). Historical usage of aqueous film forming foam: A case study of the widespread distribution of perfluoroalkyl acids from a military airport to groundwater, lakes, soils and fish. *Chemosphere*, 129, 39–45. <https://doi.org/10.1016/J.CHEMOSPHERE.2014.09.005>

Fitzgerald, N. J. M., Wargenau, A., Sorenson, C., Pedersen, J., Tufenkji, N., Novak, P. J., & Simcik, M. F. (2018). Partitioning and Accumulation of Perfluoroalkyl Substances in Model Lipid Bilayers and Bacteria. *Environmental Science and Technology*, 52(18), 10433–10440. https://doi.org/10.1021/ACS.EST.8B02912/ASSET/IMAGES/LARGE/ES-2018-02912K_0002.JPEG

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, 226(1), 497–509. [https://doi.org/10.1016/s0021-9258\(18\)64849-5](https://doi.org/10.1016/s0021-9258(18)64849-5)

France, R. L. (1995). Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Marine Ecology Progress Series*, 124, 307–312. <https://www.int-res.com/abstracts/meps/v124/p307-312/>

Fredriksson, F., Eriksson, U., Kärrman, A., & Yeung, L. W. Y. (2022). Per- and polyfluoroalkyl substances (PFAS) in sludge from wastewater treatment plants in Sweden — First findings of novel fluorinated copolymers in Europe including temporal analysis. *Science of The Total Environment*, 846, 157406. <https://doi.org/10.1016/J.SCITOTENV.2022.157406>

Fry, B., & Sherr, E. B. (1989). $\delta^{13}\text{C}$ Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. In P. W. Rundel, J. R. Ehleringer, & K. A. Nagy (Eds.), *Stable Isotopes in Ecological Research* (pp. 196–229). Springer New York.

Fry, B., Scalan, R. S., Winters, J. K., & Parker, P. L. (1982). Sulphur uptake by salt grasses, mangroves, and seagrasses in anaerobic sediments. *Geochimica et Cosmochimica Acta*, 46(6), 1121–1124. [https://doi.org/https://doi.org/10.1016/0016-7037\(82\)90063-1](https://doi.org/https://doi.org/10.1016/0016-7037(82)90063-1)

G

Gabriels, W., Lock, K., De Pauw, N., & Goethals, P. L. M. (2010). Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnologia - Ecology and Management of Inland Waters*, 40(3), 199–207. <https://doi.org/10.1016/J.LIMNO.2009.10.001>

Gagliano, E., Sgroi, M., Falciglia, P. P., Vagliasindi, F. G. A., & Roccaro, P. (2020). Removal of poly- and perfluoroalkyl substances (PFAS) from water by adsorption: Role of PFAS chain length, effect of organic matter and challenges in adsorbent regeneration. *Water Research*, 171, 115381. <https://doi.org/https://doi.org/10.1016/j.watres.2019.115381>

Gaines, L. G. T. (2023). Historical and current usage of per- and polyfluoroalkyl substances (PFAS): A literature review. *American Journal of Industrial Medicine* 66, 5, 353–378. <https://doi.org/10.1002/ajim.23362>

Galatius, A., Bossi, R., Sonne, C., Rigét, F. F., Kinze, C. C., Lockyer, C., Teilmann, J., & Dietz, R. (2013). PFAS profiles in three North Sea top predators: Metabolic differences among species? *Environmental Science and Pollution Research*. 20, 8013–8020. <https://doi.org/10.1007/s11356-013-1633-x>

Galatius, A., Dietz, R., Rigét, F. F., Sonne, C., Kinze, C. C., Lockyer, C., & Bossi, R. (2011). Temporal and life history related trends of perfluorochemicals in harbor porpoises from the Danish North Sea. *Marine Pollution Bulletin*, 62(7), 1476–1483. <https://doi.org/10.1016/j.marpolbul.2011.04.011>

Gao, Y., De Brauwere, A., Elskens, M., Croes, K., Baeyens, W., & Leermakers, M. (2012). Evolution of trace metal and organic pollutant concentrations in the Scheldt River Basin and the Belgian Coastal Zone over

- the last three decades. *Journal of Marine Systems*, 128, 52–61. <https://doi.org/https://doi.org/10.1016/j.jmarsys.2012.04.002>
- Garrett, A. B. (1962). Teflon: Roy J. Plunkett. *Journal of Chemical Education*, 39(6), 288. <https://doi.org/10.1021/ED039P288>
- Gebbink, W. A., van Asseldonk, L., & van Leeuwen, S. P. J. (2017). Presence of Emerging Per- and Polyfluoroalkyl Substances (PFASs) in River and Drinking Water near a Fluorochemical Production Plant in the Netherlands. *Environmental Science & Technology*, 51(19), 11057–11065. <https://doi.org/10.1021/acs.est.7b02488>
- Gebbink, W.A., Bignert, A., & Berger, U. (2016). Perfluoroalkyl Acids (PFAAs) and Selected Precursors in the Baltic Sea Environment: Do Precursors Play a Role in Food Web Accumulation of PFAAs? *Environmental Science and Technology* 50, 6354–6362. <https://doi.org/10.1021/acs.est.6b01197>
- Gerbersdorf, S. U., Cimadoribus, C., Class, H., Engesser, K. H., Helbich, S., Hollert, H., Lange, C., Kranert, M., Metzger, J., Nowak, W., Seiler, T. B., Steger, K., Steinmetz, H., & Wieprecht, S. (2015). Anthropogenic Trace Compounds (ATCs) in aquatic habitats - research needs on sources, fate, detection and toxicity to ensure timely elimination strategies and risk management. *Environment International*, 79, 85–105. <https://doi.org/10.1016/J.ENVINT.2015.03.011>
- Gkika, I. S., Xie, G., van Gestel, C. A. M., Ter Laak, T. L., Vonk, J. A., van Wezel, A. P., & Kraak, M. H. S. (2023). Research Priorities for the Environmental Risk Assessment of Per- and Polyfluorinated Substances. *Environmental Toxicology and Chemistry*, 42(11), 2302–2316. <https://doi.org/10.1002/ETC.5729>
- Glenn, G., Shogren, R., Jin, X., Orts, W., Hart-Cooper, W., & Olson, L. (2021). Per- and polyfluoroalkyl substances and their alternatives in paper food packaging. *Comprehensive Reviews in Food Science and Food Safety*, 20(3), 2596–2625. <https://doi.org/10.1111/1541-4337.12726>
- Glüge, J., Scheringer, M., Cousins, I. T., Dewitt, J. C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C. A., Trier, X., & Wang, Z. (2020). An overview of the uses of per- and polyfluoroalkyl substances (PFAS). *Environmental Science: Processes & Impacts*, 22(12), 2345–2373. <https://doi.org/10.1039/D0EM00291G>
- Gomis, M. I., Vestergren, R., Borg, D., & Cousins, I. T. (2018). Comparing the toxic potency in vivo of long-chain perfluoroalkyl acids and fluorinated alternatives. *Environment International*, 113, 1–9. <https://doi.org/10.1016/J.ENVINT.2018.01.011>
- Goodrow, S. M., Ruppel, B., Lippincott, R. L., Post, G. B., & Procopio, N. A. (2020). Investigation of levels of perfluoroalkyl substances in surface water, sediment and fish tissue in New Jersey, USA. *Science of the Total Environment*, 729, 138839. <https://doi.org/10.1016/J.SCITOTENV.2020.138839>
- Gordon, S. C. (2011). Toxicological evaluation of ammonium 4,8-dioxa-3H-perfluorononanoate, a new emulsifier to replace ammonium perfluorooctanoate in fluoropolymer manufacturing. *Regulatory Toxicology and Pharmacology*, 59(1), 64–80. <https://doi.org/https://doi.org/10.1016/j.yrtph.2010.09.008>
- Gourlay-Francé, C., & Tusseau-Vuillemin, M.-H. (2013). Bioavailability of contaminants. In J.-F. Férard & C. Blaise (Eds.), *Encyclopedia of Aquatic Ecotoxicology* (pp. 181–190). Springer.
- Grandjean, P., Clapp, R. (2015). Perfluorinated alkyl substances: Emerging insights into health risks. *New Solutions*, 25, 147–163. <https://doi.org/10.1177/1048291115590506>
- Grantham, T. E., Cañedo-Argüelles, M., Perrée, I., Rieradevall, M., & Prat, N. (2012). A mesocosm approach for detecting stream invertebrate community responses to treated wastewater effluent. *Environmental Pollution*, 160, 95–102. <https://doi.org/https://doi.org/10.1016/j.envpol.2011.09.014>

Groffen, T., Bervoets, L., Jeong, Y., Willems, T., Eens, M., & Prinsen, E. (2021). A rapid method for the detection and quantification of legacy and emerging per- and polyfluoroalkyl substances (PFAS) in bird feathers using UPLC-MS/MS. *Journal of Chromatography B*, 1172, 122653. <https://doi.org/10.1016/J.JCHROMB.2021.122653>

Groffen, T., Eens, M., & Bervoets, L. (2019a). Do concentrations of perfluoroalkylated acids (PFAAs) in isopods reflect concentrations in soil and songbirds? A study using a distance gradient from a fluorochemical plant. *Science of The Total Environment*, 657, 111–123. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.12.072>

Groffen, T., Keirsebelik, H., Dendievel, H., Falcou-Préfol, M., Bervoets, L., & Schoelynck, J. (2024). Are Chinese mitten crabs (*Eriocheir sinensis*) suitable as biomonitor or bioindicator of per- and polyfluoroalkyl substances (PFAS) pollution? *Journal of Hazardous Materials*, 464, 133024. <https://doi.org/10.1016/j.jhazmat.2023.133024>

Groffen, T., Lasters, R., Lemièrre, F., Willems, T., Eens, M., Bervoets, L., & Prinsen, E. (2019b). Development and validation of an extraction method for the analysis of perfluoroalkyl substances (PFASs) in environmental and biotic matrices. *Journal of Chromatography B*, 1116, 30–37. <https://doi.org/10.1016/j.jchromb.2019.03.034>

Groffen, T., Lopez-Antia, A., D'Hollander, W., Prinsen, E., Eens, M., & Bervoets, L. (2017). Perfluoroalkylated acids in the eggs of great tits (*Parus major*) near a fluorochemical plant in Flanders, Belgium. *Environmental Pollution*, 228, 140–148. <https://doi.org/10.1016/j.envpol.2017.05.007>

Groffen, T., Wepener, V., Malherbe, W., & Bervoets, L. (2018). Distribution of perfluorinated compounds (PFASs) in the aquatic environment of the industrially polluted Vaal River, South Africa. *Science of the Total Environment*, 627, 1334–1344. <https://doi.org/10.1016/j.scitotenv.2018.02.023>

Guelfo, J. L., & Higgins, C. P. (2013). Subsurface transport potential of perfluoroalkyl acids at aqueous film-forming foam (AFFF)-impacted sites. *Environmental Science and Technology*, 47(9), 4164–4171. https://doi.org/10.1021/ES3048043/SUPPL_FILE/ES3048043_SI_001.PDF

Guelinckx, J., Maes, J., de Brabandere, L., Dehairs, F., & Ollevier, F. (2006). Migration dynamics of clupeoids in the Schelde estuary: A stable isotope approach. *Estuarine, Coastal and Shelf Science*, 66(3–4), 612–623. <https://doi.org/10.1016/j.ecss.2005.11.007>

Guillette, T. C., McCord, J., Guillette, M., Polera, M. E., Rachels, K. T., Morgeson, C., Kotlarz, N., Knappe, D. R. U., Reading, B. J., Strynar, M., & Belcher, S. M. (2020). Elevated levels of per- and polyfluoroalkyl substances in Cape Fear River Striped Bass (*Morone saxatilis*) are associated with biomarkers of altered immune and liver function. *Environment International*, 136, 105358. <https://doi.org/https://doi.org/10.1016/j.envint.2019.105358>

Gust, K. A., Mylroie, J. E., Kimble, A. N., Wilbanks, M. S., Steward, C. S. C., Chapman, K. A., Jensen, K. M., Kennedy, A. J., Krupa, P. M., Waisner, S. A., Pandelides, Z., Garcia-Reyero, N., Erickson, R. J., Ankley, G. T., Conder, J., & Moore, D. W. (2024). Survival, Growth, and Reproduction Responses in a Three-Generation Exposure of the Zebrafish (*Danio rerio*) to Perfluorooctane Sulfonate. *Environmental Toxicology and Chemistry*, 43(1), 115–131. <https://doi.org/10.1002/ETC.5770>

Göckener, B., Fliedner, A., Rüdél, H., Fettig, I., & Koschorreck, J. (2021). Exploring unknown per- and polyfluoroalkyl substances in the German environment – The total oxidizable precursor assay as helpful tool in research and regulation. *Science of The Total Environment*, 782, 146825. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.146825>

H

- Habibullah-Al-Mamun, M., Ahmed, M. K., Raknuzzaman, M., Islam, M. S., Negishi, J., Nakamichi, S., Sekine, M., Tokumura, M., & Masunaga, S. (2016). Occurrence and distribution of perfluoroalkyl acids (PFAAs) in surface water and sediment of a tropical coastal area (Bay of Bengal coast, Bangladesh). *Science of The Total Environment*, 571, 1089–1104. <https://doi.org/10.1016/J.SCITOTENV.2016.07.104>
- Hagenaars, A., Vergauwen, L., Benoot, D., Laukens, K., & Knapen, D. (2013). Mechanistic toxicity study of perfluorooctanoic acid in zebrafish suggests mitochondrial dysfunction to play a key role in PFOA toxicity. *Chemosphere*, 91(6), 844–856. <https://doi.org/10.1016/J.CHEMOSPHERE.2013.01.056>
- Haimbaugh, A., Wu, C. C., Akemann, C., Meyer, D. N., Connell, M., Abdi, M., Khalaf, A., Johnson, D., & Baker, T. R. (2022). Multi- and Transgenerational Effects of Developmental Exposure to Environmental Levels of PFAS and PFAS Mixture in Zebrafish (*Danio rerio*). *Toxics*, 10(6), 334. <https://doi.org/10.3390/TOXICS10060334/S1>
- Hamed, M., Vats, A., Lim, I. E., Sapkota, B., & Abdelmoneim, A. (2024). Effects of developmental exposure to individual and combined PFAS on development and behavioral stress responses in larval zebrafish. *Environmental Pollution*, 349, 123912. <https://doi.org/10.1016/J.ENVPOL.2024.123912>
- Han, J., & Fang, Z. (2010). Estrogenic effects, reproductive impairment and developmental toxicity in ovoviparous swordtail fish (*Xiphophorus helleri*) exposed to perfluorooctane sulfonate (PFOS). *Aquatic Toxicology*, 99(2), 281–290. <https://doi.org/10.1016/J.AQUATOX.2010.05.010>
- Haukås, M., Berger, U., Hop, H., Gulliksen, B., & Gabrielsen, G.W. (2007). Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environmental Pollution*, 148, 360–371. <https://doi.org/10.1016/j.envpol.2006.09.021>
- Helsel, D. R. (2006). Fabricating data: How substituting values for nondetects can ruin results, and what can be done about it. *Chemosphere*, 65(11), 2434–2439. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2006.04.051>
- Henderson, P. A., Holmes, R. H.A. (1989). Whiting migration in the Bristol Channel: a predator-prey relationship. *Journal of Fish Biology*, 34(3), 409–416. <https://doi.org/10.1111/j.1095-8649.1989.tb03323.x>
- Henry, B. J., Carlin, J. P., Hammerschmidt, J. A., Buck, R. C., Buxton, L. W., Fiedler, H., Seed, J., & Hernandez, O. (2018). A critical review of the application of polymer of low concern and regulatory criteria to fluoropolymers. *Integrated Environmental Assessment and Management*, 14(3), 316–334. <https://doi.org/10.1002/IEAM.4035>
- Hensema, T. J., Berendsen, B. J. A., & van Leeuwen, S. P. J. (2021). Non-targeted identification of per- and polyfluoroalkyl substances at trace level in surface water using fragment ion flagging. *Chemosphere*, 265, 128599. <https://doi.org/10.1016/J.CHEMOSPHERE.2020.128599>
- Herzka, S. Z. (2005). Assessing connectivity of estuarine fishes based on stable isotope ratio analysis. *Estuarine, Coastal and Shelf Science*, 64(1), 58–69. <https://doi.org/https://doi.org/10.1016/j.ecss.2005.02.006>
- Higgins, C. P., & Luthy, R. G. (2006). Sorption of perfluorinated surfactants on sediments. *Environmental Science and Technology*, 40(23), 7251–7256. https://doi.org/10.1021/ES061000N/SUPPL_FILE/ES061000NSI20060824_095850.PDF

- Higgins, C. P., Paesani, Z. J., Chalew, T. E. A., & Halden, R. U. (2009). Bioaccumulation of triclocarban in *Lumbriculus variegatus**. *Environmental Toxicology and Chemistry*, 28(12), 2580–2586. <https://doi.org/10.1897/09-013.1>
- Hoa, N. T. Q., Lieu, T. T., Anh, H. Q., Huong, N. T. A., Nghia, N. T., Chuc, N. T., Quang, P. D., Vi, P. T., & Tuyen, L. H. (2022). Perfluoroalkyl substances (PFAS) in freshwater fish from urban lakes in Hanoi, Vietnam: concentrations, tissue distribution, and implication for risk assessment. *Environmental Science and Pollution Research*, 29(34), 52057–52069. <https://doi.org/10.1007/S11356-022-19532-0/FIGURES/5>
- Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, 120(3), 314–326. <https://doi.org/10.1007/s004420050865>
- Hobson, K. A., Fisk, A., Karnovsky, N., Holst, M., Gagnon, J. M., & Fortier, M. (2002). A stable isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. *Deep Sea Research Part II: Topical Studies in Oceanography*, 49(22–23), 5131–5150. [https://doi.org/10.1016/S0967-0645\(02\)00182-0](https://doi.org/10.1016/S0967-0645(02)00182-0)
- Hoff, P. T., Scheirs, J., Van de Vijver, K., Van Dongen, W., Esmans, E. L., Blust, R., & De Coen, W. (2004). Biochemical effect evaluation of perfluorooctane sulfonic acid-contaminated wood mice (*Apodemus sylvaticus*). *Environmental Health Perspectives*, 112(6), 681–686. <https://doi.org/10.1289/ehp.6479>
- Hoff, P. T., Van de Vijver, K., Dauwe, T., Covaci, A., Maervoet, J., Eens, M., Blust, R., & De Coen, W. (2005). Evaluation of biochemical effects related to perfluorooctane sulfonic acid exposure in organohalogen-contaminated great tit (*Parus major*) and blue tit (*Parus caeruleus*) nestlings. *Chemosphere*, 61(11), 1558–1569. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2005.04.109>
- Hoff, P. T., Van de Vijver, K., Van Dongen, W., Esmans, E. L., Blust, R., & De Coen, W. M. (2003). Perfluorooctane sulfonic acid in bib (*Trisopterus luscus*) and plaice (*Pleuronectes platessa*) from the Western Scheldt and the Belgian North Sea: Distribution and biochemical effects. *Environmental Toxicology and Chemistry*, 22(3), 608–614. [https://doi.org/10.1897/1551-5028\(2003\)022<0608:PSAIBT>2.0.CO;2](https://doi.org/10.1897/1551-5028(2003)022<0608:PSAIBT>2.0.CO;2)
- Hoke, R. A., Ferrell, B. D., Ryan, T., Sloman, T. L., Green, J. W., Nabb, D. L., Mingoia, R., Buck, R. C., & Korzeniowski, S. H. (2015). Aquatic hazard, bioaccumulation and screening risk assessment for 6:2 fluorotelomer sulfonate. *Chemosphere*, 128, 258–265. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2015.01.033>
- Hoke, R. A., Ferrell, B. D., Ryan, T., Sloman, T. L., Green, J. W., Nabb, D. L., Mingoia, R., Buck, R. C., & Korzeniowski, S. H. (2015). Aquatic hazard, bioaccumulation and screening risk assessment for 6:2 fluorotelomer sulfonate. *Chemosphere*, 128, 258–265. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2015.01.033>
- Holt, A. E., & Miller, W. S. (2010). Bioindicators: Using Organisms to Measure Environmental Impacts. *Nature Education Knowledge*, 3(10), 8.
- Houde, M., De Silva, A. O., Muir, D. C. G., & Letcher, R. J. (2011). Monitoring of Perfluorinated Compounds in Aquatic Biota: An Updated Review. *Environmental Science & Technology*, 45(19), 7962–7973. <https://doi.org/10.1021/es104326w>
- Houde, M., Martin, J. W., Letcher, R. J., Solomon, K. R., & Muir, D. C. G. (2006). Biological monitoring of polyfluoroalkyl substances: A review. In *Environmental Science and Technology*, 40(11), 3463–3473. <https://doi.org/10.1021/es052580b>

Houtz, E. F., Higgins, C. P., Field, J. A., & Sedlak, D. L. (2013). Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environmental Science and Technology*, 47(15), 8187–8195. https://doi.org/10.1021/ES4018877/SUPPL_FILE/ES4018877_SI_001.PDF

Huset, C. A., Barlaz, M. A., Barofsky, D. F., & Field, J. A. (2011). Quantitative determination of fluorochemicals in municipal landfill leachates. *Chemosphere*, 82(10), 1380–1386. <https://doi.org/10.1016/J.CHEMOSPHERE.2010.11.072>

I

Islam, N., Garcia da Fonseca, T., Vilke, J., Gonçalves, J. M., Pedro, P., Keiter, S., Cunha, S. C., Fernandes, J. O., & Bebianno, M. J. (2021). Perfluorooctane sulfonic acid (PFOS) adsorbed to polyethylene microplastics: Accumulation and ecotoxicological effects in the clam *Scrobicularia plana*. *Marine Environmental Research*, 164, 105249. <https://doi.org/https://doi.org/10.1016/j.marenvres.2020.105249>

Ivantsova, E., Lu, A., & Martyniuk, C. J. (2024). Occurrence and toxicity mechanisms of perfluorobutanoic acid (PFBA) and perfluorobutane sulfonic acid (PFBS) in fish. *Chemosphere*, 349, 140815. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2023.140815>

J

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, 80(3), 595–602. <https://doi.org/10.1111/j.1365-2656.2011.01806.x>

Janas, U., & Barańska, A. (2008). What is the diet of *Palaemon elegans* Rathke, 1837 (Crustacea, Decapoda), a non-indigenous species in the Gulf of Gdańsk (southern Baltic Sea)? *Oceanologia*, 50(2), 221–237. <http://www.iopan.gda.pl/oceanologia/>

Janousek, R. M., Mayer, J., & Knepper, T. P. (2019). Is the phase-out of long-chain PFASs measurable as fingerprint in a defined area? Comparison of global PFAS concentrations and a monitoring study performed in Hesse, Germany from 2014 to 2018. *Trends in Analytical Chemistry*, 120, 115393. <https://doi.org/10.1016/J.TRAC.2019.01.017>

Jantzen, C. E., Annunziato, K. M., & Cooper, K. R. (2016). Behavioral, morphometric, and gene expression effects in adult zebrafish (*Danio rerio*) embryonically exposed to PFOA, PFOS, and PFNA. *Aquatic Toxicology*, 180, 123–130. <https://doi.org/10.1016/J.AQUATOX.2016.09.011>

Jardine, T. D., Kidd, K. A., & Fisk, A. T. (2006). Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. *Environmental Science and Technology*, 40(24), 7501–7511. <https://doi.org/10.1021/es061263h>

Jensen, A.A., & Leffers, H. (2008). Emerging endocrine disruptors: Perfluoroalkylated substances. *International Journal of Andrology*. 31(2), 161–169. <https://doi.org/10.1111/j.1365-2605.2008.00870.x>

Jeon, J., Kannan, K., Lim, B. J., An, K. G., & Kim, S. D. (2011). Effects of salinity and organic matter on the partitioning of perfluoroalkyl acid (PFAs) to clay particles. *Journal of Environmental Monitoring*, 13(6), 1803–1810. <https://doi.org/10.1039/C0EM00791A>

Jeong, Y., Da Silva, K. M., Iturrospe, E., Fuji, Y., Boogaerts, T., van Nuijs, A. L. N., Koelmel, J., & Covaci, A. (2022). Occurrence and contamination profile of legacy and emerging per- and polyfluoroalkyl substances

(PFAS) in Belgian wastewater using target, suspect and non-target screening approaches. *Journal of Hazardous Materials*, 437, 129378. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2022.129378>

Joerss, H., Apel, C., & Ebinghaus, R. (2019). Emerging per- and polyfluoroalkyl substances (PFASs) in surface water and sediment of the North and Baltic Seas. *Science of The Total Environment*, 686, 360–369. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.05.363>

Joerss, H., Menger, F., Tang, J., Ebinghaus, R., & Ahrens, L. (2022). Beyond the Tip of the Iceberg: Suspect Screening Reveals Point Source-Specific Patterns of Emerging and Novel Per- and Polyfluoroalkyl Substances in German and Chinese Rivers. *Environmental Science & Technology*, 56(9), 5456–5465. <https://doi.org/10.1021/acs.est.1c07987>

Johansson, H., Lindström, M., & Håkanson, L. (2001). On the modelling of the particulate and dissolved fractions of substances in aquatic ecosystems — sedimentological and ecological interactions. *Ecological Modelling*, 137(2), 225–240. [https://doi.org/https://doi.org/10.1016/S0304-3800\(00\)00439-7](https://doi.org/https://doi.org/10.1016/S0304-3800(00)00439-7)

Johnson, J. K., Michael Salerno, K., Schlesinger, D. R., Le, N. Q., Ko, J. S., & Xia, Z. (2022). Removing forever chemicals via amphiphilic functionalized membranes. *Npj Clean Water* 2022 5:1, 5(1), 1–6. <https://doi.org/10.1038/s41545-022-00193-y>

Jonker, M. T. O. (2024). Per- and Polyfluoroalkyl Substances in Water (2008–2022) and Fish (2015–2022) in The Netherlands: Spatiotemporal Trends, Fingerprints, Mass Discharges, Sources, and Bioaccumulation Factors. *Environmental Toxicology and Chemistry*, 43(5), 965–975. <https://doi.org/10.1002/etc.5846>

Jou, L. J., Chen, W. Y., & Liao, C. M. (2009). Online detection of waterborne bioavailable copper by valve daily rhythms in freshwater clam *Corbicula fluminea*. *Environmental Monitoring and Assessment*, 155(1–4), 257–272. <https://doi.org/10.1007/s10661-008-0433-0>

K

Kaboré, H. A., Goeury, K., Desrosiers, M., Vo Duy, S., Liu, J., Cabana, G., Munoz, G., & Sauvé, S. (2022). Novel and legacy per- and polyfluoroalkyl substances (PFAS) in freshwater sporting fish from background and firefighting foam impacted ecosystems in Eastern Canada. *Science of the Total Environment*, 816. <https://doi.org/10.1016/j.scitotenv.2021.151563>

Kannan, K., Tao, L., Sinclair, E., Pastva, S. D., Jude, D. J., & Giesy, J. P. (2005). Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. *Archives of Environmental Contamination and Toxicology*, 48(4), 559–566. <https://doi.org/10.1007/s00244-004-0133-x>

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Surridge, B., Hoover, D., Grace, R., & Gobas, F.A.P.C. (2009). Perfluoroalkyl contaminants in an arctic marine food web: Trophic magnification and wildlife exposure. *Environmental Science and Technology*, 43, 4037–4043. <https://doi.org/10.1021/es9003894>

Khan, B., Burgess, R. M., & Cantwell, M. G. (2023). Occurrence and Bioaccumulation Patterns of Per- and Polyfluoroalkyl Substances (PFAS) in the Marine Environment. *ACS ES&T Water*, 3(5), 1243–1259. <https://doi.org/10.1021/acsestwater.2c00296>

Kidd, K. A., Burkhard, L. P., Babut, M., Borgå, K., Muir, D. C. G., Perceval, O., Ruedel, H., Woodburn, K., & Embry, M. R. (2019). Practical advice for selecting or determining trophic magnification factors for application under the European Union Water Framework Directive. *Integrated Environmental Assessment and Management*, 15(2), 266–277. <https://doi.org/10.1002/ieam.4102>

- Kissa, E. (1994). Fluorinated surfactants: Synthesis-Properties-Applications (Surfactant science series 50). Marcel Dekker.
- Knepper, T. P., & Lange, F. T. (2012). Polyfluorinated Chemicals and Transformation Products (17th ed.). The Handbook of Environmental Chemistry.
- Koban, L. A., King, T., Huff, T. B., Furst, K. E., Nelson, T. R., Pfluger, A. R., Kuppa, M. M., & Fowler, A. E. (2024). Passive biomonitoring for per- and polyfluoroalkyl substances using invasive clams, *C. fluminea*. Journal of Hazardous Materials, 472, 134463. <https://doi.org/10.1016/j.jhazmat.2024.134463>
- Korzeniowski, S. H., Buck, R. C., Newkold, R. M., Kassmi, A. El, Laganis, E., Matsuoka, Y., Dinelli, B., Beauchet, S., Adamsky, F., Weilandt, K., Soni, V. K., Kapoor, D., Gunasekar, P., Malvasi, M., Brinati, G., & Musio, S. (2023). A critical review of the application of polymer of low concern regulatory criteria to fluoropolymers II: Fluoroplastics and fluoroelastomers. Integrated Environmental Assessment and Management, 19(2), 326–354. <https://doi.org/10.1002/IEAM.4646>
- Kottelat M., & Freyhof J. (2008). Handbook of European freshwater fishes. Ichthyological Research, 55(1), 99. <https://doi.org/10.1007/s10228-007-0012-3>
- Koutsikopoulos, C., Dorel, D., & Desaunay, Y. (1995). Movement of Sole (*Solea solea*) in the Bay of Biscay: Coastal Environment and Spawning Migration. Journal of the Marine Biological Association of the United Kingdom. 75(1), 109-126. doi:10.1017/S002531540001523X
- Kowalska, D., Sosnowska, A., Zdybel, S., Stepnik, M., & Puzyn, T. (2024). Predicting bioconcentration factors (BCFs) for Per- and Polyfluoroalkyl Substances (PFAS). Chemosphere, 364, 143146. <https://doi.org/10.1016/j.CHEMOSPHERE.2024.143146>
- Kumar, E., Koponen, J., Rantakokko, P., Airaksinen, R., Ruokojärvi, P., Kiviranta, H., Vuorinen, P. J., Myllylä, T., Keinänen, M., Raitaniemi, J., Mannio, J., Junttila, V., Nieminen, J., Venäläinen, E., & Jestoi, M. (2022). Distribution of perfluoroalkyl acids in fish species from the Baltic Sea and freshwaters in Finland. Chemosphere. 291, 132688. <https://doi.org/10.1016/j.chemosphere.2021.132688>
- Kurwadkar, S., Dane, J., Kanel, S. R., Nadagouda, M. N., Cawdrey, R. W., Ambade, B., Struckhoff, G. C., & Wilkin, R. (2022). Per- and polyfluoroalkyl substances in water and wastewater: A critical review of their global occurrence and distribution. Science of the Total Environment, 809, 151003. <https://doi.org/10.1016/j.scitotenv.2021.151003>
- Kwadijk, C.J.A.F., Korytár, P., & Koelmans, A.A. (2010). Distribution of perfluorinated compounds in aquatic systems in the Netherlands. Environmental Science and Technology, 44, 3746–3751. <https://doi.org/10.1021/es100485e>
- Kördel, W., Garelick, H., Gawlik, B. M., Kandile, N. G., Peijnenburg, W. J. G. M., & Rüdell, H. (2013). Substance-related environmental monitoring strategies regarding soil, groundwater and surface water - an overview. Environmental Science and Pollution Research, 20(5), 2810–2827. <https://doi.org/10.1007/S11356-013-1531-2/FIGURES/8>

L

- Labadie, P., & Chevreuil, M. (2011). Partitioning behaviour of perfluorinated alkyl contaminants between water, sediment and fish in the Orge River (nearby Paris, France). Environmental Pollution, 159(2), 391–397. <https://doi.org/https://doi.org/10.1016/j.envpol.2010.10.039>

- Lacroix, C., Richard, G., Segueineau, C., Guyomarch, J., Moraga, D., & Auffret, M. (2015). Active and passive biomonitoring suggest metabolic adaptation in blue mussels (*Mytilus* spp.) chronically exposed to a moderate contamination in Brest harbor (France). *Aquatic Toxicology*, 162, 126–137. <https://doi.org/10.1016/J.AQUATOX.2015.03.008>
- Lam, N. H., Cho, C. R., Lee, J. S., Soh, H. Y., Lee, B. C., Lee, J. A., Tatarozako, N., Sasaki, K., Saito, N., Iwabuchi, K., Kannan, K., & Cho, H. S. (2014). Perfluorinated alkyl substances in water, sediment, plankton and fish from Korean rivers and lakes: A nationwide survey. *Science of The Total Environment*, 491–492, 154–162. <https://doi.org/10.1016/J.SCITOTENV.2014.01.045>
- Land, M., De Wit, C. A., Bignert, A., Cousins, I. T., Herzke, D., Johansson, J. H., & Martin, J. W. (2018). What is the effect of phasing out long-chain per- and polyfluoroalkyl substances on the concentrations of perfluoroalkyl acids and their precursors in the environment? A systematic review. *Environmental Evidence*, 7, 4. <https://doi.org/10.1186/s13750-017-0114-y>
- Lang, J. R., Allred, B. M. K., Field, J. A., Levis, J. W., & Barlaz, M. A. (2017). National Estimate of Per- and Polyfluoroalkyl Substance (PFAS) Release to U.S. Municipal Landfill Leachate. *Environmental Science and Technology*, 51(4), 2197–2205. https://doi.org/10.1021/ACS.EST.6B05005/SUPPL_FILE/ES6B05005_SI_002.XLSX
- Langberg, H. A., Breedveld, G. D., Grønning, H. M., Kvennås, M., Jenssen, B. M., & Hale, S. E. (2019). Bioaccumulation of Fluorotelomer Sulfonates and Perfluoroalkyl Acids in Marine Organisms Living in Aqueous Film-Forming Foam Impacted Waters. *Environmental Science and Technology*, 53(18), 10951–10960. <https://doi.org/10.1021/acs.est.9b00927>
- Langberg, H. A., Hale, S. E., Breedveld, G. D., Jenssen, B. M., & Jartun, M. (2022). A review of PFAS fingerprints in fish from Norwegian freshwater bodies subject to different source inputs. *Environmental Science: Processes Impacts* 24, 330–342. <https://doi.org/10.1039/D1EM00408E>
- Lasier, P. J., Washington, J. W., Hassan, S. M., & Jenkins, T. M. (2011). Perfluorinated chemicals in surface waters and sediments from northwest Georgia, USA, and their bioaccumulation in *Lumbriculus variegatus*. *Environmental Toxicology and Chemistry*, 30(10), 2194–2201. <https://doi.org/10.1002/ETC.622>
- Lee, H., Deon, J., & Mabury, S. A. (2010). Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment. *Environmental Science and Technology*, 44(9), 3305–3310. https://doi.org/10.1021/ES9028183/SUPPL_FILE/ES9028183_SI_001.PDF
- Lee, J. W., Choi, K., Park, K., Seong, C., Yu, S. Do, & Kim, P. (2020). Adverse effects of perfluoroalkyl acids on fish and other aquatic organisms: A review. *Science of the Total Environment*, 707, 135334. <https://doi.org/10.1016/j.scitotenv.2019.135334>
- Lee, Y. M., Lee, J. Y., Kim, M. K., Yang, H., Lee, J. E., Son, Y., Kho, Y., Choi, K., & Zoh, K. D. (2020). Concentration and distribution of per- and polyfluoroalkyl substances (PFAS) in the Asan Lake area of South Korea. *Journal of Hazardous Materials*, 381, 120909. <https://doi.org/10.1016/J.JHAZMAT.2019.120909>
- Lemos, L., Gantiva, L., Kaylor, C., Sanchez, A., & Quinete, N. (2022). American oysters as bioindicators of emerging organic contaminants in Florida, United States. *Science of The Total Environment*, 835, 155316. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.155316>
- Lenka, S. P., Kah, M., & Padhye, L. P. (2021). A review of the occurrence, transformation, and removal of poly- and perfluoroalkyl substances (PFAS) in wastewater treatment plants. *Water Research*, 199, 117187. <https://doi.org/10.1016/J.WATRES.2021.117187>

- Luebker, D. J., Hansen, K. J., Bass, N. M., Butenhoff, J. L., & Seacat, A. M. (2002). Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology*, 176(3), 175–185. [https://doi.org/https://doi.org/10.1016/S0300-483X\(02\)00081-1](https://doi.org/https://doi.org/10.1016/S0300-483X(02)00081-1)
- Leung, S. C. E., Wanninayake, D., Chen, D., Nguyen, N.-T., & Li, Q. (2023). Physicochemical properties and interactions of perfluoroalkyl substances (PFAS) - Challenges and opportunities in sensing and remediation. *Science of The Total Environment*, 905, 166764. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.166764>
- Lewis, A. J., Yun, X., Spooner, D. E., Kurz, M. J., McKenzie, E. R., & Sales, C. M. (2022). Exposure pathways and bioaccumulation of per- and polyfluoroalkyl substances in freshwater aquatic ecosystems: Key considerations. *Science of the Total Environment*, 822, 153561. <https://doi.org/10.1016/j.scitotenv.2022.153561>
- Liang, X., Yang, X., Jiao, W., Zhou, J., & Zhu, L. (2022). Simulation modelling the structure related bioaccumulation and biomagnification of per- and polyfluoroalkyl substances in aquatic food web. *Science of The Total Environment*, 838, 156397. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.156397>
- Li, F., Duan, J., Tian, S., Ji, H., Zhu, Y., Wei, Z., & Zhao, D. (2020). Short-chain per- and polyfluoroalkyl substances in aquatic systems: Occurrence, impacts and treatment. *Chemical Engineering Journal*, 380, 122506. <https://doi.org/10.1016/J.CEJ.2019.122506>
- Li, L., Zheng, B., & Liu, L. (2010). Biomonitoring and Bioindicators Used for River Ecosystems: Definitions, Approaches and Trends. *Procedia Environmental Sciences*, 2, 1510–1524. <https://doi.org/https://doi.org/10.1016/j.proenv.2010.10.164>
- Li, Y., Oliver, D. P., & Kookana, R. S. (2018). A critical analysis of published data to discern the role of soil and sediment properties in determining sorption of per and polyfluoroalkyl substances (PFASs). *Science of The Total Environment*, 628–629, 110–120. <https://doi.org/10.1016/J.SCITOTENV.2018.01.167>
- Lindgaard, C. (1995). Classification of water-bodies and pollution. *The Chironomidae*, 385–404. https://doi.org/10.1007/978-94-011-0715-0_15
- Lin, Q., Zhang, Y., Marrs, R., Sekar, R., Luo, X., & Wu, N. (2020). Evaluating ecosystem functioning following river restoration: the role of hydromorphology, bacteria, and macroinvertebrates. *The Science of the Total Environment*, 743. <https://doi.org/10.1016/J.SCITOTENV.2020.140583>
- Liu, C., Yu, K., Shi, X., Wang, J., Lam, P. K. S., Wu, R. S. S., & Zhou, B. (2007). Induction of oxidative stress and apoptosis by PFOS and PFOA in primary cultured hepatocytes of freshwater tilapia (*Oreochromis niloticus*). *Aquatic Toxicology*, 82(2), 135–143. <https://doi.org/https://doi.org/10.1016/j.aquatox.2007.02.006>
- Liu, J., & Lee, L. S. (2005). Solubility and sorption by soils of 8:2 fluorotelomer alcohol in water and cosolvent systems. *Environmental Science and Technology*, 39(19), 7535–7540. <https://doi.org/10.1021/ES051125C>
- Liu, J., & Mejia Avendaño, S. (2013). Microbial degradation of polyfluoroalkyl chemicals in the environment: A review. *Environment International*, 61, 98–114. <https://doi.org/10.1016/J.ENVINT.2013.08.022>
- Liu, J., Zhao, X., Liu, Y., Qiao, X., Wang, X., Ma, M., Jin, X., Liu, C., Zheng, B., Shen, J., & Guo, R. (2019). High contamination, bioaccumulation and risk assessment of perfluoroalkyl substances in multiple

- environmental media at the Baiyangdian Lake. *Ecotoxicology and Environmental Safety*, 182, 109454. <https://doi.org/10.1016/J.ECOENV.2019.109454>
- Liu, M., Wei, C., Tan, L., Xu, W., Li, L., & Zhang, G. (2024). Respiration rate and intestinal microbiota as promising indicators for assessing starvation intensity and health status in Pacific oyster (*Crassostrea gigas*). *Aquaculture*, 593, 741330. <https://doi.org/https://doi.org/10.1016/j.aquaculture.2024.741330>
- Liu, M., Yi, S., Chen, P., Chen, M., Zhong, W., Yang, J., Sun, B., & Zhu, L. (2019). Thyroid endocrine disruption effects of perfluoroalkyl phosphinic acids on zebrafish at early development. *Science of The Total Environment*, 676, 290–297. <https://doi.org/10.1016/J.SCITOTENV.2019.04.177>
- Liu, W., Li, J., Gao, L., Zhang, Z., Zhao, J., He, X., & Zhang, X. (2018). Bioaccumulation and effects of novel chlorinated polyfluorinated ether sulfonate in freshwater alga *Scenedesmus obliquus*. *Environmental Pollution*, 233, 8–15. <https://doi.org/https://doi.org/10.1016/j.envpol.2017.10.039>
- Liu, Y., Junaid, M., Xu, P., Zhong, W., Pan, B., & Xu, N. (2020). Suspended sediment exacerbates perfluorooctane sulfonate mediated toxicity through reactive oxygen species generation in freshwater clam *Corbicula fluminea*. *Environmental Pollution*, 267, 115671. <https://doi.org/https://doi.org/10.1016/j.envpol.2020.115671>
- Liu, Z., Lu, Y., Wang, T., Wang, P., Li, Q., Johnson, A. C., Sarvajayakesavalu, S., & Sweetman, A. J. (2016). Risk assessment and source identification of perfluoroalkyl acids in surface and ground water: Spatial distribution around a mega-fluorochemical industrial park, China. *Environment International*, 91, 69–77. <https://doi.org/10.1016/J.ENVINT.2016.02.020>
- Lohmann, R., & Letcher, R. J. (2023). The universe of fluorinated polymers and polymeric substances and potential environmental impacts and concerns. *Current Opinion in Green and Sustainable Chemistry*, 41, 100795. <https://doi.org/https://doi.org/10.1016/j.cogsc.2023.100795>
- Loos, R., Locoro, G., Huber, T., Wollgast, J., Christoph, E. H., de Jager, A., Manfred Gawlik, B., Hanke, G., Umlauf, G., & Zaldívar, J. M. (2008). Analysis of perfluorooctanoate (PFOA) and other perfluorinated compounds (PFCs) in the River Po watershed in N-Italy. *Chemosphere*, 71(2), 306–313. <https://doi.org/10.1016/J.CHEMOSPHERE.2007.09.022>
- Lopez-Antia, A., Dauwe, T., Meyer, J., Maes, K., Bervoets, L., & Eens, M. (2017). High levels of PFOS in eggs of three bird species in the neighbourhood of a fluoro-chemical plant. *Ecotoxicology and Environmental Safety*, 139, 165–171. <https://doi.org/10.1016/j.ecoenv.2017.01.040>
- Luoma, S. N., Cain, D. J., & Rainbow, P. S. (2010). Calibrating biomonitors to ecological disturbance: A new technique for explaining metal effects in natural waters. *Integrated Environmental Assessment and Management*, 6(2), 199–209. <https://doi.org/10.1897/IEAM-2009-067.1>
- Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, 101(1), 13–30. <https://doi.org/https://doi.org/10.1016/j.aquatox.2010.10.006>
- Lyle, A. A., & Maitland, P. S. (1997). The spawning migration and conservation of smelt *Osmerus eperlanus* in the River Cree, southwest Scotland. *Biological Conservation*, 80(3), 303–311. [https://doi.org/https://doi.org/10.1016/S0006-3207\(96\)00039-0](https://doi.org/https://doi.org/10.1016/S0006-3207(96)00039-0)
- López-López, E., & Sedeño-Díaz, J. E. (2015). Biological indicators of water quality: The role of fish and macroinvertebrates as indicators of water quality. *Environmental Indicators*, 643–661. https://doi.org/10.1007/978-94-017-9499-2_37/COVER

M

- Mackie, G. L., & Schloesser, D. W. (1996). Comparative Biology of Zebra Mussels in Europe and North America: An Overview. *Integrative and Comparative Biology*, 36(3), 244–258. <https://doi.org/10.1093/ICB/36.3.244>
- Maes, J., Stevens, M., & Ollevier, F. (2005). The composition and community structure of the ichthyofauna of the upper Scheldt estuary: Synthesis of a 10-year data collection (1991-2001). *Journal of Applied Ichthyology*, 21(2), 86–93. <https://doi.org/10.1111/j.1439-0426.2004.00628.x>
- Mahoney, H., Xie, Y., Brinkmann, M., & Giesy, J. P. (2022). Next generation per- and poly-fluoroalkyl substances: Status and trends, aquatic toxicity, and risk assessment. *Eco-Environment and Health*, 1(2), 117–131. <https://doi.org/https://doi.org/10.1016/j.eehl.2022.05.002>
- Manojkumar, Y., Pilli, S., Rao, P. V., & Tyagi, R. D. (2023). Sources, occurrence and toxic effects of emerging per- and polyfluoroalkyl substances (PFAS). *Neurotoxicology and Teratology*, 97, 107174. <https://doi.org/https://doi.org/10.1016/j.ntt.2023.107174>
- Martin, J. W., Asher, B. J., Beesoon, S., Benskin, J. P., & Ross, M. S. (2010). PFOS or PreFOS? Are perfluorooctane sulfonate precursors (PreFOS) important determinants of human and environmental perfluorooctane sulfonate (PFOS) exposure? *Journal of Environmental Monitoring*, 12(11), 1979–2004. <https://doi.org/10.1039/C0EM00295J>
- Martin, J. W., Mabury, S. A., Solomon, K. R., & Muir, D. C. G. (2003). Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry*, 22(1), 196–204. <https://doi.org/10.1002/ETC.5620220126>
- Marziali, L., Rosignoli, F., Valsecchi, S., Polesello, S., & Stefani, F. (2019). Effects of Perfluoroalkyl Substances on a Multigenerational Scale: A Case Study with *Chironomus riparius* (Diptera, Chironomidae). *Environmental Toxicology and Chemistry*, 38(5), 988–999. <https://doi.org/10.1002/ETC.4392>
- Mastrantonio, M., Bai, E., Uccelli, R., Cordiano, V., Screpanti, A., & Crosignani, P. (2018). Drinking water contamination from perfluoroalkyl substances (PFAS): An ecological mortality study in the Veneto Region, Italy. *European Journal of Public Health* 28, 180–185. <https://doi.org/10.1093/eurpub/ckx066>
- Mathieson, S., Cattrijsse, A., Costa, M.J., Drake, P., Elliott, M., Gardner, J., & Marchand, J. (2000). Fish assemblages of European tidal marshes: A comparison based on species, families and functional guilds. *Marine Ecology Progress Series*, 204, 225–242. <https://doi.org/10.3354/meps204225>
- Maulvault, A. L., Camacho, C., Barbosa, V., Alves, R., Anacleto, P., Fogaça, F., Kwadijk, C., Kotterman, M., Cunha, S. C., Fernandes, J. O., Rasmussen, R. R., Sloth, J. J., Aznar-Alemany, Ò., Eljarrat, E., Barceló, D., & Marques, A. (2018). Assessing the effects of seawater temperature and pH on the bioaccumulation of emerging chemical contaminants in marine bivalves. *Environmental Research*, 161, 236–247. <https://doi.org/10.1016/j.envres.2017.11.017>
- Mazzoni, M., Polesello, S., Rusconi, M., & Valsecchi, S. (2016). Liquid chromatography mass spectrometry determination of perfluoroalkyl acids in environmental solid extracts after phospholipid removal and on-line turbulent flow chromatography purification. *Journal of Chromatography A*, 1453, 62–70. <https://doi.org/10.1016/J.CHROMA.2016.05.047>
- Mazzoni, M., Ferrario, C., Bettinetti, R., Piscia, R., Cicala, D., Volta, P., Borgå, K., Valsecchi, S., & Polesello, S. (2020). Trophic magnification of legacy (PCB, DDT and Hg) and emerging pollutants (PFAS) in the fish

community of a small protected southern alpine lake (Lake Mergozzo, Northern Italy). *Water*, 12(6). <https://doi.org/10.3390/W12061591>

McGoran, A. R., Clark, P. F., & Morritt, D. (2017). Presence of microplastic in the digestive tracts of European flounder, *Platichthys flesus*, and European smelt, *Osmerus eperlanus*, from the River Thames. *Environmental Pollution*, 220, 744–751. <https://doi.org/10.1016/j.envpol.2016.09.078>

McLachlan, M. S., Holmstrom, K. E., Reth, M., & Berger, U. (2007). Riverine discharge of perfluorinated carboxylates from the European continent. *Environmental Science and Technology*, 41(21), 7260–7265. https://doi.org/10.1021/ES071471P/SUPPL_FILE/ES071471PSI20070813_082042.PDF

McMahon, K. W., Hamady, L. L., & Thorrold, S. R. (2013). A review of ecogeochemistry approaches to estimating movements of marine animals. *Limnology and Oceanography*, 58(2), 697–714. <https://doi.org/https://doi.org/10.4319/lo.2013.58.2.0697>

Meire, P., Ysebaert, T., Van Damme, S., Van Den Bergh, E., Maris, T., & Struyf, E. (2005). The Scheldt estuary: A description of a changing ecosystem. *Hydrobiologia*, 540(1–3), 1–11. <https://doi.org/10.1007/s10750-005-0896-8>

Menegatto, M., Lezzi, S., Musolino, M., & Zamperini, A. (2022). The Psychological Impact of Per- and Poly-Fluoroalkyl Substances (PFAS) Pollution in the Veneto Region, Italy: A Qualitative Study with Parents. *International Journal of Environmental Research and Public Health*, 19(22), 14761. <https://doi.org/10.3390/IJERPH192214761>

Mersch, J., Wagner, P., & Pihan, J.-C. (1996). Copper in indigenous and transplanted zebra mussels in relation to changing water concentrations and body weight. *Environmental Toxicology and Chemistry*, 15(6), 886–893. <https://doi.org/10.1002/ETC.5620150610>

Middelburg, J. J., & Nieuwenhuize, J. (1998). Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. *Marine Chemistry*, 60(3–4), 217–225. [https://doi.org/10.1016/S0304-4203\(97\)00104-7](https://doi.org/10.1016/S0304-4203(97)00104-7)

Milinic, J., Lacorte, S., Vidal, M., & Rigol, A. (2015). Sorption behaviour of perfluoroalkyl substances in soils. *Science of the Total Environment*, 511, 63–71. <https://doi.org/10.1016/J.SCITOTENV.2014.12.017>

Minier, C., Abarnou, A., Jaouen-Madoulet, A., le Guellec, A. M., Tutundjian, R., Bocquené, G., & Leboulenger, F. (2006). A pollution-monitoring pilot study involving contaminant and biomarker measurements in the Seine Estuary, France, using zebra mussels (*Dreissena polymorpha*). *Environmental Toxicology and Chemistry*, 25(1), 112–119. <https://doi.org/10.1897/05-161R.1>

Miranda, D. A., Benskin, J. P., Awad, R., Lepoint, G., Leonel, J., & Hatje, V. (2021). Bioaccumulation of Per- and polyfluoroalkyl substances (PFASs) in a tropical estuarine food web. *Science of the Total Environment*, 754, 142–146. <https://doi.org/10.1016/j.scitotenv.2020.142146>

Miranda, D. de A., Peaslee, G. F., Zachritz, A. M., & Lamberti, G. A. (2022). A worldwide evaluation of trophic magnification of per- and polyfluoroalkyl substances in aquatic ecosystems. *Integrated Environmental Assessment and Management*, 18(6), 1500–1512. <https://doi.org/10.1002/ieam.4579>

Moens, T., Bouillon, S., & Gallucci, F. (2005). Dual stable isotope abundances unravel trophic position of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom*, 85(6), 1401–1407. <https://doi.org/10.1017/S0025315405012580>

- Mousavi, S. K., Primicerio, R., & Amundsen, P. A. (2003). Diversity and structure of Chironomidae (Diptera) communities along a gradient of heavy metal contamination in a subarctic watercourse. *Science of The Total Environment*, 307(1–3), 93–110. [https://doi.org/10.1016/S0048-9697\(02\)00465-5](https://doi.org/10.1016/S0048-9697(02)00465-5)
- Moschino, V., Del Negro, P., De Vittor, C., & Da Ros, L. (2016). Biomonitoring of a polluted coastal area (Bay of Muggia, Northern Adriatic Sea): A five-year study using transplanted mussels. *Ecotoxicology and Environmental Safety*, 128, 1–10. <https://doi.org/10.1016/J.ECOENV.2016.02.006>
- Mubiana, V. K., Vercauteren, K., & Blust, R. (2006). The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*. *Environmental Pollution*, 144(1), 272–279. <https://doi.org/https://doi.org/10.1016/j.envpol.2005.12.017>
- Muir, D., & Miaz, L. T. (2021). Spatial and Temporal Trends of Perfluoroalkyl Substances in Global Ocean and Coastal Waters. *Environmental Science and Technology*, 55(14), 9527–9537. <https://doi.org/10.1021/acs.est.0c08035>
- Munoz, G., Budzinski, H., & Labadie, P. (2017a). Influence of environmental factors on the fate of legacy and emerging per- and polyfluoroalkyl substances along the salinity/turbidity gradient of a macrotidal estuary. *Environmental Science and Technology*, 51(21). https://doi.org/10.1021/ACS.EST.7B03626/SUPPL_FILE/ES7B03626_SI_001.PDF
- Munoz, G., Budzinski, H., Babut, M., Drouineau, H., Lauzent, M., Menach, K. Le, Lobry, J., Selleslagh, J., Simonnet-Laprade, C., & Labadie, P. (2017b). Evidence for the Trophic Transfer of Perfluoroalkylated Substances in a Temperate Macrotidal Estuary. *Environmental Science and Technology*, 51, 8450–8459. <https://doi.org/10.1021/acs.est.7b02399>
- Munoz, G., Fechner, L. C., Geneste, E., Pardon, P., Budzinski, H., & Labadie, P. (2018). Spatio-temporal dynamics of per and polyfluoroalkyl substances (PFASs) and transfer to periphytic biofilm in an urban river: case-study on the River Seine. *Environmental Science and Pollution Research*, 25(24), 23574–23582. <https://doi.org/10.1007/S11356-016-8051-9/FIGURES/3>
- Munoz, G., Giraudel, J.-L., Botta, F., Lestremau, F., Dévier, M.-H., Budzinski, H., & Labadie, P. (2015). Spatial distribution and partitioning behavior of selected poly- and perfluoroalkyl substances in freshwater ecosystems: A French nationwide survey. *Science of The Total Environment*, 517, 48–56. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2015.02.043>
- Munoz, G., Liu, J., Vo Duy, S., & Sauvé, S. (2019). Analysis of F-53B, Gen-X, NaDONA, and emerging fluoroalkylether substances in environmental and biomonitoring samples: A review. *Trends in Environmental Analytical Chemistry*, 23, e00066–e00066. <https://doi.org/https://doi.org/10.1016/j.teac.2019.e00066>
- Munoz, G., Mercier, L., Duy, S. V., Liu, J., Sauvé, S., & Houde, M. (2022). Bioaccumulation and trophic magnification of emerging and legacy per- and polyfluoroalkyl substances (PFAS) in a St. Lawrence River food web. *Environmental Pollution*, 309, 119739. <https://doi.org/https://doi.org/10.1016/j.envpol.2022.119739>
- Munsch, C., Bely, N., Pollono, C., & Aminot, Y. (2019). Perfluoroalkyl substances (PFASs) in the marine environment: Spatial distribution and temporal profile shifts in shellfish from French coasts. *Chemosphere*, 228, 640–648. <https://doi.org/10.1016/j.chemosphere.2019.04.205>
- Murphy, P. M., & Hewat, T. (2008). Fluorosurfactants in Enhanced Oil Recovery. *The Open Petroleum Engineering Journal*, 1, 58–61. <http://dx.doi.org/10.2174/1874834100801010058>

Murshed, R., Lopez-Lauri, F., & Sallanon, H. (2008). Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. *Analytical Biochemistry*, 383(2), 320–322. <https://doi.org/https://doi.org/10.1016/j.ab.2008.07.020>

Muus, B.J. & Dahlmstrom, P. (1978). Meeresfische in Farben. Die bekanntesten Fische der Nordsee, Ostsee und des Atlantiks. Biologie, Fang, Wirtschaftliche Bedeutung. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, 52, 460–460. <https://doi.org/10.1002/iroh.19670520320>

Muylaert, K., Sabbe, K., & Vyverman, W. (2009). Changes in phytoplankton diversity and community composition along the salinity gradient of the Schelde estuary (Belgium/The Netherlands). *Estuarine, Coastal and Shelf Science*, 82(2), 335–340. <https://doi.org/https://doi.org/10.1016/j.ecss.2009.01.024>

Möller, A., Ahrens, L., Surm, R., Westerveld, J., Van Der Wielen, F., Ebinghaus, R., & De Voogt, P. (2010). Distribution and sources of polyfluoroalkyl substances (PFAS) in the River Rhine watershed. *Environmental Pollution*, 158(10), 3243–3250. <https://doi.org/10.1016/j.envpol.2010.07.019>

N

Nakagawa, H., Hirata, T., Terada, T., Jutabha, P., Miura, D., Harada, K. H., Inoue, K., Anzai, N., Endou, H., Inui, K., Kanai, Y., & Koizumi, A. (2008). Roles of Organic Anion Transporters in the Renal Excretion of Perfluorooctanoic Acid. *Basic & Clinical Pharmacology & Toxicology*, 103(1), 1–8. <https://doi.org/https://doi.org/10.1111/j.1742-7843.2007.00155.x>

Navarro, D. A., Oliver, D. P., Simpson, S. L., & Kookana, R. S. (2022). Organic carbon and salinity affect desorption of PFAS from estuarine sediments. *Journal of Soils and Sediments*, 22(4), 1302–1314. <https://doi.org/10.1007/S11368-022-03172-5/FIGURES/5>

Ng, C.A., & Hungerbühler, K. (2013). Bioconcentration of perfluorinated alkyl acids: How important is specific binding? *Environmental Science and Technology*, 47, 7214–7223. <https://doi.org/10.1021/es400981a>

Nieto, C., Ovando, X. M. C., Loyola, R., Izquierdo, A., Romero, F., Molineri, C., Rodríguez, J., Rueda Martín, P., Fernández, H., Manzo, V., & Miranda, M. J. (2017). The role of macroinvertebrates for conservation of freshwater systems. *Ecology and Evolution*, 7(14), 5502–5513. <https://doi.org/10.1002/ECE3.3101>

Nilin, J., Pestana, J. L. T., Ferreira, N. G., Loureiro, S., Costa-Lotufo, L. V., & Soares, A. M. V. M. (2012). Physiological responses of the European cockle *Cerastoderma edule* (Bivalvia: Cardidae) as indicators of coastal lagoon pollution. *Science of The Total Environment*, 435–436, 44–52. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2012.06.107>

Nobles, T., & Zhang, Y. (2015). Survival, growth and condition of freshwater mussels: Effects of municipal wastewater effluent. *PLOS ONE*, 10(6). <https://doi.org/10.1371/journal.pone.0128488>

Norton, G. M. (2021). Polyethylene - The Material of Chance . In *Ten Materials That Shaped Our World* (pp. 145–159). Springer, Cham.

O

Ohoru, C. R., Amaku, J. F., Conradie, J., Olisah, C., Akpomie, K. G., Malloum, A., Akpotu, S. O., Adegoke, K. A., Okeke, E. S., & Omotola, E. O. (2024). Effect of physicochemical parameters on the occurrence of per-

and polyfluoroalkyl substances (PFAS) in aquatic environment. *Marine Pollution Bulletin*, 208, 117040. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2024.117040>

Oliver, D. P., Li, Y., Orr, R., Nelson, P., Barnes, M., McLaughlin, M. J., & Kookana, R. S. (2019). The role of surface charge and pH changes in tropical soils on sorption behaviour of per- and polyfluoroalkyl substances (PFASs). *Science of The Total Environment*, 673, 197–206. <https://doi.org/10.1016/J.SCITOTENV.2019.04.055>

O’Leary, M. H. (1988). Carbon Isotopes in Photosynthesis: Fractionation techniques may reveal new aspects of carbon dynamics in plants. *BioScience*, 38(5), 328–336. <https://doi.org/10.2307/1310735>

P

Pan, C.G., Zhao, J.L., Liu, Y.S., Zhang, Q.Q., Chen, Z.F., Lai, H.J., Peng, F.J., Liu, S.S., & Ying, G.G. (2014). Bioaccumulation and risk assessment of per- and polyfluoroalkyl substances in wild freshwater fish from rivers in the Pearl River Delta region, South China. *Ecotoxicology and Environmental Safety*, 107, 192–199. <https://doi.org/10.1016/j.ecoenv.2014.05.031>

Pan, Y., Zhang, H., Cui, Q., Sheng, N., Yeung, L. W. Y., Sun, Y., Guo, Y., & Dai, J. (2018). Worldwide Distribution of Novel Perfluoroether Carboxylic and Sulfonic Acids in Surface Water. *Environmental Science and Technology*, 52(14), 7621–7629. <https://doi.org/10.1021/acs.est.8b00829>

Peltier, G. L., Wright, M. S., Hopkins, W. A., & Meyer, J. L. (2009). Accumulation of trace elements and growth responses in *Corbicula fluminea* downstream of a coal-fired power plant. *Ecotoxicology and Environmental Safety*, 72(5), 1384–1391. <https://doi.org/https://doi.org/10.1016/j.ecoenv.2009.01.011>

Penland, T. N., Cope, W. G., Kwak, T. J., Strynar, M. J., Grieshaber, C. A., Heise, R. J., & Sessions, F. W. (2020). Trophodynamics of Per-and Polyfluoroalkyl Substances in the Food Web of a Large Atlantic Slope River. *Environmental Science and Technology*, 54(11), 6800–6811. https://doi.org/10.1021/ACS.EST.9B05007/ASSET/IMAGES/LARGE/ES9B05007_0005.JPEG

Perkins, M. J., McDonald, R. A., van Veen, F. J. F., Kelly, S. D., Rees, G., & Bearhop, S. (2014). Application of Nitrogen and Carbon Stable Isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) to Quantify Food Chain Length and Trophic Structure. *PLOQ ONE* 9(3), e93281. <https://doi.org/10.1371/journal.pone.0093281>

Persson, A., & Nilsson, E. (2007). Foraging Behavior of Benthic Fish as an Indicator of Ecosystem State in Shallow Lakes. *Israel Journal of Ecology and Evolution*, 53(3–4), 407–421. <https://doi.org/https://doi.org/10.1560/IJEE.53.3.407>

Peterson, B. J., & Fry, B. (1987). Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics*, 18, 293–320. <https://doi.org/10.1146/annurev.es.18.110187.001453>

Peterson, B. J., & Howarth, R. W. (1987). Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography*, 32(6), 1195–1213. <https://doi.org/10.4319/lo.1987.32.6.1195>

Pi, N., Ng, J. Z., & Kelly, B. C. (2017). Uptake and elimination kinetics of perfluoroalkyl substances in submerged and free-floating aquatic macrophytes: Results of mesocosm experiments with *Echinodorus horemanii* and *Eichhornia crassipes*. *Water Research*, 117, 167–174. <https://doi.org/10.1016/J.WATRES.2017.04.003>

Pietropoli, E., Bardhi, A., Simonato, V., Zanella, M., Iori, S., Barbarossa, A., Giantin, M., Dacasto, M., De Liguoro, M., & Pauletto, M. (2024). Comparative toxicity assessment of alternative versus legacy PFAS: Implications for two primary trophic levels in freshwater ecosystems. *Journal of Hazardous Materials*, 477, 135269. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2024.135269>

Pinnegar, J. K., Jennings, S., O'Brien, C. M., & Polunin, N. V. C. (2002). Long-term changes in the trophic level of the Celtic Sea fish community and fish market price distribution. *Journal of Applied Ecology*, 39(3), 377–390. <https://doi.org/10.1046/j.1365-2664.2002.00723.x>

Pinzone, M., Damseaux, F., Michel, L. N., & Das, K. (2019). Stable isotope ratios of carbon, nitrogen and sulphur and mercury concentrations as descriptors of trophic ecology and contamination sources of Mediterranean whales. *Chemosphere*, 237. <https://doi.org/10.1016/j.chemosphere.2019.124448>

Pitter, G., Da Re, F., Canova, C., Barbieri, G., Jeddi, M.Z., Daprà, F., Manea, F., Zolin, R., Bettega, A.M., Stopazzolo, G., Vittorii, S., Zambelli, L., Martuzzi, M., Mantoan, D., & Russo, F. (2020). Serum levels of perfluoroalkyl substances (PFAS) in adolescents and young adults exposed to contaminated drinking water in the Veneto region, Italy: A cross-sectional study based on a health surveillance program. *Environmental Health Perspectives*, 128(2), 027007. <https://doi.org/10.1289/EHP5337>

Piva, E., Schumann, S., Dotteschini, S., Brocca, G., Radaelli, G., Marion, A., Irato, P., Bertotto, D., & Santovito, G. (2022). Antioxidant Responses Induced by PFAS Exposure in Freshwater Fish in the Veneto Region. *Antioxidants*, 11(6), 1115. <https://doi.org/10.3390/antiox11061115>

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, 83(3), 703–718. [https://doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montaña, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152(1), 179–189. <https://doi.org/10.1007/s00442-006-0630-x>

Powley, C. R., George, S. W., Ryan, T. W., & Buck, R. C. (2005). Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. *Analytical Chemistry*, 77(19), 6353–6358. <https://doi.org/10.1021/ac0508090>

Prevedouros, K., Cousins, I. T., Buck, R. C., & Korzeniowski, S. H. (2006). Sources, fate and transport of perfluorocarboxylates. *Environmental Science and Technology*, 40(1), 32–44. https://doi.org/10.1021/ES0512475/SUPPL_FILE/ES0512475.PDF

Prosser, R. S., Mahon, K., Sibley, P. K., Poirier, D., & Watson-Leung, T. (2016). Bioaccumulation of perfluorinated carboxylates and sulfonates and polychlorinated biphenyls in laboratory-cultured *Hexagenia* spp., *Lumbriculus variegatus* and *Pimephales promelas* from field-collected sediments. *Science of The Total Environment*, 543, 715–726. <https://doi.org/10.1016/J.SCITOTENV.2015.11.062>

Q

Quinete, N., Wu, Q., Zhang, T., Yun, S. H., Moreira, I., & Kannan, K. (2009). Specific profiles of perfluorinated compounds in surface and drinking waters and accumulation in mussels, fish, and dolphins from southeastern Brazil. *Chemosphere*, 77(6), 863–869. <https://doi.org/10.1016/J.CHEMOSPHERE.2009.07.079>

R

- Rainbow, P. S., Hildrew, A. G., Smith, B. D., Geatches, T., & Luoma, S. N. (2012). Caddisflies as biomonitors identifying thresholds of toxic metal bioavailability that affect the stream benthos. *Environmental Pollution*, 166, 196–207. <https://doi.org/10.1016/j.envpol.2012.03.017>
- Rao, D. G. V. P., & Khan, M. A. Q. (2000). Zebra Mussels: Enhancement of Copper Toxicity by High Temperature and Its Relationship with Respiration and Metabolism. *Water Environment Research*, 72(2), 175–178. <https://doi.org/10.2175/106143000x137257>
- Reif, D., Zoboli, O., Wolfram, G., Amann, A., Saracevic, E., Riedler, P., Hainz, R., Hintermaier, S., Krampe, J., & Zessner, M. (2022). Pollutant source or sink? Adsorption and mobilization of PFOS and PFOA from sediments in a large shallow lake with extended reed belt. *Journal of Environmental Management*, 320, 115871. <https://doi.org/10.1016/J.JENVMAN.2022.115871>
- Reinikainen, J., Bouhoule, E., & Sorvari, J. (2024). Inconsistencies in the EU regulatory risk assessment of PFAS call for readjustment. *Environment International*, 186. <https://doi.org/10.1016/j.envint.2024.108614>
- Ren, J., Point, A. D., Fakouri Baygi, S., Fernando, S., Hopke, P. K., Holsen, T. M., & Crimmins, B. S. (2022). Bioaccumulation of polyfluoroalkyl substances in the Lake Huron aquatic food web. *Science of The Total Environment*, 819, 152974. <https://doi.org/10.1016/j.scitotenv.2022.152974>
- Rericha, Y., Cao, D., Truong, L., Simonich, M. T., Field, J. A., & Tanguay, R. L. (2022). Sulfonamide functional head on short-chain perfluorinated substance drives developmental toxicity. *Science*, 25(2), 103789. <https://doi.org/10.1016/j.isci.2022.103789>
- Rericha, Y., Cao, D., Truong, L., Simonich, M., Field, J. A., & Tanguay, R. L. (2021). Behavior Effects of Structurally Diverse Per- And Polyfluoroalkyl Substances in Zebrafish. *Chemical Research in Toxicology*, 34(6), 1409–1416. https://doi.org/10.1021/ACS.CHEMRESTOX.1C00101/SUPPL_FILE/TX1C00101_SI_003.XLSX
- Resh, V. H., Norris, R. H., & Barbour, M. T. (1995). Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. *Australian Journal of Ecology*, 20(1), 108–121. <https://doi.org/10.1111/J.1442-9993.1995.TB00525.X>
- Riva, C., Binelli, A., & Provini, A. (2008). Evaluation of several priority pollutants in zebra mussels (*Dreissena polymorpha*) in the largest Italian subalpine lakes. *Environmental Pollution (Barking, Essex : 1987)*, 151(3), 652–662. <https://doi.org/10.1016/J.ENVPOL.2007.03.016>
- Rochette, S., Rivot, E., Morin, J., Mackinson, S., Riou, P., & Le Pape, O. (2010). Effect of nursery habitat degradation on flatfish population: application to *Solea solea* in the Eastern Channel (Western Europe). *Journal of Sea Research*, 64(1–2), 34–44. <https://doi.org/10.1007/s10980-021-01394-5>
- Romer, C. D. (1988). World War I and the postwar depression A reinterpretation based on alternative estimates of GNP. *Journal of Monetary Economics*, 22(1), 91–115. [https://doi.org/10.1016/0304-3932\(88\)90171-7](https://doi.org/10.1016/0304-3932(88)90171-7)
- Roméo, M., Mourgaud, Y., Geffard, A., Gnassia-Barelli, M., Amiard, J. C., & Budzinski, H. (2003). Multimarker approach in transplanted mussels for evaluating water quality in Charentes, France, coast areas exposed to different anthropogenic conditions. *Environmental Toxicology*, 18(5), 295–305. <https://doi.org/10.1002/tox.10128>
- Rosewarne, P. J., Mortimer, R. J. G., Newton, R. J., Grocock, C., Wing, C. D., & Dunn, A. M. (2016). Feeding behaviour, predatory functional responses and trophic interactions of the invasive Chinese mitten crab

(*Eriocheir sinensis*) and signal crayfish (*Pacifastacus leniusculus*). *Freshwater Biology*, 61(4), 426–443. <https://doi.org/https://doi.org/10.1111/fwb.12717>

Rusconi, M., Marziali, L., Stefani, F., Valsecchi, S., Bettinetti, R., Mazzoni, M., Rosignoli, F., & Polesello, S. (2015). Evaluating the impact of a fluoropolymer plant on a river macrobenthic community by a combined chemical, ecological and genetic approach. *Science of The Total Environment*, 538, 654–663. <https://doi.org/10.1016/J.SCITOTENV.2015.08.086>

S

Saether, O. A. (1979). Chironomid communities as water quality indicators. *Ecography*, 2(2), 65–74. <https://doi.org/10.1111/J.1600-0587.1979.TB00683.X>

Santana, M. S., Yamamoto, F. Y., Sandrini-Neto, L., Filipak Neto, F., Ortolani-Machado, C. F., Oliveira Ribeiro, C. A., & Prodocimo, M. M. (2018). Diffuse sources of contamination in freshwater fish: Detecting effects through active biomonitoring and multi-biomarker approaches. *Ecotoxicology and Environmental Safety*, 149, 173–181. <https://doi.org/10.1016/J.ECOENV.2017.11.036>

Sant, K. E., Sinno, P. P., Jacobs, H. M., & Timme-Laragy, A. R. (2018). Nrf2a modulates the embryonic antioxidant response to perfluorooctanesulfonic acid (PFOS) in the zebrafish, *Danio rerio*. *Aquatic Toxicology*, 198, 92–102. <https://doi.org/https://doi.org/10.1016/j.aquatox.2018.02.010>

SCHEER. (2022). Final Opinion on Draft Environmental Quality Standards for Priority Substances under the Water Framework Directive - PFAS.

Schiffer, C., Muller, A., Egeberg, D.L., Alvarez, L., Brenker, C., Rehfeld, A., Frederiksen, H., Waschle, B., Kaupp, U.B., Balbach, M., Wachten, D., Skakkebaek, N.E., Almstrup, K., & Strunker, T. (2014). Direct action of endocrine disrupting chemicals on human sperm. *EMBO Reports*, 15, 758–765. <https://doi.org/10.15252/embr.201438869>

Schuetze, A., Heberer, T., Effkemann, S., & Juergensen, S., 2010. Occurrence and assessment of perfluorinated chemicals in wild fish from Northern Germany. *Chemosphere* 78, 647–652. <https://doi.org/10.1016/j.chemosphere.2009.12.015>

Schymanski, E. L., Zhang, J., Thiessen, P. A., Chirsir, P., Kondic, T., & Bolton, E. E. (2023). Per- and polyfluoroalkyl substances (PFAS) in PubChem: 7 million and growing. *Environmental Science and Technology*, 57(44), 16918–16928. <https://doi.org/10.1021/acs.est.3c04855>

Science History Institute. (n.d.). Roy J. Plunkett. <https://www.sciencehistory.org/education/scientific-biographies/roy-j-plunkett/>

Semerád, J., Horká, P., Filipová, A., Kukla, J., Holubová, K., Musilová, Z., Jandová, K., Frouz, J., & Cajthaml, T. (2022). The driving factors of per- and polyfluorinated alkyl substance (PFAS) accumulation in selected fish species: The influence of position in river continuum, fish feed composition, and pollutant properties. *Science of The Total Environment*, 816, 151662. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.151662>

Shi, Y., Vestergren, R., Nost, T. H., Zhou, Z., & Cai, Y. (2018). Probing the Differential Tissue Distribution and Bioaccumulation Behavior of Per- and Polyfluoroalkyl Substances of Varying Chain-Lengths, Isomeric Structures and Functional Groups in Crucian Carp. *Environmental Science and Technology*, 52(8), 4592–4600. <https://doi.org/10.1021/acs.est.7b06128>

- Shi, Y., Vestergren, R., Zhou, Z., Song, X., Xu, L., Liang, Y., & Cai, Y. (2015). Tissue Distribution and Whole Body Burden of the Chlorinated Polyfluoroalkyl Ether Sulfonic Acid F-53B in Crucian Carp (*Carassius carassius*): Evidence for a Highly Bioaccumulative Contaminant of Emerging Concern. *Environmental Science and Technology*, 49(24), 14156–14165. <https://doi.org/10.1021/acs.est.5b04299>
- Shi, Y., Wang, J., Pan, Y., & Cai, Y. (2012). Tissue distribution of perfluorinated compounds in farmed freshwater fish and human exposure by consumption. *Environmental Toxicology and Chemistry*, 31(4), 717–723. <https://doi.org/10.1002/etc.1758>
- Shoeib, M., Harner, T., Wilford, B.H., Jones, K.C., & Zhu, J. (2005). Perfluorinated sulfonamides in indoor and outdoor air and indoor dust: Occurrence, partitioning, and human exposure. *Environmental Science and Technology* 39, 6599–6606. <https://doi.org/10.1021/es048340y>
- Simonnet-Laprade, C., Budzinski, H., Maciejewski, K., Le Menach, K., Santos, R., Alliot, F., Goutte, A., & Labadie, P. (2019). Biomagnification of perfluoroalkyl acids (PFAAs) in the food web of an urban river: assessment of the trophic transfer of targeted and unknown precursors and implications. *Environmental Science: Processes & Impacts*, 21(11), 1864–1874. <https://doi.org/10.1039/C9EM00322C>
- Sinclair, G. M., Long, S. M., & Jones, O. A. H. (2020). What are the effects of PFAS exposure at environmentally relevant concentrations? *Chemosphere*, 258, 127340. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2020.127340>
- Smolders, R., Bervoets, L., & Blust, R. (2002). Transplanted zebra mussels (*Dreissena polymorpha*) as active biomonitors in an effluent-dominated river. *Environmental Toxicology and Chemistry*, 21(9), 1889–1896. <https://doi.org/10.1002/etc.5620210916>
- Smolders, R., Bervoets, L., Wepener, V., & Blust, R. (2003). A Conceptual Framework for Using Mussels as Biomonitors in Whole Effluent Toxicity. *Human and Ecological Risk Assessment: An International Journal*, 9(3), 741–760. <https://doi.org/10.1080/713609965>
- So, M. K., Taniyasu, S., Lam, P. K. S., Zheng, G. J., Giesy, J. P., & Yamashita, N. (2006). Alkaline digestion and solid phase extraction method for perfluorinated compounds in mussels and oysters from south China and Japan. *Archives of Environmental Contamination and Toxicology*, 50(2), 240–248. <https://doi.org/10.1007/S00244-005-7058-X/FIGURES/3>
- Sousa, R., Antunes, C., & Guilhermino, L. (2008). Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. *Annales de Limnologie - International Journal of Limnology*, 44(2), 85–94. <https://doi.org/DOI: 10.1051/limn:2008017>
- Stevenson, C. N., MacManus-Spencer, L. A., Luckenbach, T., Luthy, R. G., & Epel, D. (2006). New Perspectives on Perfluorochemical Ecotoxicology: Inhibition and Induction of an Efflux Transporter in the Marine Mussel, *Mytilus californianus*. *Environmental Science & Technology*, 40(17), 5580–5585. <https://doi.org/10.1021/es0602593>
- Stock, N. L., Furdui, V. I., Muir, D. C. G., & Mabury, S. A. (2007). Perfluoroalkyl contaminants in the Canadian Arctic: evidence of atmospheric transport and local contamination. *Environmental Science & Technology*, 41(10), 3529–3536. <https://doi.org/10.1021/ES062709X>
- Stronkhorst, J. (1993). The environmental risks of pollution in the Scheldt estuary. *Netherlands Journal of Aquatic Ecology*, 27(2), 383–393. <https://doi.org/10.1007/BF02334800>

Su, L., Cai, H., Kolandhasamy, P., Wu, C., Rochman, C. M., & Shi, H. (2018). Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. *Environmental Pollution*, 234, 347–355. <https://doi.org/https://doi.org/10.1016/j.envpol.2017.11.075>

Sukhotin, A. A. (1992). Respiration and energetics in mussels (*Mytilus edulis* L.) cultured in the White Sea. *Aquaculture*, 101(1–2), 41–57. [https://doi.org/10.1016/0044-8486\(92\)90231-9](https://doi.org/10.1016/0044-8486(92)90231-9)

Summers, R. W. (1979). Life cycle and population ecology of the flounder *Platichthys flesus* (L.) in the Ythan estuary, Scotland. *Journal of Natural History*, 13(6), 703–723. <https://doi.org/10.1080/00222937900770531>

Sun, J. M., Kelly, B. C., Gobas, F. A. P. C., & Sunderland, E. M. (2022). A food web bioaccumulation model for the accumulation of per- and polyfluoroalkyl substances (PFAS) in fish: how important is renal elimination?. *Environmental science. Processes & impacts*, 24(8), 1152–1164. <https://doi.org/10.1039/d2em00047d>

Suski, J. G., Salice, C. J., Chanov, M. K., Ayers, J., Rewerts, J., & Field, J. (2021). Sensitivity and Accumulation of Perfluorooctanesulfonate and Perfluorohexanesulfonic Acid in Fathead Minnows (*Pimephales promelas*) Exposed over Critical Life Stages of Reproduction and Development. *Environmental Toxicology and Chemistry*, 40(3), 811–819. <https://doi.org/10.1002/ETC.4936>

T

Taal, I., Saks, L., Nedolgora, S., Verliin, A., Kesler, M., Jürgens, K., Svirgsden, R., Vetemaa, M., & Saat, T. (2014). Diet composition of smelt *Osmerus eperlanus* (Linnaeus) in brackish near-shore ecosystem (Eru Bay, Baltic Sea). *Ecology of Freshwater Fish*, 23(2), 121–128. <https://doi.org/10.1111/eff.12044>

Teal, L.R., de Leeuw, J.J., van der Veer, H.W., & Rijnsdorp, A.D. (2008). Effects of climate change on growth of 0- group sole and plaice. *Marine Ecology Progress Series* 358, 219–230. <https://doi.org/10.3354/meps07367>

Teichert, N., Lizé, A., Tabouret, H., Roussel, J. M., Bareille, G., Trancart, T., Acou, A., Virag, L. S., Pécheyran, C., Carpentier, A., & Feunteun, E. (2022). European flounder foraging movements in an estuarine nursery seascape inferred from otolith microchemistry and stable isotopes: Fish foraging movements in nursery seascape. *Marine Environmental Research*, 182. <https://doi.org/10.1016/j.marenvres.2022.105797>

Teunen, L., Bervoets, L., Belpaire, C., De Jonge, M., & Groffen, T. (2021). PFAS accumulation in indigenous and translocated aquatic organisms from Belgium, with translation to human and ecological health risk. *Environmental Sciences Europe*, 33(1). <https://doi.org/10.1186/s12302-021-00477-z>

Teunen, L., De Jonge, M., Malarvannan, G., Covaci, A., Belpaire, C., Focant, J. F., Blust, R., & Bervoets, L. (2022). The relevance of European Biota Quality Standards on the ecological water quality as determined by the multimetric macro-invertebrate index: A Flemish case study. *Ecotoxicology and Environmental Safety*, 231, 113222. <https://doi.org/10.1016/j.ecoenv.2022.113222>

Tomy, G. T., Budakowski, W., Halldorson, T., Helm, P. A., Stern, G. A., Friesen, K., Pepper, K., Tittlemier, S. A., & Fisk, A. T. (2004). Fluorinated organic compounds in an Eastern arctic marine food web. *Environmental Science and Technology*, 38(24), 6475–6481. <https://doi.org/10.1021/es049620g>

Tomy, G. T., Pleskach, K., Ferguson, S. H., Hare, J., Stern, G., Macinnis, G., Marvin, C. H., & Loseto, L. (2009). Trophodynamics of some PFCs and BFRs in a western Canadian Arctic marine food web. *Environmental Science and Technology*, 43(11), 4076–4081. <https://doi.org/10.1021/es900162n>

Touaylia, S., Khazri, A., Mezni, A., & Bejaoui, M. (2019). Effects of emerging persistent organic pollutant perfluorooctane sulfonate (PFOS) on the Crustacean *Gammarus insensibilis*. *Human and Ecological Risk Assessment: An International Journal*, 25(8), 2133–2141. <https://doi.org/10.1080/10807039.2018.1489717>

Trigos, S., García-March, J. R., Vicente, N., Tena, J., & Torres, J. (2015). Respiration rates of the fan mussel *Pinna nobilis* at different temperatures. *Journal of Molluscan Studies*, 81(2), 217–222. <https://doi.org/10.1093/mollus/eyu075>

Trueman, C. N., Mackenzie, K. M., & Palmer, M. R. (2012). Identifying migrations in marine fishes through stable-isotope analysis. *Journal of Fish Biology*, 81(2), 826–847. <https://doi.org/10.1111/j.1095-8649.2012.03361.x>

Tsaridou, C., & Karabelas, A. J. (2021). Drinking water standards and their implementation— a critical assessment. *Water (Switzerland)*, 13(20). <https://doi.org/10.3390/w13202918>

Turner, A., & Rawling, M. C. (2001). The influence of salting out on the sorption of neutral organic compounds in estuaries. *Water Research*, 35(18), 4379–4389. [https://doi.org/https://doi.org/10.1016/S0043-1354\(01\)00163-4](https://doi.org/https://doi.org/10.1016/S0043-1354(01)00163-4)

U

UAntwerpen, VITO, PIH, UHasselt, & VUB. (2023). Jongerenstudie HBM - omgeving 3M – Resultatenrapport, in opdracht van het Departement Omgeving, Vlaams Planbureau voor Omgeving.

UNEP, 2019. The Stockholm convention on persistent organic pollutants 11, 329–342. <https://doi.org/10.2307/2668517>

UNEP. (2011). Guidance on alternatives to perfluorooctane sulfonic acid and its derivatives.

Ukasha, T., Faisal, N. U. H., Adjji, B. K., & Nugroho, A. P. (2024). Active biomonitoring in streams by using multimarker approaches of mussels. *Global Journal of Environmental Science and Management*, 10(3), 1171–1196. <https://doi.org/10.22034/gjesm.2024.03.15>

Ullah, S., Huber, S., Bignert, A., & Berger, U. (2014). Temporal trends of perfluoroalkane sulfonic acids and their sulfonamide-based precursors in herring from the Swedish west coast 1991-2011 including isomer-specific considerations. *Environ. Intern.* 65, 63–72. <https://doi.org/10.1016/j.envint.2014.01.005>

V

VITO, PIH, & Agentschap Zorg en Gezondheid. (2022). Bevolkingsonderzoek PFAS bij omwonenden van de 3M site in Zwijndrecht.

Van Ael, E., Blust, R., & Bervoets, L. (2017). Metals in the Scheldt estuary: From environmental concentrations to bioaccumulation. *Environmental Pollution*, 228, 82–91. <https://doi.org/10.1016/j.envpol.2017.05.028>

van Ael, E., Covaci, A., Das, K., Lepoint, G., Blust, R., & Bervoets, L. (2013). Factors influencing the bioaccumulation of persistent organic pollutants in food webs of the scheldt estuary. *Environmental Science and Technology*, 47(19), 11221–11231. <https://doi.org/10.1021/es400307s>

van Beek, F.A., Rijnsdorp, A.D., & de Clerck, R. (1989). Monitoring juvenile stocks of flatfish in the Wadden Sea and the coastal areas of the southeastern North Sea. *Helgoländer Meeresuntersuchungen* 43, 461–477. <https://doi.org/10.1007/BF02365904>

Van De Vijver, K. I., Hoff, P. T., Van Dongen, W., Esmans, E. L., Blust, R., & De Coen, W. M. (2003). Exposure patterns of perfluorooctane sulfonate in aquatic invertebrates from the Western Scheldt estuary and the southern North Sea. *Environmental Toxicology and Chemistry*, 22(9), 2037–2041. <https://doi.org/10.1897/02-385>

Van De Vijver, K.I., Holsbeek, L., Das, K., Blust, R., Joiris, C., & De Coen, W. (2007). Occurrence of perfluorooctane sulfonate and other perfluorinated alkylated substances in harbor porpoises from the Black Sea. *Environmental Science and Technology*. 41, 315–320. <https://doi.org/10.1021/es060827e>

van Dijk, W. M., Cox, J. R., Leuven, J. R. F. W., Cleveringa, J., Taal, M., Hiatt, M. R., Sonke, W., Verbeek, K., Speckmann, B., & Kleinhans, M. G. (2021). The vulnerability of tidal flats and multi-channel estuaries to dredging and disposal. *Anthropocene Coasts*, 4(1), 36–60. <https://doi.org/10.1139/anc-2020-0006>

Van Leeuwen, S., Strub, M., Cofino, W., & Bavel, B. Van (2011). Third interlaboratory study on perfluorinated compounds in environmental and human matrices. IVM Institute for Environmental Studies.

Van Liefferinge, C., Dillen, A., Ide, C., Herrel, A., Belpaire, C., Mouton, A., De Deckere, E., & Meire, P. (2012). The role of a freshwater tidal area with controlled reduced tide as feeding habitat for European eel (*Anguilla anguilla*, L.). *Journal of Applied Ichthyology*, 28(4), 572–581. <https://doi.org/10.1111/J.1439-0426.2012.01963.X>

Vanderborght, J.-P., Folmer, I. M., Aguilera, D. R., Uhrenholdt, T., & Regnier, P. (2007). Reactive-transport modelling of C, N, and O₂ in a river–estuarine–coastal zone system: Application to the Scheldt estuary. *Marine Chemistry*, 106(1), 92–110. [https://doi.org/https://doi.org/10.1016/j.marchem.2006.06.006](https://doi.org/10.1016/j.marchem.2006.06.006)

Verhaert, V., Newmark, N., D’Hollander, W., Covaci, A., Vlok, W., Wepener, V., Addo-Bediako, A., Jooste, A., Teuchies, J., Blust, R., & Bervoets, L. (2017). Persistent organic pollutants in the Olifants River Basin, South Africa: Bioaccumulation and trophic transfer through a subtropical aquatic food web. *Science of the Total Environment*, 586, 792–806. <https://doi.org/10.1016/j.scitotenv.2017.02.057>

Vidal, A., Lafay, F., Daniele, G., Vulliet, E., Rochard, E., Garric, J., & Babut, M. (2019). Does water temperature influence the distribution and elimination of perfluorinated substances in rainbow trout (*Oncorhynchus mykiss*)? *Environmental Science and Pollution Research*, 26(16), 16355–16365. <https://doi.org/10.1007/s11356-019-05029-w>

Vieira, V.M., Hoffman, K., Shin, H.M., Weinberg, J.M., Webster, T.F., & Fletcher, T. (2013). Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: A geographic analysis. *Environmental Health Perspective*, 121, 318–323. <https://doi.org/10.1289/ehp.1205829>

Villanueva, P. (2005). MLE-based procedure for left-censored data Excel spreadsheet. Unpublished Report, US Environmental Protection Agency, Office of Pesticide Programs, Washington, DC.

Vlaamse Milieumaatschappij. (2022). Kwaliteit van het drinkwater.

W

- Wallace, J. B., & Webster, J. R. (2003). The Role of Macroinvertebrates in Stream Ecosystem Function. *Annual Review of Entomology*, 41(1), 115–139. <https://doi.org/10.1146/ANNUREV.EN.41.010196.000555>
- Wallington, T. J., Hurley, M. D., Xia, J., Wuebbles, D. J., Sillman, S., Ito, A., Penner, J. E., Ellis, D. A., Martin, J., Mabury, S. A., Nielsen, O. J., & Sulbaek Andersen, M. P. (2006). Formation of C7F15COOH (PFOA) and other perfluorocarboxylic acids during the atmospheric oxidation of 8:2 fluorotelomer alcohol. *Environmental Science & Technology*, 40(3), 924–930. <https://doi.org/10.1021/ES051858X>
- Wang, H., Hu, D., Wen, W., Lin, X., & Xia, X. (2023). Warming Affects Bioconcentration and Bioaccumulation of Per- and Polyfluoroalkyl Substances by Pelagic and Benthic Organisms in a Water–Sediment System. *Environmental Science and Technology*, 57(9), 3612–3622. <https://doi.org/10.1021/acs.est.2c07631>
- Wang, N., Liu, J., Buck, R. C., Korzeniowski, S. H., Wolstenholme, B. W., Folsom, P. W., & Sulecki, L. M. (2011). 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere*, 82(6), 853–858. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2010.11.003>
- Wang, P., Zhang, M., Lu, Y., Meng, J., Li, Q., & Lu, X. (2019). Removal of perfluoroalkyl acids (PFAAs) through fluorochemical industrial and domestic wastewater treatment plants and bioaccumulation in aquatic plants in river and artificial wetland. *Environment International*, 129, 76–85. <https://doi.org/10.1016/J.ENVINT.2019.04.072>
- Wang, S., Hong, H., & Wang, X. (2005). Bioenergetic responses in green lipped mussels (*Perna viridis*) as indicators of pollution stress in Xiamen coastal waters, China. *Marine Pollution Bulletin*, 51(8), 738–743. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2005.02.024>
- Wang, S., Huang, J., Yang, Y., Hui, Y., Ge, Y., Larssen, T., Yu, G., Deng, S., Wang, B., & Harman, C. (2013). First Report of a Chinese PFOS Alternative Overlooked for 30 Years: Its Toxicity, Persistence, and Presence in the Environment. *Environmental Science & Technology*, 47(18), 10163–10170. <https://doi.org/10.1021/es401525n>
- Wang, X., Shi, X., Zheng, S., Zhang, Q., Peng, J., Tan, W., & Wu, K. (2022). Perfluorooctane sulfonic acid (PFOS) exposures interfere with behaviors and transcription of genes on nervous and muscle system in zebrafish embryos. *Science of the Total Environment*, 848, 157816. <https://doi.org/10.1016/J.SCITOTENV.2022.157816>
- Wang, Y.-Q., Hu, L.-X., Liu, T., Zhao, J.-H., Yang, Y.-Y., Liu, Y.-S., & Ying, G.-G. (2022). Per- and polyfluoroalkyl substances (PFAS) in drinking water system: Target and non-target screening and removal assessment. *Environment International*, 163, 107219. <https://doi.org/https://doi.org/10.1016/j.envint.2022.107219>
- Wang, Z., Buser, A. M., Cousins, I. T., Demattio, S., Drost, W., Johansson, O., Ohno, K., Patlewicz, G., Richard, A. M., Walker, G. W., White, G. S., & Leinälä, E. (2021). A New OECD Definition for Per- And Polyfluoroalkyl Substances. *Environmental Science and Technology*, 55, 15575–15578. <https://doi.org/10.1021/acs.est.1c06896>
- Wang, Z., Cousins, I. T., Scheringer, M., & Hungerbühler, K. (2013). Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFASs) and their potential precursors. *Environment International*, 60, 242–248. <https://doi.org/10.1016/J.ENVINT.2013.08.021>
- Wang, Z., Cousins, I. T., Scheringer, M., Buck, R. C., & Hungerbühler, K. (2014). Global emission inventories for C4–C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and

emissions from quantifiable sources. *Environment International*, 70, 62–75. <https://doi.org/10.1016/J.ENVINT.2014.04.013>

Wang, Z., Dewitt, J. C., Higgins, C. P., & Cousins, I. T. (2017). A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)? *Environmental Science and Technology*, 51(5), 2508–2518. <https://doi.org/10.1021/acs.est.6b04806>

Wen, W., Xia, X., Chen, X., Wang, H., Zhu, B., Li, H., & Li, Y. (2016). Bioconcentration of perfluoroalkyl substances by *Chironomus plumosus* larvae in water with different types of dissolved organic matters. *Environmental Pollution*, 213, 299–307. <https://doi.org/10.1016/J.ENVPOL.2016.02.018>

Wen, W., Xia, X., Hu, D., Zhou, D., Wang, H., Zhai, Y., & Lin, H. (2017). Long-Chain Perfluoroalkyl acids (PFAAs) Affect the Bioconcentration and Tissue Distribution of Short-Chain PFAAs in Zebrafish (*Danio rerio*). *Environmental Science and Technology*, 51(21), 12358–12368. https://doi.org/10.1021/ACS.EST.7B03647/ASSET/IMAGES/ES-2017-036478_M003.GIF

Wen, W., Xiao, L., Hu, D., Zhang, Z., Xiao, Y., Jiang, X., Zhang, S., & Xia, X. (2023). Fractionation of perfluoroalkyl acids (PFAAs) along the aquatic food chain promoted by competitive effects between longer and shorter chain PFAAs. *Chemosphere*, 318, 137931. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2023.137931>

Wepener, V. (2013). Active Biomonitoring. In *Encyclopedia of Aquatic Ecotoxicology*. Springer. https://doi.org/https://doi.org/10.1007/978-94-007-5704-2_3

Werner, R. A., & Brand, W. A. (2001). Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry*, 15(7), 501–519. <https://doi.org/https://doi.org/10.1002/rcm.258>

Whitehead, P.J.P. (1985). Clupeoid fishes of the world (suborder *Clupeoidei*). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies and wolf-herrings. *FAO Species Catalogue*, 7(2) 42–50.

Wilman, B., Bełdowska, M., Rychter, A., & Popławska, A. (2023). Factors determining bioaccumulation of neurotoxicant Hg in the zebra mussels (*Dreissena polymorpha*): Influence of biometric parameters, sex and storage of shell. *Marine Pollution Bulletin*, 197, 115718. <https://doi.org/10.1016/j.marpolbul.2023.115718>

X

Xia, J., Fu, S., Cao, Z., Peng, Jianglan, Peng, Jing, Dai, T., & Cheng, L. (2013). Ecotoxicological effects of waterborne PFOS exposure on swimming performance and energy expenditure in juvenile goldfish (*Carassius auratus*). *Journal of Environmental Science (China)* 25, 1672–1679. [https://doi.org/10.1016/S1001-0742\(12\)60219-8](https://doi.org/10.1016/S1001-0742(12)60219-8)

Xia, X., Rabearisoa, A. H., Dai, Z., Jiang, X., Zhao, P., & Wang, H. (2015). Inhibition effect of Na⁺ and Ca²⁺ on the bioaccumulation of perfluoroalkyl substances by *Daphnia magna* in the presence of protein. *Environmental Toxicology and Chemistry*, 34(2), 429–436. <https://doi.org/10.1002/ETC.2823>

Xiong, J., & Li, Z. (2024). Predicting PFAS fate in fish: Assessing the roles of dietary, respiratory, and dermal uptake in bioaccumulation modeling. *Environmental Research*, 252, 119036. <https://doi.org/https://doi.org/10.1016/j.envres.2024.119036>

Xu, L., Shi, Y., Li, C., Song, X., Qin, Z., Cao, D., & Cai, Y. (2017). Discovery of a Novel Polyfluoroalkyl Benzenesulfonic Acid around Oilfields in Northern China. *Environmental Science & Technology*, 51(24), 14173–14181. <https://doi.org/10.1021/ACS.EST.7B04332>

Y

Yamashita, N., Kannan, K., Taniyasu, S., Horii, Y., Petrick, G., & Gamo, T. (2005). A global survey of perfluorinated acids in oceans. *Marine Pollution Bulletin*, 51(8–12), 658–668. <https://doi.org/10.1016/j.marpolbul.2005.04.026>

Yang, X., Huang, J., Zhang, K., Yu, G., Deng, S., & Wang, B. (2014). Stability of 6:2 fluorotelomer sulfonate in advanced oxidation processes: Degradation kinetics and pathway. *Environmental Science and Pollution Research*, 21(6), 4634–4642. <https://doi.org/10.1007/s11356-013-2389-z>

Yeung, L. W. Y., & Mabury, S. A. (2013). Bioconcentration of Aqueous Film-Forming Foam (AFFF) in Juvenile Rainbow Trout (*Oncorhynchus mykiss*). *Environmental Science & Technology*, 47(21), 12505–12513. <https://doi.org/10.1021/es403170f>

Yeung, L. W. Y., Dassuncao, C., Mabury, S., Sunderland, E. M., Zhang, X., & Lohmann, R. (2017). Vertical Profiles, Sources, and Transport of PFASs in the Arctic Ocean. *Environmental Science and Technology*, 51(12), 6735–6744. https://doi.org/10.1021/ACS.EST.7B00788/SUPPL_FILE/ES7B00788_SI_001.PDF

Yeung, L. W. Y., & Mabury, S. A. (2013). Bioconcentration of Aqueous Film-Forming Foam (AFFF) in Juvenile Rainbow Trout (*Oncorhynchus mykiss*). *Environmental Science & Technology*, 47(21), 12505–12513. <https://doi.org/10.1021/es403170f>

You, C., Jia, C., & Pan, G. (2010). Effect of salinity and sediment characteristics on the sorption and desorption of perfluorooctane. *Environmental Pollution*, 158, 1343–1347. <https://doi.org/10.1016/j.envpol.2010.01.009>

Young, C. J., Furdul, V. I., Franklin, J., Koerner, R. M., Muir, D. C. G., & Mabury, S. A. (2007). Perfluorinated acids in Arctic snow: new evidence for atmospheric formation. *Environmental Science & Technology*, 41(10), 3455–3461. <https://doi.org/10.1021/ES0626234>

Yun, X., Lewis, A. J., Stevens-King, G., Sales, C. M., Spooner, D. E., Kurz, M. J., Suri, R., & McKenzie, E. R. (2023). Bioaccumulation of per- and polyfluoroalkyl substances by freshwater benthic macroinvertebrates: Impact of species and sediment organic carbon content. *Science of The Total Environment*, 866, 161208. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.161208>

Z

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

Zhang, C., Hopkins, Z. R., McCord, J., Strynar, M. J., & Knappe, D. R. U. (2019). Fate of Per- and Polyfluoroalkyl Ether Acids in the Total Oxidizable Precursor Assay and Implications for the Analysis of Impacted Water. *Environmental Science and Technology Letters*. 6, 662–668. <https://doi.org/10.1021/acs.estlett.9b00525>

- Zhang, C., Yan, H., Li, F., Hu, X., & Zhou, Q. (2013). Sorption of short- and long-chain perfluoroalkyl surfactants on sewage sludges. *Journal of Hazardous Materials*, 260, 689–699. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2013.06.022>
- Zhang, D. Q., Wang, M., He, Q., Niu, X., & Liang, Y. (2020). Distribution of perfluoroalkyl substances (PFASs) in aquatic plant-based systems: From soil adsorption and plant uptake to effects on microbial community. *Environmental Pollution*, 257, 113575. <https://doi.org/10.1016/J.ENVPOL.2019.113575>
- Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A., & Barrow, C. J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology*, 18(3–5), 445–450. <https://doi.org/10.1007/s10811-006-9048-4>
- Zhang, S., Guo, X., Lu, S., Sang, N., Li, G., Xie, P., Liu, C., Zhang, L., & Xing, Y. (2018). Exposure to PFDoA causes disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. *Environmental Pollution*, 235, 974–982. <https://doi.org/https://doi.org/10.1016/j.envpol.2018.01.015>
- Zhang, W., Zhang, Y., Taniyasu, S., Yeung, L. W. Y., Lam, P. K. S., Wang, J., Li, X., Yamashita, N., & Dai, J. (2013). Distribution and fate of perfluoroalkyl substances in municipal wastewater treatment plants in economically developed areas of China. *Environmental Pollution*, 176, 10–17. <https://doi.org/10.1016/J.ENVPOL.2012.12.019>
- Zhao, L., Teng, M., Zhao, X., Li, Y., Sun, J., Zhao, W., Ruan, Y., Leung, K. M. Y., & Wu, F. (2023). Insight into the binding model of per- and polyfluoroalkyl substances to proteins and membranes. *Environment International*, 175, 107951. <https://doi.org/https://doi.org/10.1016/j.envint.2023.107951>
- Zhao, P., Xia, X., Dong, J., Xia, N., Jiang, X., Li, Y., & Zhu, Y. (2016). Short- and long-chain perfluoroalkyl substances in the water, suspended particulate matter, and surface sediment of a turbid river. *Science of The Total Environment*, 568, 57–65. <https://doi.org/10.1016/J.SCITOTENV.2016.05.221>
- Zhao, S., Wang, B., Zhu, L., Liang, T., Chen, M., Yang, L., Lv, J., Liu, L., Zhao, P., Xia, X., Dong, J., Xia, N., Jiang, X., Li, Y. Y., Zhu, Y., Zabaleta, I., Bizkarguenaga, E., Nunoo, D. B. O., Schultes, L., ...& Josefsson, S. (2018). Trophic magnification of poly- and perfluorinated compounds in a subtropical food web. *Environmental Science and Technology*, 45(4), 598–678. <https://doi.org/10.1080/10643389.2011.627016>
- Zhao, Y. G., Wan, H. T., Law, A. Y. S., Wei, X., Huang, Y. Q., Giesy, J. P., Wong, M. H., & Wong, C. K. C. (2011). Risk assessment for human consumption of perfluorinated compound-contaminated freshwater and marine fish from Hong Kong and Xiamen. *Chemosphere*, 85(2), 277–283. <https://doi.org/10.1016/j.chemosphere.2011.06.002>
- Zhao, Z., Xie, Z., Möller, A., Sturm, R., Tang, J., Zhang, G., & Ebinghaus, R. (2012). Distribution and long-range transport of polyfluoroalkyl substances in the Arctic, Atlantic Ocean and Antarctic coast. *Environmental Pollution (Barking, Essex : 1987)*, 170, 71–77. <https://doi.org/10.1016/J.ENVPOL.2012.06.004>
- Zhu, Z., Wang, T., Wang, P., Lu, Y., & Giesy, J. P. (2014). Perfluoroalkyl and polyfluoroalkyl substances in sediments from South Bohai coastal watersheds, China. *Marine Pollution Bulletin*, 85(2), 619–627. <https://doi.org/10.1016/J.MARPOLBUL.2013.12.042>

Supplementary Information

Appendix A: Chapter II

Tables:

Table A.1. Average total length (cm) for each species per location. Length at first maturity (L_{mat}) available on Fishbase is also presented below (www.fishbase.se). Species which were not sampled at a certain location are indicated by '-'. In case L_{mat} was not available on Fishbase, the common length was used, indicated by '*'. Per location and per species the number of replicates ranges from 3 - 4.

Loc.	<i>S. solea</i>	<i>G. vulgaris</i>	<i>P. platessa</i>	<i>M. merlangus</i>	<i>C. harengus</i>	<i>M. surmuletus</i>	<i>A. laterna</i>
1	15.9	16.5	14.4	19.3	-	-	-
2	10.9	-	15.0	19.7	-	-	-
3		16.2	-	22.3	-	-	-
4	20.1	15.8	17.5	19.3	-	-	-
5	19.9	-	13.5	20.1	8.4	-	-
6	15.8	-	10.5	19.9	8.9	-	-
7	10.1	-	13.9	17.9	-	-	-
8	16.5	-	14.8	21.1	-	10.3	12.7
9	18.9	-	21.0	-	10.3	13.0	11.2
10	-	-	20.1	-	-	11.0	9.9
L_{mat}	30.3	25.0*	30.8	27.8	16.7	16.1	12*

Table A.2. Average weight (g) for each species per location. Species who were not sampled at a certain location are indicated by '-'. Per location and per species the number of replicates ranges from 3 - 4.

Location	<i>S. solea</i>	<i>G. vulgaris</i>	<i>P. platessa</i>	<i>M. merlangus</i>	<i>C. harengus</i>	<i>M. surmuletus</i>	<i>A. laterna</i>
1	35.9	68.3	31.4	62.6	-	-	-
2	10.9	-	47.9	69.8	-	-	-
3	-	34.8	-	113.3	-	-	-
4	90.6	39.5	54.1	81.4	-	-	-
5	17.1	-	25.2	56.4	5.2	-	-
6	34.1	-	9.8	71.8	3.4	-	-
7	7.4	-	23.8	52.1	-	-	-
8	43.1	-	43.1	82.4	-	22.1	17.9
9	58.1	-	31.7	-	9.1	29.2	11.3
10	-	-	80.4	-	-	13.4	9.2

Table A.3. MRM transitions, internal standards (ISTDs), cone voltage (V) and collision energies (eV) for the target perfluoroalkyl substances and their internal standards. Table adopted from Groffen et al. (2019b).

Compound	Precursor ion (m/z)	Product ion (m/z)		Cone Voltage (V)	Collision energy (eV) for diagnostic transition1	Collision energy (eV) for diagnostic transition 2	Internal standard (ISTD) used for quantification
		Diagnostic product ion 1	Diagnostic product ion 2				
PFBA	213	169	169	19	19	50	¹³ C ₄ -PFBA
PFPeA	263	219	219	15	10	45	¹³ C ₄ -PFBA
PFHxA	313	269	119	19	21	65	[1,2- ¹³ C ₂]PFHxA
PFHpA	363	319	169	24	40	30	[1,2- ¹³ C ₂]PFHxA
PFOA	413	369	169	22	13	60	[1,2,3,4- ¹³ C ₄]PFOA
PFNA	463	419	169	28	17	20	[1,2,3,4,5- ¹³ C ₅]PFNA
PFDA	513	469	219	25	29	29	[1,2- ¹³ C ₂]PFDA
PFUnDA	563	519	169	18	30	35	[1,2- ¹³ C ₂]PFUnDA
PFDoDA	613	569	319	22	21	30	[1,2- ¹³ C ₂]PFDoDA
PFTeDA	663	619	319	26	21	30	[1,2- ¹³ C ₂]PFDoDA
PFTeDA	713	669	169	28	21	21	[1,2- ¹³ C ₂]PFDoDA
PFBS	299	80	99	40	65	45	¹⁸ O ₂ -PFHxS
PFHxS	399	80	99	22	30	60	¹⁸ O ₂ -PFHxS
PFOS	499	80	99	60	58	58	[1,2,3,4- ¹³ C ₄]PFOS
PFDS	599	80	99	29	63	63	[1,2,3,4- ¹³ C ₄]PFOS
¹³ C ₄ -PFBA	217	172	172	19	19	50	
[1,2- ¹³ C ₂]PFHxA	315	269	119	19	21	65	
[1,2,3,4- ¹³ C ₄]PFOA	417	372	172	22	13	60	
[1,2,3,4,5- ¹³ C ₅]PFNA	468	423	172	28	17	20	
[1,2- ¹³ C ₂]PFDA	515	470	220	25	29	29	
[1,2- ¹³ C ₂]PFUnDA	565	520	170	18	32	35	
[1,2- ¹³ C ₂]PFDoDA	615	570	320	22	21	30	
¹⁸ O ₂ -PFHxS	403	84	103	22	30	60	
[1,2,3,4- ¹³ C ₄]PFOS	503	80	99	60	58	58	

Table A.4: Comparison of average reference concentrations (ng/g ww) measured in the present study with the average, median and min. – max. ranges of the interlaboratory study on the certified reference material (ng/g ww) (Van Leeuwen et al. 2011). NA: Not assessed.

Fish	Assigned value	Average	Median	Min.	Max.	present study
PFBA	NA	0.16	0.16	NA	NA	<LOQ
PFPeA	NA	0.24	0.24	NA	NA	<LOQ
PFHxA	NA	2.07	2.15	0.09	3.90	<LOQ
PFHpA	NA	1.28	1.00	0.80	2.05	<LOQ
PFOA	NA	3.80	0.39	0.09	33.0	0.25
PFNA	0.52	1.23	0.60	0.23	6.75	0.61
PFDA	2.62	4.04	2.69	0.66	27.0	2.93
PFUnDA	1.43	1.73	1.40	0.38	4.70	1.82
PFDoDA	0.27	1.05	0.30	0.20	5.30	0.32
PFTTrDA	NA	0.73	0.39	0.10	2.16	0.27
PFTeDA	NA	0.08	0.08	NA	NA	<LOQ
PFHxDA	NA	NA	NA	NA	NA	NA
PFODA	NA	NA	NA	NA	NA	NA
L-PFBS	NA	0.10	0.10	0.00	0.00	<LOQ
L-PFHxS	NA	0.89	0.11	0.00	5.10	<LOQ
L-PFOS	65.4	61.6	67.0	2.48	110	61.4
L-PFDS	NA	1.93	0.29	0.14	5.35	<LOQ
PFOSA	1.44	1.80	1.60	0.88	3.60	NA

Table A.5: Average limit of quantification (LOQ) per analyte and per matrix (ng/g ww).

	PFOS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA
Fish liver	0.05	0.12	0.04	0.08	0.08	0.01	0.01
Fish muscle	0.008	0.01	0.007	0.02	0.02	0.005	0.002
crustaceans	0.07	0.06	0.04	0.08	0.11	0.19	0.12

Table A.6: Overview of the number of replicates (N) per species (fish and crustaceans) and per location. This number of replicates was used to pool the samples for the Stable isotope analysis. '-' indicates when a certain species was not collected at that location.

Location:	1	2	3	4	5	6	7	8	9	10
Fish species:										
<i>S. solea</i>	3	4	-	3	3	3		3	3	3
<i>G. vulgaris</i>	4	-	3	4	-	-	-	-	-	-
<i>M. merlangus</i>	3	4	3	3	4	3	3	3	-	-

<i>P. platessa</i>	3	4	-	3	4	3	3	3	3	3
<i>C. harengus</i>	-	-	-	-	3	3			3	
<i>M. surmuletus</i>	-	-	-	-	-	-	-	3	3	3
<i>A. laterna</i>	-	-	-	-	-	-	-	-	3	-
Crustacean species:										
<i>C. crangon</i>	3	3	3	3	-	3	3	3	-	-
<i>L. holsatus</i>	4	4	4	4	3	4	4	4	3	3

Table A.7: PFAS concentrations in muscle tissue of fish species (*S. solea*, *G. vulgaris*, *P. platessa*, *M. merlangus*, *P. platessa*, *C. harengus* and *M. surmuletus*) for the 10 locations. PFAS concentrations are expressed in ng/g ww (wet weight). 'NA' is Not Detected and 'LOQ' is Limit of Quantification.

Location	Species	PFOS	PFOA	PFNA	PFDA	PFunDA	PFDODA	PFTDA
	LOQ:	0.008	0.012	0.007	0.022	0.023	0.005	0.002
1	<i>G. vulgaris</i>	0.126	0.271	ND	ND	ND	ND	0.72
1	<i>G. vulgaris</i>	0.778	0.156	ND	ND	0.446	0.168	ND
1	<i>G. vulgaris</i>	0.085	0.242	ND	0.329	0.385	ND	ND
1	<i>G. vulgaris</i>	0.186	0.314	0.09	0.273	0.404	0.067	0.020
1	<i>M. merlangus</i>	1.19	0.223	0.09	0.374	0.397	0.084	0.026
1	<i>M. merlangus</i>	2.97	0.142	0.099	0.612	0.792	0.148	0.062
1	<i>M. merlangus</i>	0.704	0.134	0.034	0.418	0.359	0.071	0.028
1	<i>P. platessa</i>	3.61	0.113	0.360	1.18	0.431	0.120	0.091
1	<i>P. platessa</i>	2.36	0.226	0.225	0.867	0.417	0.062	0.030
1	<i>P. platessa</i>	2.50	0.114	0.423	0.990	0.541	0.079	0.050
1	<i>S. solea</i>	1.11	0.161	0.158	0.627	0.484	0.144	0.078
1	<i>S. solea</i>	2.33	0.162	0.218	0.749	0.218	0.157	0.130
1	<i>S. solea</i>	4.00	0.213	0.344	0.699	0.651	0.193	0.230
2	<i>M. merlangus</i>	0.835	0.356	0.164	0.601	0.511	0.086	0.040
2	<i>M. merlangus</i>	0.299	0.282	0.143	0.584	0.429	0.101	0.060
2	<i>M. merlangus</i>	0.377	0.254	ND	0.468	ND	ND	ND
2	<i>M. merlangus</i>	0.455	0.169	0.053	0.399	0.302	0.066	0.041
2	<i>P. platessa</i>	0.647	0.243	ND	0.124	0.204	ND	ND
2	<i>P. platessa</i>	0.617	0.315	0.108	0.425	0.531	0.066	0.030
2	<i>P. platessa</i>	4.47	0.183	0.060	0.698	0.589	0.104	0.049
2	<i>P. platessa</i>	1.28	0.213	0.108	0.693	0.445	ND	0.046
2	<i>S. solea</i>	24.3	0.362	0.616	1.01	0.662	0.144	0.084
2	<i>S. solea</i>	1.08	0.390	0.125	0.602	0.411	0.084	0.032
2	<i>S. solea</i>	1.21	0.226	0.231	0.631	0.552	0.041	0.040
2	<i>S. solea</i>	1.28	0.448	0.167	0.534	0.374	0.054	0.042
3	<i>G. vulgaris</i>	0.167	0.179	ND	0.311	0.519	ND	ND
3	<i>G. vulgaris</i>	0.250	0.159	ND	0.365	0.493	0.035	ND
3	<i>G. vulgaris</i>	0.396	0.392	0.653	0.417	1.03	0.089	ND
3	<i>M. merlangus</i>	0.524	0.298	0.040	0.177	0.512	0.067	0.032
3	<i>M. merlangus</i>	0.451	0.133	0.070	0.408	0.436	0.070	0.024
3	<i>M. merlangus</i>	0.519	0.265	0.065	0.263	0.272	0.100	0.026
4	<i>G. vulgaris</i>	0.402	0.254	ND	0.284	0.338	0.038	ND
4	<i>G. vulgaris</i>	0.334	ND	ND	ND	ND	ND	ND
4	<i>G. vulgaris</i>	0.181	ND	ND	0.394	0.332	ND	ND

4	<i>G. vulgaris</i>	0.286	0.198	ND	ND	0.440	ND	ND
4	<i>M. merlangus</i>	1.55	0.234	0.110	0.528	0.417	0.050	0.035
4	<i>M. merlangus</i>	1.38	0.185	0.066	0.503	0.375	0.090	0.047
4	<i>M. merlangus</i>	1.51	0.190	0.164	0.518	0.621	0.071	0.047
4	<i>P. platessa</i>	4.65	1.263	0.529	1.07	0.792	0.045	0.089
4	<i>P. platessa</i>	11.9	0.602	1.04	1.70	0.866	0.142	0.062
4	<i>P. platessa</i>	4.61	0.616	0.368	1.08	0.725	0.074	0.029
4	<i>S. solea</i>	1.95	0.333	0.389	1.20	0.384	<LOQ	<LOQ
4	<i>S. solea</i>	6.84	0.666	0.786	1.63	0.585	0.135	0.033
5	<i>C. harengus</i>	3.14	0.320	0.102	0.199	0.344	0.045	<LOQ
5	<i>C. harengus</i>	0.442	0.226	0.021	0.260	0.548	0.076	0.049
5	<i>C. harengus</i>	0.216	0.250	ND	0.340	0.378	0.050	0.034
5	<i>M. merlangus</i>	0.592	0.280	0.136	0.507	0.429	0.090	ND
5	<i>M. merlangus</i>	1.03	0.154	0.072	0.474	0.461	0.073	0.027
5	<i>M. merlangus</i>	0.333	0.191	0.057	0.243	0.490	0.060	ND
5	<i>M. merlangus</i>	1.86	0.318	0.201	0.579	0.293	0.062	0.034
5	<i>P. platessa</i>	3.46	0.279	0.321	0.551	0.755	0.102	0.036
5	<i>P. platessa</i>	1.56	0.194	0.093	0.587	0.727	0.104	0.051
5	<i>P. platessa</i>	1.04	0.237	0.111	0.492	0.454	0.067	0.030
5	<i>P. platessa</i>	11.1	1.18	1.16	1.17	0.835	0.124	0.048
5	<i>S. solea</i>	2.53	0.408	0.238	0.409	0.404	0.100	0.107
5	<i>S. solea</i>	0.82	0.181	0.168	0.438	0.533	0.076	0.030
6	<i>C. harengus</i>	1.41	0.220	0.128	0.195	0.129	0.032	<LOQ
6	<i>C. harengus</i>	1.74	0.121	ND	0.377	0.986	0.079	0.050
6	<i>C. harengus</i>	0.83	0.357	0.053	ND	0.281	0.067	<LOQ
6	<i>M. merlangus</i>	1.44	0.273	0.200	0.647	0.416	0.089	0.057
6	<i>M. merlangus</i>	0.485	0.220	ND	ND	0.652	ND	0.019
6	<i>M. merlangus</i>	0.62	0.247	0.082	0.231	0.351	0.047	0.033
6	<i>P. platessa</i>	3.71	0.175	0.287	0.615	0.251	0.084	0.015
6	<i>P. platessa</i>	0.959	0.060	0.356	0.292	0.203	0.054	0.018
6	<i>P. platessa</i>	2.48	0.190	0.231	0.633	0.257	0.053	0.023
6	<i>S. solea</i>	0.704	0.260	0.136	0.340	ND	ND	ND
6	<i>S. solea</i>	1.47	0.134	0.132	0.497	0.254	0.032	0.016
6	<i>S. solea</i>	1.35	0.239	0.153	0.805	0.476	0.090	0.060
7	<i>M. merlangus</i>	0.566	0.100	ND	0.617	0.471	0.091	0.038
7	<i>M. merlangus</i>	1.34	0.179	0.189	0.478	0.554	0.076	0.044
7	<i>M. merlangus</i>	1.85	0.202	0.099	0.655	0.741	0.072	0.037
7	<i>P. platessa</i>	2.44	0.146	0.171	0.945	0.618	0.114	0.025
7	<i>P. platessa</i>	2.90	0.308	0.199	0.645	0.483	0.055	0.021
7	<i>P. platessa</i>	2.63	0.137	0.186	0.583	0.208	0.093	ND
7	<i>S. solea</i>	1.04	0.250	0.259	0.497	0.439	0.038	0.052
7	<i>S. solea</i>	0.78	0.199	0.094	0.417	0.567	0.110	0.017
8	<i>A. laterna</i>	2.02	0.155	0.107	0.492	0.203	0.040	0.031
8	<i>A. laterna</i>	2.68	0.141	0.138	0.719	0.525	0.053	0.020
8	<i>A. laterna</i>	1.80	0.160	0.174	0.636	0.375	0.136	0.043
8	<i>M. merlangus</i>	0.469	0.241	0.057	0.199	0.519	0.051	0.028
8	<i>M. merlangus</i>	0.861	0.144	0.032	0.331	0.367	0.072	<LOQ
8	<i>M. merlangus</i>	0.694	0.292	ND	ND	0.631	0.073	ND
8	<i>M. surmuletus</i>	0.663	0.202	0.209	0.314	0.649	0.056	0.010
8	<i>M. surmuletus</i>	0.684	0.335	0.128	0.485	0.421	0.077	0.013
8	<i>M. surmuletus</i>	1.03	0.598	0.191	0.312	0.303	0.113	0.014

8	<i>P. platessa</i>	2.12	0.185	0.126	0.582	0.387	0.083	0.008
8	<i>P. platessa</i>	1.50	0.269	0.042	0.603	0.750	0.099	0.014
8	<i>P. platessa</i>	2.75	0.260	0.220	0.377	0.195	0.152	0.007
8	<i>S. solea</i>	0.858	0.187	0.284	0.781	0.367	0.103	0.022
8	<i>S. solea</i>	0.302	0.388	0.122	0.272	0.119	0.052	0.023
8	<i>S. solea</i>	1.20	0.267	0.289	0.850	0.630	0.066	0.019
9	<i>A. laterna</i>	2.96	0.303	0.391	0.332	0.660	0.080	0.073
9	<i>A. laterna</i>	3.19	0.269	0.063	0.696	0.259	0.174	0.076
9	<i>A. laterna</i>	3.75	0.308	0.225	0.527	0.623	0.121	0.101
9	<i>C. harengus</i>	4.65	0.299	0.172	0.451	0.352	0.119	0.084
9	<i>C. harengus</i>	5.29	0.332	0.172	0.334	0.686	0.090	0.049
9	<i>C. harengus</i>	1.52	0.232	0.050	0.380	0.481	0.109	0.064
9	<i>M. surmuletus</i>	2.69	0.684	0.739	0.879	0.337	0.100	0.066
9	<i>M. surmuletus</i>	2.46	0.494	0.616	0.463	0.604	0.088	0.058
9	<i>M. surmuletus</i>	2.16	0.462	0.444	0.320	0.497	0.044	0.044
9	<i>P. platessa</i>	2.83	0.274	0.395	0.774	0.466	0.068	0.040
9	<i>P. platessa</i>	1.83	0.231	0.094	0.552	0.157	0.035	0.023
9	<i>P. platessa</i>	1.32	0.297	0.149	0.351	0.200	0.083	0.033
9	<i>S. solea</i>	0.835	0.144	0.050	0.692	0.576	0.053	0.023
9	<i>S. solea</i>	4.16	0.395	0.694	1.06	0.363	0.087	0.017
9	<i>S. solea</i>	0.668	0.213	0.048	0.296	0.210	0.061	0.027
10	<i>A. laterna</i>	2.90	0.360	0.311	0.560	0.506	0.145	0.092
10	<i>A. laterna</i>	4.66	0.211	0.572	0.623	0.611	0.118	0.048
10	<i>A. laterna</i>	1.56	0.222	0.145	0.220	0.510	0.059	0.052
10	<i>M. surmuletus</i>	1.53	0.534	0.453	0.405	0.401	0.106	0.094
10	<i>M. surmuletus</i>	1.30	0.223	0.190	0.322	0.332	0.072	0.059
10	<i>M. surmuletus</i>	1.55	0.425	0.182	0.351	0.403	0.086	0.045
10	<i>P. platessa</i>	1.84	0.193	0.745	0.486	0.424	0.087	0.024
10	<i>P. platessa</i>	1.10	0.178	0.064	0.382	0.383	0.072	0.047
10	<i>P. platessa</i>	3.67	0.233	0.291	0.901	0.723	0.072	0.049

Table A.8: PFAS concentrations in liver tissue of fish species (*S. solea*, *G. vulgaris*, *P. platessa*, *M. merlangus*, *P. platessa*, *C. harengus* and *M. surmuletus*) for the 10 locations. PFAS concentrations are expressed in ng/g ww (wet weight). 'ND' is Not Detected and 'LOQ' is Limit of Quantification.

Location	Species	PFOS	PFOA	PFNA	PFDA	PFunDA	PFDoDA	PFTrDA
	LOQ:	0.05	0.12	0.04	0.08	0.08	0.01	0.01
1	<i>S. solea</i>	14.4	3.08	ND	4.81	4.78	0.81	0.59
1	<i>S. solea</i>	39.7	0.93	2.42	6.60	3.93	1.41	1.09
1	<i>S. solea</i>	39.7	0.74	2.80	5.80	3.37	1.15	1.11
1	<i>P. platessa</i>	39.6	1.35	4.31	9.14	5.14	0.59	0.49
1	<i>P. platessa</i>	26.2	1.44	2.73	5.21	2.53	0.76	0.33
1	<i>P. platessa</i>	17.9	0.45	2.14	4.78	2.10	0.45	0.10
1	<i>G. vulgaris</i>	0.83	0.64	0.16	0.71	0.74	0.18	0.11
1	<i>G. vulgaris</i>	3.57	0.561	<LOQ	0.780	0.867	0.205	0.049
1	<i>G. vulgaris</i>	0.691	0.491	<LOQ	0.502	0.580	0.107	0.020
1	<i>G. vulgaris</i>	0.950	0.424	ND	0.511	0.722	0.045	0.038
1	<i>M. merlangus</i>	4.76	0.449	0.298	1.09	0.911	0.229	0.059

1	<i>M. merlangus</i>	18.4	0.773	0.705	2.87	1.85	0.599	0.355
1	<i>M. merlangus</i>	2.87	0.725	0.109	0.826	1.14	0.237	0.165
2	<i>P. platessa</i>	5.59	1.12	0.347	ND	2.10	0.276	18.4
2	<i>P. platessa</i>	4.70	0.483	0.195	1.62	1.98	ND	ND
2	<i>P. platessa</i>	12.5	0.850	0.821	2.94	2.13	0.423	31.8
2	<i>P. platessa</i>	19.0	0.627	0.281	2.01	1.85	0.301	9.07
2	<i>S. solea</i>	7.39	1.90	0.850	1.71	1.82	0.406	19.9
2	<i>S. solea</i>	11.9	1.94	2.07	3.85	3.88	ND	23.7
2	<i>S. solea</i>	11.0	2.07	1.40	2.21	2.19	0.523	27.4
2	<i>M. merlangus</i>	2.79	0.783	0.396	1.27	1.09	0.256	14.2
2	<i>M. merlangus</i>	2.75	0.343	0.272	1.55	1.34	0.338	2.49
2	<i>M. merlangus</i>	4.68	0.888	0.685	0.970	1.15	0.286	7.81
2	<i>M. merlangus</i>	2.41	0.610	0.347	0.984	1.13	0.151	13.4
3	<i>G. vulgaris</i>	0.76	0.471	0.150	0.433	0.454	0.091	0.026
3	<i>M. merlangus</i>	5.84	0.512	0.529	1.86	1.93	0.448	0.403
3	<i>M. merlangus</i>	2.10	0.932	0.208	1.73	1.42	0.182	0.071
3	<i>M. merlangus</i>	3.30	0.691	0.311	1.26	1.01	0.215	0.181
4	<i>G. vulgaris</i>	2.03	0.931	0.220	0.805	0.888	0.189	5.26
4	<i>G. vulgaris</i>	2.48	1.23	0.305	0.587	0.661	0.162	5.73
4	<i>G. vulgaris</i>	0.73	0.508	ND	0.518	0.505	0.106	ND
4	<i>G. vulgaris</i>	1.40	0.656	ND	0.661	0.729	0.067	ND
4	<i>S. solea</i>	78.6	4.03	6.91	9.00	2.74	0.396	29.1
4	<i>S. solea</i>	14.2	1.59	2.90	3.36	3.19	0.574	68.2
4	<i>P. platessa</i>	18.3	3.55	1.80	2.95	1.57	0.287	116
4	<i>P. platessa</i>	60.8	1.77	4.45	6.81	4.44	0.714	13.1
4	<i>P. platessa</i>	23.8	2.56	2.47	3.21	1.74	0.228	13.1
4	<i>M. merlangus</i>	9.26	0.763	1.35	2.15	1.70	0.359	7.98
4	<i>M. merlangus</i>	5.12	0.487	0.301	1.25	0.754	0.278	9.30
4	<i>M. merlangus</i>	7.00	0.563	0.984	2.10	1.05	0.266	10.6
5	<i>S. solea</i>	13.1	2.65	0.769	3.88	4.14	0.776	0.20
5	<i>S. solea</i>	38.4	2.80	3.38	3.20	2.32	0.887	0.788
5	<i>P. platessa</i>	40.2	2.17	3.51	10.0	6.30	0.769	0.265
5	<i>P. platessa</i>	17.8	1.62	1.22	4.53	4.99	0.964	0.343
5	<i>P. platessa</i>	14.4	3.16	0.920	1.58	1.44	0.433	0.138
5	<i>P. platessa</i>	107	11.3	12.7	11.2	4.88	0.756	0.304
5	<i>M. merlangus</i>	4.70	0.637	0.461	1.42	1.27	0.143	0.298
5	<i>M. merlangus</i>	3.70	0.496	0.308	1.28	1.01	0.234	0.169
5	<i>M. merlangus</i>	4.46	0.669	0.312	1.72	1.35	0.406	0.468
5	<i>M. merlangus</i>	10.6	0.732	0.794	2.15	1.46	0.163	0.222
6	<i>S. solea</i>	9.51	2.15	1.29	4.77	2.42	0.558	0.609
6	<i>S. solea</i>	18.4	1.08	1.59	4.19	2.57	0.955	1.74
6	<i>P. platessa</i>	64.3	3.37	4.96	8.49	7.65	0.857	0.543
6	<i>P. platessa</i>	7.44	1.16	<LOQ	1.28	1.14	0.233	0.358
6	<i>P. platessa</i>	<LOQ	<LOQ	ND	<LOQ	<LOQ	<LOQ	ND
6	<i>M. merlangus</i>	10.7	1.72	0.928	4.12	3.50	0.720	0.356
6	<i>M. merlangus</i>	6.23	1.29	0.568	1.38	1.33	0.302	<LOQ
6	<i>M. merlangus</i>	4.20	0.283	0.278	0.570	0.257	0.205	0.130
7	<i>P. platessa</i>	33.3	1.53	1.88	4.46	2.32	0.582	0.155
7	<i>P. platessa</i>	73.1	4.59	4.28	10.9	7.32	1.56	0.417
7	<i>P. platessa</i>	9.68	0.772	0.546	2.93	2.24	0.304	<LOQ

7	<i>M. merlangus</i>	21.3	3.40	0.738	3.95	5.20	1.05	0.124
7	<i>M. merlangus</i>	7.95	1.02	0.858	2.06	1.21	0.259	0.178
7	<i>M. merlangus</i>	19.0	1.46	1.83	4.29	3.83	0.852	0.367
8	<i>M. merlangus</i>	5.36	0.813	0.868	0.974	1.01	ND	0.613
8	<i>M. merlangus</i>	3.66	0.504	0.290	1.14	0.931	0.205	0.136
8	<i>M. merlangus</i>	8.65	0.458	0.409	1.68	1.21	0.355	0.211
8	<i>M. surmuletus</i>	10.8	2.59	1.60	4.19	2.29	0.643	0.327
8	<i>M. surmuletus</i>	13.2	4.38	2.40	4.26	2.85	0.983	0.328
8	<i>p. Platessa</i>	7.70	0.762	0.610	1.91	1.41	0.459	<LOQ
8	<i>P. platessa</i>	40.1	1.39	2.61	6.77	3.42	0.668	0.197
8	<i>P. platessa</i>	15.4	1.52	1.22	3.82	2.81	0.917	<LOQ
8	<i>S. solea</i>	15.2	1.60	4.42	8.80	4.73	0.838	0.295
8	<i>S. solea</i>	9.72	2.69	1.80	4.95	2.25	0.568	0.230
8	<i>S. solea</i>	12.2	1.94	2.34	5.40	3.62	0.819	0.446
9	<i>S. solea</i>	13.8	1.83	1.02	3.81	1.66	0.660	0.357
9	<i>M. surmuletus</i>	13.6	4.15	4.51	4.74	3.70	0.621	0.224
9	<i>M. surmuletus</i>	37.3	8.16	6.91	11.0	6.74	1.04	0.450
9	<i>C. harengus</i>	0.258	25.8	<LOQ	<LOQ	0.11	ND	ND
9	<i>C. harengus</i>	16.7	6.14	1.83	5.52	9.23	1.81	0.899
9	<i>C. harengus</i>	4.18	1.53	0.459	1.38	2.31	0.452	0.225
9	<i>S. solea</i>	9.97	1.33	1.01	2.58	2.30	0.619	0.167
9	<i>S. solea</i>	107	3.81	11.2	17.8	8.14	0.908	0.573
9	<i>S. solea</i>	11.7	2.31	1.07	3.98	4.40	0.565	0.943
9	<i>P. platessa</i>	40.1	2.15	4.54	4.00	2.50	0.521	0.671
9	<i>P. platessa</i>	37.6	2.00	2.29	4.77	3.00	0.642	0.463
9	<i>P. platessa</i>	15.2	1.30	0.883	1.78	1.26	0.411	0.185
10	<i>M. surmuletus</i>	9.87	3.87	ND	6.00	5.26	0.969	0.488
10	<i>M. surmuletus</i>	2.03	0.704	0.439	0.660	1.18	0.140	0.060
10	<i>P. platessa</i>	21.8	0.822	3.03	3.20	2.07	0.561	0.225
10	<i>P. platessa</i>	21.9	0.657	1.89	1.15	1.61	0.545	0.298
10	<i>P. platessa</i>	35.3	0.727	2.63	5.74	2.30	0.516	ND
10	<i>A. laterna</i>	24.5	1.46	2.00	2.11	3.10	0.491	ND
10	<i>A. laterna</i>	13.7	2.93	1.46	3.86	3.97	0.588	ND

Table A.9: PFAS concentrations in whole body tissue of crustaceans (*L. holsatus* and *C. crangon*) for the 10 locations. PFAS concentrations are expressed in ng/g ww (wet weight). 'NA' is Not Detected and 'LOQ' is Limit of Quantification.

Location	Species	PFOS	PFOA	PFNA	PFDA	PFUnDA
	LOQ:	0.070	0.060	0.040	0.080	0.110
1	<i>L. holsatus</i>	0.723	0.128	0.172	1.11	0.895
1	<i>L. holsatus</i>	1.27	0.439	0.333	0.520	0.742
1	<i>L. holsatus</i>	1.12	0.528	0.185	0.730	0.543
1	<i>C. crangon</i>	1.59	0.798	<LOQ	0.474	0.925
1	<i>C. crangon</i>	3.86	0.205	0.471	1.47	0.860
1	<i>C. crangon</i>	1.52	0.132	0.429	0.990	0.312
2	<i>L. holsatus</i>	0.526	0.280	0.071	0.487	0.604
2	<i>L. holsatus</i>	5.61	3.49	0.629	4.87	3.17
2	<i>L. holsatus</i>	5.00	4.35	1.35	4.67	2.85

2	<i>C. crangon</i>	3.26	0.797	0.434	0.919	0.866
2	<i>C. crangon</i>	0.928	0.641	<LOQ	0.795	0.603
2	<i>C. crangon</i>	3.12	0.268	0.524	1.48	0.774
3	<i>L. holsatus</i>	3.82	0.767	0.339	1.14	0.822
3	<i>L. holsatus</i>	1.81	0.486	0.161	0.745	0.808
3	<i>L. holsatus</i>	0.437	0.212	0.047	0.388	0.534
3	<i>C. crangon</i>	4.55	0.793	0.688	2.16	1.12
3	<i>C. crangon</i>	1.59	1.11	0.146	0.744	0.655
3	<i>C. crangon</i>	3.32	0.602	0.189	0.983	1.15
4	<i>L. holsatus</i>	2.13	2.20	0.714	0.868	1.54
4	<i>L. holsatus</i>	0.351	0.927	0.732	1.21	1.82
4	<i>L. holsatus</i>	2.44	0.710	0.090	0.889	1.55
4	<i>C. crangon</i>	1.34	1.21	<LOQ	0.542	ND
4	<i>C. crangon</i>	1.66	0.384	0.111	0.819	ND
5	<i>L. holsatus</i>	2.16	0.703	0.237	1.27	1.02
5	<i>L. holsatus</i>	1.24	0.462	0.145	0.680	0.614
5	<i>L. holsatus</i>	1.72	0.496	0.158	0.680	0.660
6	<i>L. holsatus</i>	0.301	0.342	0.159	0.787	0.609
6	<i>L. holsatus</i>	3.53	1.00	0.586	1.72	1.35
6	<i>C. crangon</i>	2.30	0.487	0.382	0.727	ND
6	<i>C. crangon</i>	1.31	0.561	0.576	0.494	ND
6	<i>C. crangon</i>	0.670	0.594	0.110	0.818	ND
7	<i>L. holsatus</i>	4.62	2.73	1.80	4.44	3.26
7	<i>L. holsatus</i>	2.71	1.43	<LOQ	4.52	4.59
7	<i>L. holsatus</i>	1.46	2.41	0.795	1.75	3.173
7	<i>C. crangon</i>	4.55	0.702	0.713	2.43	0.913
7	<i>C. crangon</i>	1.85	1.58	<LOQ	1.47	0.715
7	<i>C. crangon</i>	1.01	1.29	<LOQ	0.722	2.49
8	<i>L. holsatus</i>	1.79	0.882	0.101	1.18	1.35
8	<i>L. holsatus</i>	0.909	1.11	0.175	1.15	1.22
8	<i>L. holsatus</i>	2.12	0.495	0.185	1.65	1.13
8	<i>C. crangon</i>	1.77	0.360	0.066	0.599	ND
8	<i>C. crangon</i>	0.292	0.706	<LOQ	0.735	ND
8	<i>C. crangon</i>	2.83	0.794	0.619	0.813	ND
9	<i>L. holsatus</i>	2.95	0.691	0.653	1.33	0.705
9	<i>L. holsatus</i>	3.10	1.55	0.155	2.04	0.716
9	<i>L. holsatus</i>	2.23	2.36	4.55	1.30	6.14
10	<i>L. holsatus</i>	4.77	2.56	0.877	0.760	1.35
10	<i>L. holsatus</i>	4.72	1.62	1.18	1.81	3.33
10	<i>L. holsatus</i>	3.84	1.98	1.38	2.40	1.41

Table A.10: Test statistics of comparison between locations for PFTrDA concentrations in fish liver tissue. 'NA' indicates that the species was not collected at the corresponding location. Not significant differences are indicated by 'ns'.

locations	<i>P. platessa</i>	<i>S. solea</i>	<i>M. merlangus</i>
1 vs. 4	p<0.001	p<0.001	p<0.001
2 vs. 4	p=0.0198	p=0.0032	ns

3 vs. 4	NA	NA	p<0.001
5 vs. 4	p<0.001	p<0.001	p<0.001
6 vs. 4	p<0.001	p<0.001	p<0.001
7 vs. 4	p<0.001	NA	p<0.001
8 vs. 4	p<0.001	p<0.001	p<0.001
9 vs. 4	p<0.001	p<0.001	NA
10 vs. 4	p<0.001	NA	NA
1 vs. 2	ns	ns	p<0.001
3 vs. 2	NA	NA	p<0.001
5 vs. 2	ns	ns	p<0.001
6 vs. 2	ns	ns	p<0.001
7 vs. 2	ns	NA	p<0.001
8 vs. 2	ns	ns	p<0.001

Table A.11. Tukey post hoc comparisons tested for differences in PFAS among fish species in liver samples. Significant differences are indicated by the corresponding p-values, 'ns' indicates non-significant differences and 'NA' is used in case compound was not detected in the corresponding samples.

LIVER	PFOS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA
<i>P. platessa</i> vs. <i>S. solea</i>	ns	ns	ns	ns	ns	ns	ns
<i>P. platessa</i> vs. <i>M. surmuletus</i>	ns	ns	ns	ns	ns	ns	ns
<i>P. platessa</i> vs. <i>A. laterna</i>	ns	ns	ns	ns	ns	ns	NA
<i>P. platessa</i> vs. <i>C. harengus</i>	ns	<0.001	<0.001	ns	<0.001	ns	ns
<i>P. platessa</i> vs. <i>M. merlangus</i>	<0.001	ns	<0.001	0.017	ns	<0.001	ns
<i>P. platessa</i> vs. <i>G. vulgaris</i>	0.002	ns	0.007	<0.001	0.005	<0.001	ns
<i>S. solea</i> vs. <i>M. surmuletus</i>	ns	ns	ns	ns	ns	ns	ns
<i>S. solea</i> vs. <i>A. laterna</i>	ns	ns	ns	ns	ns	ns	NA
<i>S. solea</i> vs. <i>C. harengus</i>	ns	<0.001	<0.001	ns	<0.001	ns	ns
<i>S. solea</i> vs. <i>M. merlangus</i>	0.014	ns	<0.001	<0.001	0.002	ns	ns
<i>S. solea</i> vs. <i>G. vulgaris</i>	0.028	ns	0.002	<0.001	<0.001	0.011	0.0414
<i>M. surmuletus</i> vs. <i>A. laterna</i>	ns	ns	ns	ns	ns	ns	NA
<i>M. surmuletus</i> vs. <i>C. harengus</i>	ns	0.005	0.002	ns	<0.001	ns	ns
<i>M. surmuletus</i> vs. <i>M. merlangus</i>	ns	ns	0.023	ns	ns	ns	ns
<i>M. surmuletus</i> vs. <i>G. vulgaris</i>	ns	ns	0.017	0.009	0.007	0.010	ns
<i>A. laterna</i> vs. <i>C. harengus</i>	ns	0.001	ns	ns	<0.001	ns	NA
<i>A. laterna</i> vs. <i>M. merlangus</i>	ns	ns	ns	ns	0.023	ns	NA
<i>A. laterna</i> vs. <i>G. vulgaris</i>	ns	ns	ns	0.033	0.002	0.009	NA
<i>C. harengus</i> vs. <i>M. merlangus</i>	ns	<0.001	ns	ns	0.033	ns	ns
<i>C. harengus</i> vs. <i>G. vulgaris</i>	ns	<0.001	ns	ns	ns	ns	ns

<i>M. merlangus</i> vs. <i>G. vulgaris</i>	ns	ns	ns	ns	ns	ns	ns
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Table A.12. Tukey post hoc comparisons tested for differences in PFAS among fish species in muscle samples. Significant differences are indicated by the corresponding p-value and 'ns' non-significant.

MUSCLE	PFOS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA
<i>P. platessa</i> vs. <i>S. solea</i>	ns	ns	ns	ns	ns	ns	ns
<i>P. platessa</i> vs. <i>M. surmuletus</i>	0.006	ns	ns	ns	ns	ns	ns
<i>P. platessa</i> vs. <i>A. laterna</i>	ns	ns	ns	ns	ns	ns	ns
<i>P. platessa</i> vs. <i>C. harengus</i>	ns	ns	ns	ns	ns	ns	ns
<i>P. platessa</i> vs. <i>M. merlangus</i>	<0.001	ns	ns	ns	ns	ns	ns
<i>P. platessa</i> vs. <i>G. vulgaris</i>	<0.001	ns	ns	ns	ns	ns	ns
<i>S. solea</i> vs. <i>M. surmuletus</i>	ns	ns	ns	ns	ns	ns	ns
<i>S. solea</i> vs. <i>A. laterna</i>	ns	ns	ns	ns	ns	ns	ns
<i>S. solea</i> vs. <i>C. harengus</i>	ns	ns	ns	ns	ns	ns	ns
<i>S. solea</i> vs. <i>M. merlangus</i>	<0.001	ns	ns	ns	ns	ns	ns
<i>S. solea</i> vs. <i>G. vulgaris</i>	<0.001	ns	ns	ns	ns	ns	ns
<i>M. surmuletus</i> vs. <i>A. laterna</i>	ns	ns	ns	ns	ns	ns	ns
<i>M. surmuletus</i> vs. <i>C. harengus</i>	ns	ns	ns	ns	ns	ns	ns
<i>M. surmuletus</i> vs. <i>M. merlangus</i>	ns	ns	ns	ns	ns	ns	ns
<i>M. surmuletus</i> vs. <i>G. vulgaris</i>	ns	ns	ns	ns	ns	ns	ns
<i>A. laterna</i> vs. <i>C. harengus</i>	ns	ns	ns	ns	ns	ns	ns
<i>A. laterna</i> vs. <i>M. merlangus</i>	<0.001	ns	ns	ns	ns	ns	ns
<i>A. laterna</i> vs. <i>G. vulgaris</i>	<0.001	ns	ns	ns	ns	ns	ns
<i>C. harengus</i> vs. <i>M. merlangus</i>	ns	ns	ns	ns	ns	ns	ns
<i>C. harengus</i> vs. <i>G. vulgaris</i>	0.002	ns	ns	ns	ns	ns	ns
<i>M. merlangus</i> vs. <i>G. vulgaris</i>	ns	ns	ns	ns	ns	ns	ns

Table A.13: $\delta^{15}\text{N}$ stable isotope analysis for each species per location. N=1, because samples were pooled per location.

	<i>S. solea</i>	<i>G. vulgaris</i>	<i>P. platessa</i>	<i>M. merlangus</i>	<i>C. harengus</i>	<i>M. surmuletus</i>	<i>A. laterna</i>	<i>C. crangon</i>	<i>L. holtsatus</i>
1	16.5	16.3	14.7	17.8				16.4	15.4
2	16.1		15.3	17.2				15.6	15.3

3	17.4		17.9				15.4	15.3
4	16.2	17	14.5	17.9			15.8	15
5	16.9	14.7		18	16.1	16.2		14.7
6	15.3	14.7		17.9	16.6	16.2		14.3
7	15.3	15.1		17			15.5	14.2
8	15.9	14.7		17.8	15.2	16.1	15.4	14.6
9	15.1	14.9		14.7	15.4	15.9	15.4	
10			14.4			13.2	14.4	13.3

Table A.14: $\delta^{13}\text{C}$ stable isotope analysis for each species per location. $N=1$, because samples were pooled per location.

	<i>S. solea</i>	<i>G. vulgaris</i>	<i>P. platessa</i>	<i>M. merlangus</i>	<i>C. harengus</i>	<i>M. surmuletus</i>	<i>A. laterna</i>	<i>C. crangon</i>	<i>L. holsatus</i>
1	-17.1	-17	-16.7	-16.7				-17	-18.2
2	-16.9		-16.9	-17.1				-16.2	-18.2
3		-16.2		-17.5				-16	-18.4
4	-16.5	-16.4	-17.3	-16.9				-16.1	-19.1
5	-18.2		-17.5	-17.4	-19.9			-16.1	-18.0
6	-17.2		-18.7	-17.3	-18.8			-16.6	-17.8
7	-16.6		-17.3	-16.7				-16.6	-18.7
8	-17.1		-17.8	-17.2		-18.6	-17.5	-15.8	-17.6
9	-17.4		-17.7		-19.4	-19.5	-17.5		-19.3
10			-17.5			-18.4	-18.3		-17.8

Table A.15: Relative trophic levels calculated based on $\delta^{15}\text{N}$ stable isotope analysis for each species per location. $N=1$, because samples were pooled per location.

	<i>S. solea</i>	<i>G. vulgaris</i>	<i>P. platessa</i>	<i>M. merlangus</i>	<i>C. harengus</i>	<i>M. surmuletus</i>	<i>A. laterna</i>	<i>C. crangon</i>	<i>L. holsatus</i>
1	3.1	3.1	2.6	3.4				3.1	2.9
2	3.2		3	3.7				2.9	2.8
3		3.6		3.6				2.9	2.8
4	3.2	3.4	2.7	3.7				3.0	2.7
5	3.3		2.6	3.6	3.1				2.6
6	2.8		2.6	3.6	3.2			3.1	2.5

7	3	2.9	3.7			2.9	2.5
8	3.2	2.9	3.5	3	3.3	2.9	2.6
9	3	2.9		2.9	3.1	3.2	2.9
10		2.8			2.4	2.8	2.2

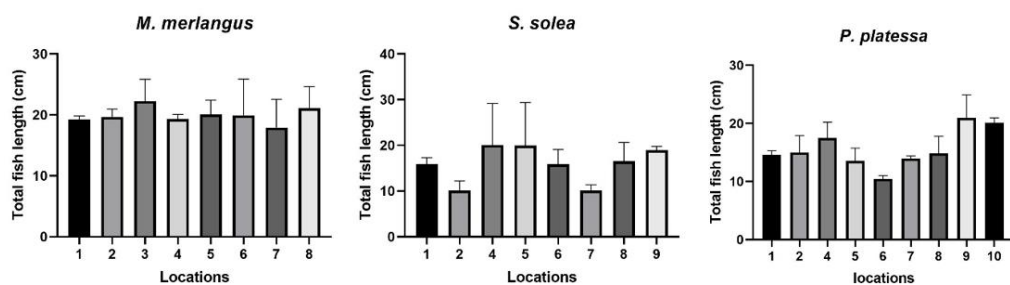
Figures:

Figure A.1: Overview of the mean total fish lengths and standard deviation of *M. merlangus*, *S. solea* and *P. platessa* per locations.

Appendix B: Chapter III

Tables:

Table B.1: Sampling dates and x,y coordinates of all sampling sites

site nr.	Location	Sampling date	X	Y
1	Doel	28/09/2020	51,311509	4,273670
2	Antwerp	1/10/2020	51,205511	4,370938
3	Kruikebeke	15/10/2020	51,171913	4,323302
4	Steendorp	29/09/2020; 30/09/2020	51,115267	4,246973
5	Branst	29/09/2020	51,077055	4,189723
6	Kastel	28/09/2020; 30/09/2020	51,051347	4,189778
7	Appels	28/09/2020	51,048703	4,069242
8	Wetteren	28/09/2020	51,003272	3,867594

Table B.2: Overview of abiotic water parameters measured by INBO (AM: above measurement range).

Site	Date	Temp. (°C)	O ₂ (mg/l)	O ₂ (%)	pH	Turbidity (NTU)	Salinity (‰)	Conductivity (µS/cm)
Doel	2/09/2020	21.5	8.01	91	7.98	AM	17.03	25800
Doel	3/09/2020	21.6	8.21	92.6	8.07	AM	17.59	26600
Antwerpen	15/09/2020	20	7.27	80.4	7.67	AM	6.86	10900
Antwerpen	16/09/2020	20.4	7.66	86	7.81	AM	7.49	11940
Steendorp	15/09/2020	20.1	6.61	73.3	7.81	83.7	2.84	4790
Steendorp	16/09/2020	20.9	7.07	79.6	7.89	52.9	3.14	5310
Kastel	29/09/2020	15.4	7.25	73.2	8.12	4.49	0.41	670
Kastel	30/09/2020	16	7.31	74.7	7.71	AM	0.4	664
Appels	29/09/2020	16.2	7.54	77.4	7.99	AM	0.43	719
Appels	30/09/2020	16.7	7.08	73.8	7.95	AM	0.42	719
Wetteren	29/09/2020	16.7	7.59	78.8	7.71	52.5	0.44	749
Wetteren	30/09/2020	16.4	7.82	81	8.06	AM	0.43	727

Table B.3: List of PFAS target analytes

Abbreviation	Compound
PFBA	Perfluorobutanoic acid
PFPeA	Perfluoropentanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PFDoDA	Perfluorododecanoic acid
PFTTrDA	Perfluorotridecanoic acid

PFTeDA	Perfluorotetradecanoic acid
PFBS	Perfluorobutanesulfonic acid
PFPeS	Perfluoropentanesulfonic acid
PFHxS	Perfluorohexanesulfonic acid
PFHpS	Perfluoroheptanesulfonic acid
PFOS	Perfluorooctanesulfonic acid
PFDS	Perfluorodecanesulfonic acid
11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid
9CL-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane--sulfonic acid
8:2 FTS	1H,1H,2H,2H-Perfluorodecane sulfonic acid
6:2 FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid
NaDONA	Sodium dodecafluoro-3H-4,8-dioxanonanoate
4:2 FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid
PFEESA	Potassium perfluoro(2-ethoxyethane)sulfonate
HFPO-DA	Hexafluoropropylene oxide dimer acid
PF5OHxA	Perfluoro-5-oxahexanoic acid
PF4OPeA	Perfluoro-4-oxapentanoic acid
3,6-OFHpA	Perfluoro-3,6-dioxaheptanoic acid
FBSA	Perfluorobutane sulfonamide

Table B.4: average PFAS concentrations (av.) and min-max ranges (ng/g ww) detected in muscle tissue of all species and all sampling sites ("<LOQ": below limit of quantification; "ND" = Not Detected; "N"= number of replicates). The average LOQs (ng/g ww) are also shown below each compound.

species	site		PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTeDA	FBSA	PFBS	PFPeS	PFHpS	PFOS	8:2 FTS	6:2 FTS	NaDONA
		LOQ	0.28	0.19	0.25	0.27	0.65	0.39	1.3	0.45	0.84	0.56	0.42	0.21	0.67	0.60	0.09
European eel (N=6)	2	av.	0.60	<LOQ	1.4	1.1	15	13	19	1.3	7.6	<LOQ	ND	18	ND	1.4	1.5
		min	0.40	<LOQ	1.1	0.88	11	11	14	0.60	4.5	<LOQ	ND	9.2	ND	<LOQ	0.42
		max	0.78	<LOQ	1.6	1.7	20	16	23	2.3	12	1.1	ND	26	ND	2.2	2.3
Bream (N=3)	2	av.	0.84	<LOQ	3.7	1.0	8.3	2.7	1.3	12	ND	ND	<LOQ	14	1.9	170	6.1
		min	<LOQ	<LOQ	1.8	0.74	7.1	2.5	<LOQ	4.2	ND	ND	<LOQ	8.0	<LOQ	109	3.1
		max	1.48	0.20	5.4	1.3	11	3.0	3.9	27	ND	ND	<LOQ	22	4.0	280	10.5
Atlantic herring (N=10)	2	av.	0.36	<LOQ	0.35	<LOQ	2.2	1.3	<LOQ	43	ND	5.9	ND	3.4	ND	<LOQ	18
		min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.6	ND	1.9	ND	0.87	ND	<LOQ	4.2
		max	1.1	<LOQ	0.64	0.37	4.7	2.7	1.9	95	ND	11	ND	7.0	ND	<LOQ	45
European seabass (N=10)	2	av.	<LOQ	<LOQ	0.91	0.60	7.9	7.2	4.8	21	ND	2.1	ND	11	ND	ND	5.2
		min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.59	<LOQ	7.6	ND	<LOQ	ND	2.4	ND	ND	1.3
		max	0.59	<LOQ	1.8	0.97	13	11	8.9	34	ND	9.6	ND	17	ND	ND	27
common sole (N=22)	2	av.	0.38	0.86	1.5	1.3	12	6.8	7.0	27	ND	ND	<LOQ	33	ND	<LOQ	1.4
		min	<LOQ	<LOQ	0.31	0.39	2.1	<LOQ	<LOQ	7.1	ND	ND	<LOQ	ND	ND	<LOQ	<LOQ
		max	0.73	2.0	6.5	1.8	24	15	14	52	ND	ND	1.2	106	ND	<LOQ	8.8

pikeperch (N=10)	2	av.	<LOQ	<LOQ	7.1	0.64	5.8	4.8	3.86	71	ND	<LOQ	ND	7.2	ND	<LOQ	0.84
		min	<LOQ	<LOQ	2.6	0.33	2.7	2.7	2.3	26	ND	<LOQ	ND	3.3	ND	<LOQ	0.19
		max	0.44	<LOQ	19	0.88	8.5	7.0	5.7	108	ND	0.70	ND	14	ND	<LOQ	1.5
common shrimp (N=9)	2	av.	0.71	1.2	3.2	1.8	12	11	15.4	33	ND	ND	0.85	30	ND	<LOQ	0.29
		min	0.28	0.25	0.45	0.54	2.0	7.3	9.5	14	ND	ND	<LOQ	3.7	ND	<LOQ	<LOQ
		max	1.1	2.0	8.4	4.2	23	14	20	50	ND	ND	3.0	113	ND	<LOQ	0.50
rockpool shrimp (N=7)	2	av.	1.1	<LOQ	0.29	<LOQ	3.2	4.2	6.0	8.8	ND	ND	ND	1.1	ND	<LOQ	0.12
		min	0.67	<LOQ	<LOQ	<LOQ	1.9	1.4	2.0	4.2	ND	ND	ND	0.38	ND	<LOQ	<LOQ
		max	1.7	<LOQ	1.1	<LOQ	5.6	15	19	14	ND	ND	ND	2.3	ND	<LOQ	0.21
European flounder (N=5)	5	av.	0.59	0.20	3.0	1.2	7.9	4.6	4.8	4.7	ND	ND	ND	18	ND	ND	1.0
		min	<LOQ	<LOQ	2.2	0.63	3.8	2.4	<LOQ	<LOQ	ND	ND	ND	16	ND	ND	0.33
		max	1.2	0.36	4.1	2.0	13	6.7	7.1	16	ND	ND	ND	19	ND	ND	2.4
European flounder (N=5)	6	av.	0.31	0.33	2.7	1.2	8.2	5.1	4.2	4.5	ND	ND	ND	17	ND	ND	0.22
		min	<LOQ	<LOQ	0.51	<LOQ	1.1	0.63	<LOQ	<LOQ	ND	ND	ND	2.6	ND	ND	<LOQ
		max	0.83	0.62	4.5	2.9	19	11	9	9.4	ND	ND	ND	23	ND	ND	0.37
European flounder (N=10)	8	av.	0.54	<LOQ	2.3	0.88	7.0	5.2	4.2	1.5	ND	ND	ND	15.	ND	ND	<LOQ
		min	<LOQ	<LOQ	0.39	<LOQ	0.75	0.64	<LOQ	<LOQ	ND	ND	ND	1.8	ND	ND	<LOQ
		max	1.7	0.46	3.4	2.0	12	8.2	10	8.5	ND	ND	ND	25	ND	ND	0.21

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European flounder (N=10)	7	av.	0.48	<LOQ	3.0	1.3	11	9.1	8.8	1.7	ND	ND	ND	19	ND	5.9	0.16
		min	<LOQ	<LOQ	1.5	<LOQ	4.3	3.5	<LOQ	<LOQ	ND	ND	ND	8.4	ND	2.8	<LOQ
		max	1.4	0.46	4.7	2.8	14	13	15	8.3	ND	ND	ND	24	ND	8.3	0.61
European flounder (N=6)	4	av.	0.73	0.62	3.5	1.9	12	7.6	6.8	18	ND	ND	ND	28	ND	12	0.64
		min	0.54	0.46	1.9	0.99	4.0	3.9	<LOQ	3.4	ND	ND	ND	22	ND	<LOQ	0.28
		max	0.94	0.74	4.4	2.5	18	12	10	39	ND	ND	ND	32	ND	18	0.90
European flounder (N=5)	2	av.	0.47	0.38	2.3	1.2	6.0	3.3	3.9	12	ND	ND	ND	22	ND	<LOQ	0.32
		min	<LOQ	<LOQ	1.8	0.73	3.0	0.96	<LOQ	<LOQ	ND	ND	ND	8.3	ND	<LOQ	0.20
		max	1.4	0.61	2.9	2.1	9.5	6.4	5.6	33	ND	ND	ND	49	ND	0.82	0.57
Chinese mitten crab (N=8)	7	av.	<LOQ	<LOQ	1.3	0.43	7.4	7.7	5.2	<LOQ	ND	ND	ND	2.7	ND	8.5	<LOQ
		min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.1	<LOQ	<LOQ	ND	ND	ND	0.84	ND	2.5	<LOQ
		max	0.72	0.25	3.3	2.1	28	23	28	<LOQ	ND	ND	ND	7.0	ND	16	<LOQ
Chinese mitten crab (N=10)	8	av.	0.77	<LOQ	0.96	0.55	5.8	5.2	8.8	<LOQ	ND	ND	ND	1.2	ND	7.8	ND
		min	0.38	<LOQ	0.42	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	<LOQ	ND
		max	1.6	0.26	1.8	1.0	16	17	43	<LOQ	ND	ND	ND	3.4	ND	39	ND
Chinese mitten crab (N=10)	6	av.	0.43	<LOQ	0.85	<LOQ	2.9	3.8	2.5	<LOQ	2.00	ND	ND	1.6	ND	47	<LOQ

		min	<LOQ	<LOQ	0.43	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.84	ND	ND	<LOQ	ND	<LOQ	<LOQ
		max	1.1	<LOQ	1.6	0.86	9.9	20	14	1.3	6.8	ND	ND	4.1	ND	161	<LOQ
Chinese mitten crab (N=10)	4	av.															
			0.49	<LOQ	1.9	0.75	7.4	5.2	7.2	2.8	ND	ND	ND	5.4	ND	17	0.82
		min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND	ND	1.2	ND	<LOQ	<LOQ
		max	0.72	0.62	8.9	4.3	21	14	21	8.0	ND	ND	ND	20	ND	49	1.6
Chinese mitten crab (N=10)	2	av.															
			0.58	<LOQ	0.67	0.31	2.9	1.8	2.1	3.6	ND	ND	ND	1.3	ND	10	<LOQ
		min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	ND	ND	0.30	ND	1.5	<LOQ
		max	0.94	0.91	1.3	1.1	6.3	3.7	5.1	7.4	ND	ND	ND	2.2	ND	65	<LOQ
Chinese mitten crab (N=10)	3	av.															
			0.31	<LOQ	0.76	0.43	5.2	3.0	1.8	20	ND	ND	ND	1.4	ND	ND	<LOQ
		min	<LOQ	<LOQ	<LOQ	<LOQ	2.4	<LOQ	<LOQ	2.9	ND	ND	ND	<LOQ	ND	ND	<LOQ
		max	0.78	<LOQ	1.6	1.3	6.9	7.6	4.5	42	ND	ND	ND	3.4	ND	ND	<LOQ
European smelt (N=4)	2	av.															
			0.51	<LOQ	0.88	0.53	3.9	1.9	<LOQ	22	ND	ND	ND	4.6	ND	4.3	6.1
		min	0.41	<LOQ	0.46	<LOQ	2.3	0.83	<LOQ	9.1	ND	ND	ND	2.6	ND	1.1	2.5
		max	0.68	<LOQ	1.5	1.0	6.3	3.1	2.1	42	ND	ND	ND	7.3	ND	13	9.6
European smelt (N=9)	1	av.															
			0.46	<LOQ	1.1	0.61	4.0	2.6	<LOQ	41	ND	ND	ND	7.7	ND	1.1	6.6

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		min	<LOQ	<LOQ	<LOQ	<LOQ	1.8	1.8	<LOQ	18	ND	ND	ND	4.2	ND	<LOQ	1.1
		max	1.3	0.24	1.9	1.3	5.7	3.3	2.5	62	ND	ND	ND	12	ND	2.2	14
European smelt (N=8)	4	av.	0.31	<LOQ	1.2	0.66	6.1	3.5	1.7	23	ND	ND	ND	8.1	ND	<LOQ	11
		min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.5	<LOQ	6.4	ND	ND	ND	4.2	ND	<LOQ	0.73
		max	0.72	<LOQ	2.5	1.1	12	5.9	6.1	51	ND	ND	ND	11	ND	<LOQ	24
European smelt (N=12)	6	av.	0.32	<LOQ	1.9	0.73	6.9	2.8	2.2	8.0	ND	ND	ND	9.5	ND	11	7.3
		min	<LOQ	<LOQ	1.0	<LOQ	3.5	0.70	<LOQ	4.5	ND	ND	ND	5.3	ND	<LOQ	1.5
		max	0.82	0.49	3.7	2.3	18	3.9	4.0	13	ND	ND	ND	12	ND	36	20
European smelt (N=6)	7	av.	0.89	<LOQ	3.1	1.9	14	7.1	6.8	8.5	ND	ND	ND	14	ND	12	2.0
		min	0.34	<LOQ	1.3	0.66	3.4	2.5	2.0	1.5	ND	ND	ND	5.3	ND	1.1	0.47
		max	2.8	0.53	9.9	7.0	47	26	24	18	ND	ND	ND	44	ND	26	3.8
European smelt (N=8)	5	av.	0.35	<LOQ	1.9	0.98	9.2	4.3	3.8	21	ND	ND	ND	11	ND	ND	10.0
		min	<LOQ	<LOQ	1.1	0.54	4.5	3.3	2.1	4.2	ND	ND	ND	7.6	ND	ND	1.8
		max	1.2	0.25	2.9	1.7	12	6.0	4.8	37	ND	ND	ND	15	ND	ND	15
European perch (N=3)	2	av.	<LOQ	0.21	2.9	1.3	41	11	13	28	ND	ND	ND	24	2.0	<LOQ	3.2
		min	<LOQ	<LOQ	2.5	0.74	4.5	6.8	<LOQ	1.4	ND	ND	ND	8.0	1.2	<LOQ	0.95
		max	0.55	0.55	3.2	1.7	72	13	23	61	ND	ND	<LOQ	36	2.9	<LOQ	6.3
European perch (N=3)	4	av.	0.41	0.41	3.5	1.4	32	11	12	47	ND	ND	0.56	44	1.6	<LOQ	3.0
		min	0.29	0.29	1.8	0.70	4.9	8.0	4.8	19	ND	ND	<LOQ	42	0.79	<LOQ	2.6
		max	0.50	0.50	5.4	1.8	87	15	20	69	ND	ND	0.95	46	2.6	<LOQ	3.8

European perch (N=3)	1	av.	0.78	0.78	2.9	0.62	2.8	19	29	1.8	ND	ND	<LOQ	16	2.6	56	1.6
		min	0.60	0.60	2.3	0.52	1.2	14	18	1.5	ND	ND	<LOQ	12	1.9	<LOQ	1.1
		max	0.98	0.98	3.8	0.82	4.9	28	48	2.0	ND	ND	<LOQ	22	3.7	167	1.9
European perch (N=3)	3	av.	0.69	0.69	1.8	0.96	6.2	14	11	95	ND	ND	<LOQ	29	<LOQ	130	0.90
		min	0.30	0.30	1.4	0.76	4.7	12	9.1	89	ND	ND	<LOQ	25	<LOQ	96	0.60
		max	0.92	0.92	2.3	1.2	8.7	17	14	98	ND	ND	<LOQ	34	<LOQ	164	1.2
European perch (N=4)	6	av.	0.70	0.70	2.5	0.71	4.1	8.1	14	0.80	ND	ND	<LOQ	8.5	0.80	2.7	2.3
		min	0.47	0.47	1.5	0.41	1.6	6.0	12	<LOQ	ND	ND	<LOQ	6.5	<LOQ	<LOQ	1.8
		max	0.96	0.96	3.8	0.92	5.5	11	16	1.3	ND	ND	<LOQ	12	2.0	4.2	2.8
European perch (N=8)	7	av.	0.44	0.44	2.2	0.73	3.5	11	14	0.70	ND	ND	<LOQ	6.3	<LOQ	ND	3.6
		min	<LOQ	<LOQ	0.81	0.32	0.87	5.6	<LOQ	<LOQ	ND	ND	<LOQ	4.6	<LOQ	ND	1.2
		max	0.70	0.70	4.2	2.0	8.3	22	25	1.2	ND	ND	1.2	8.9	1.8	ND	7.7
European perch (N=3)	8	av.	<LOQ	0.31	1.6	0.77	7.7	12	11	1.6	ND	ND	<LOQ	11	<LOQ	ND	0.55
		min	<LOQ	<LOQ	1.2	0.63	6.2	12	9.1	1.3	ND	ND	<LOQ	9.3	<LOQ	ND	0.23
		max	0.67	0.67	2.0	0.98	8.7	13	13	2.0	ND	ND	0.61	13	<LOQ	ND	0.79
European perch (N=5)	5	av.	0.76	0.79	3.5	1.2	7.8	17	18	1.7	ND	0.88	1.1	17	0.91	<LOQ	2.2
		min	<LOQ	0.23	1.5	0.68	2.6	10	<LOQ	<LOQ	ND	<LOQ	<LOQ	7.6	<LOQ	<LOQ	0.93
		max	1.5	1.5	8.1	2.8	15	35	30	5.2	ND	1.8	1.9	54	1.6	<LOQ	3.5

Table B5: Average PFAS concentrations (av.) and min-max ranges (ng/g ww) detected in liver tissue from a number of species sampled at site two (Antwerp) (" $<LOQ$ ": below limit of quantification; "ND" = Not Detected; "N" = number of replicates). The average LOQs (ng/g ww) are also shown below each compound.

Species		PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTeDA	FBSA	PFBS	PFPeS	PFHpS	PFOS	8:2 FTS	6:2 FTS	NaDONA
	LOQ	1.9	1.2	1.7	1.1	5.1	2.3	6.4	4.2	7.9	2.9	3.3	2.5	3.5	3.9	1.1
European smelt (N=3)	av.	<LOQ	<LOQ	5.4	3.9	25	12	7.21	107	ND	3.5	ND	66	ND	ND	72
	min	<LOQ	<LOQ	3.4	2.4	7.7	4.3	<LOQ	47	ND	<LOQ	ND	33	ND	ND	46
	max	<LOQ	<LOQ	9.2	6.9	53	26	21.53	227	ND	6.5	ND	130	ND	ND	87
European flounder (N=4)	av.	4.6	<LOQ	20	9.9	83	34	20.00	149	ND	<LOQ	ND	449	<LOQ	ND	13
	min	<LOQ	<LOQ	13	5.3	44	12	<LOQ	<LOQ	ND	<LOQ	ND	153	<LOQ	ND	8.6
	max	16	2.9	37	13	102	49	59.94	300	ND	<LOQ	ND	1181	<LOQ	ND	17
Atlantic herring (N=10)	av.	4.1	<LOQ	7.1	<LOQ	23	9.3	<LOQ	422	ND	10	ND	47	<LOQ	ND	38
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	<LOQ	ND	14	<LOQ	ND	<LOQ
	max	9.1	<LOQ	25	4.0	51	20	<LOQ	1221	ND	18	ND	127	<LOQ	ND	71
pikeperch (N=10)	av.	<LOQ	<LOQ	8.1	9.6	106	51	35.13	358	ND	<LOQ	ND	150	ND	ND	1.6
	min	<LOQ	<LOQ	3.7	4.8	50	22	18.31	136	ND	<LOQ	ND	71	ND	ND	<LOQ
	max	4.3	2.9	14	16	162	76	46.93	537	ND	<LOQ	ND	292	ND	ND	4.7
European eel (N=4)	av.	2.0	<LOQ	9.3	8.3	130	91	65.34	11	91	<LOQ	ND	184	<LOQ	<LOQ	<LOQ

	min	<LOQ	6.8	5.2	75	57	47	<LOQ	32	<LOQ	ND	106	<LOQ	<LOQ	ND	
	max	2.9	<LOQ	13	11	165	123	84.90	16	150	<LOQ	ND	321	<LOQ	<LOQ	1.2
European seabass (N=8)	av.	4.5	<LOQ	24	6.6	70	60	54.42	175	ND	<LOQ	ND	189	ND	ND	12
	min	<LOQ	<LOQ	7.9	4.9	49	36	<LOQ	104	ND	<LOQ	ND	117	ND	ND	1.4
	max	7.3	<LOQ	41	8.5	107	80	89	259	ND	<LOQ	ND	283	ND	ND	30
common sole (N=16)	av.	3.5	9.4	107	14	114	72	73	209	ND	<LOQ	5.8	1057	<LOQ	<LOQ	25
	min	<LOQ	<LOQ	12	1.7	12	8.1	8.4	29	ND	<LOQ	<LOQ	153	<LOQ	<LOQ	<LOQ
	max	12	22	225	21	200	130	134	700	ND	<LOQ	15	2465	3.6	5.5	44

Table B.6: Overview of isotopic niche areas (SEAc) (‰).

Species	$\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$	$\delta^{15}\text{N}$ vs. $\delta^{34}\text{S}$	$\delta^{13}\text{C}$ vs. $\delta^{34}\text{S}$	ΣPFAS vs. $\delta^{15}\text{N}$	ΣPFAS vs. $\delta^{13}\text{C}$	ΣPFAS vs. $\delta^{34}\text{S}$
Chinese mitten crab	8.9	11	7.6	202	95	211
Atlantic herring	2.3	3.6	11	676	1546	250
European seabass	11	5.3	6.7	247	409	316
common sole	1.3	1.5	2.6	76	190	169
pikeperch	4.7	10	7.7	235	155	418
common shrimp	2.8	4.3	7.7	143	177	342
European eel	0.22	0.96	0.16	22	9.1	34
European flounder	1.4	29	13	158	57	208
European smelt	7.5	3.7	19	49	184	147
rockpool shrimp	0.99	1.8	2.9	25	33	45

Table B.7: mean Total lenght (mm) and weight (g) with Standard Deviation for fish and invertebrate species sampled at Antwerp sampling station

Species	(carapax) Length (mm)	Weight (g)
European smelt	160 ± 15	24 ± 8.3
European perch	92 ± 13	9.0 ± 3.7
bream	144 ± 47	7.0 ± 0.67
Pikeperch	142 ± 21	24 ± 14
European eel	495 ± 119	294 ± 151
European flounder	75 ± 11	4.6 ± 2.5
common sole	139 ± 72	58 ± 122.3
European seabass	85 ± 20	6.8 ± 5.0
Atlantic herring	101 ± 8.3	6.5 ± 1.4
common shrimp	52 ± 2.1	0.87 ± 0.08
rockpool shrimp	51 ± 6.5	1.0 ± 0.24
Chinese mitten crab	43 ± 5.1	32 ± 13

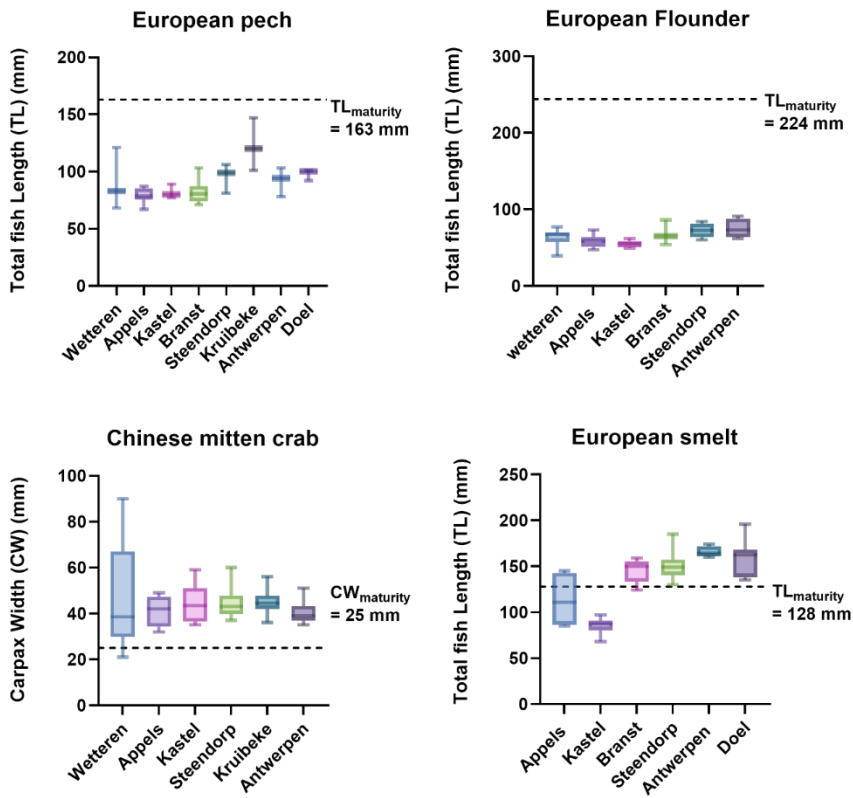
Figures:

Figure B.1: Mean Total Length (TL) (mm) and Carapax Width (CW) (mm) with Standard Deviation (SD) of European perch, European flounder, Chinese mitten crab and European smelt per sampling site. The TLs at maturity were sourced from www.fishbase.org and the CW at maturity was sourced from Dittel et al. (2009).

Appendix C: Chapter IV

PFAS extraction procedure biotic and abiotic samples:

The extraction protocols for both biotic and abiotic samples followed the methodologies outlined by Powley et al. (2005) and Groffen et al. (2019b). Clam tissues were homogenized using a mortar and pestle and transferred into 50 mL polypropylene (PP) tubes. The tissue mass and sediment were measured using a precision balance (Mettler AT261 DeltaRange balance) to ensure a minimum of 30 mg of tissue was available for analysis. For the water samples 10 mL was transferred into 50 mL PP tubes. To each sample, 80 μ L of an isotopically mass-labelled internal standard solution (ISTD) mix solution with a concentration of 125 pg/L was added. The ISTD (MPFAC-MXA, Wellington laboratories, Guelph, Canada) was diluted in a 1:1 acetonitrile (ACN). For the extraction, 10 mL of ACN was added to the clam and sediment samples. Then, all samples were vortexed and subjected to three 10-minute cycles in an ultrasonic bath (Branson 2510), with vortexing between cycles. Subsequently, the samples were placed on a shaking plate overnight at 135 rpm. The following day, the samples were centrifuged (Eppendorf 5804R) at 2400 rpm for 10 minutes at 4°C. The supernatant was then transferred to 15 mL PP tubes for further processing.

For clam tissue samples, the supernatant was concentrated using a vacuum concentrator (RVC 2-25) at 20°C until approximately 0.5 mL remained. Concurrently, 0.1 mL of activated carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, USA) was prepared in Eppendorf tubes with 50 μ L of glacial acetic acid (100%) and left to soak for at least 15 minutes. The concentrated extract was transferred to the Eppendorf tubes containing the activated carbon. To ensure complete transfer, the 15 mL tubes were rinsed twice with 250 μ L of ACN (100%), vortexed, and the rinses were added to the Eppendorf tubes. The samples were vortexed for at least one minute to ensure thorough mixing of the activated carbon and the sample. Following this, the samples were centrifuged (Eppendorf 5804R) at 2400 rpm for 10 minutes at 4°C, and the supernatant was transferred to new Eppendorf tubes. The supernatant was then concentrated again using the rotational vacuum concentrator at 20°C until almost dry. Finally, the samples were reconstituted with 200 μ L of a 2% ammonium hydroxide solution in ACN (100%) and prepared for filtration.

For abiotic samples, HR-XAW cartridges were conditioned with 5 mL of acetonitrile, followed by equilibration with 5 mL of Milli-Q water. Once the cartridges were nearly dry, the samples

were loaded. The cartridges were then washed sequentially with 5 mL of 25 mM ammonium acetate and 2 mL of ACN (100%). The elution was carried out using two successive 1 mL aliquots of 2% ammonium hydroxide solution in ACN. The eluents were dried completely and then reconstituted with 200 µL of the same ammonium hydroxide solution in ACN, after which they were transferred to 6 mL PP tubes for the final step.

Both biotic and abiotic extracts underwent filtration to remove any solid particles. The extracts were passed through a 10 mL Braun syringe connected to an Ion Chromatography Acrodisc 13 mm syringe filter with a 0.2 µm Supor (PES) membrane. The filtered samples were then transferred into PP injector vials, ready for subsequent analysis.

UPLC-TQD analysis:

The analysis of per- and polyfluoroalkyl substances (PFASs) was performed using ultra-performance liquid chromatography coupled with tandem electrospray ionization mass spectrometry in negative mode (UPLC-MS/MS, ACQUITY TQD, Waters, Milford, MA, USA), following the methodology described by Groffen et al. (2019b). Separation of the analytes was achieved using an ACQUITY BEH C18 column (2.1 × 50 mm, 1.7 µm; Waters, USA). The mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (ACN), with a sample injection volume of 6 µL and a flow rate of 450 µL/min. PFAS compounds were identified and quantified using multiple reaction monitoring (MRM). Diagnostic transitions, cone voltages, and collision energies for each analyte were referenced from Groffen et al. (2019b, 2021a, Table 1). The resulting data were processed using MassLynx software (Waters) to extract the peak response areas of the target analytes and internal standards (ISTDs).

Tables:

Table C.1: List of PFAS target analytes

Abbreviation	Compound
PFBA	Perfluorobutanoic acid
PFPeA	Perfluoropentanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PFDoDA	Perfluorododecanoic acid

PFTrDA	Perfluorotridecanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFBS	Perfluorobutanesulfonic acid
PFPeS	Perfluoropentanesulfonic acid
PFHxS	Perfluorohexanesulfonic acid
PFHpS	Perfluoroheptanesulfonic acid
PFOS	Perfluorooctanesulfonic acid
PFDS	Perfluorodecanesulfonic acid
11Cl-PF3OUdS	11-Chloroeicosafuoro-3-oxaundecane-1-sulfonic acid
9Cl-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane--sulfonic acid
8:2 FTS	1H,1H,2H,2H-Perfluorodecane sulfonic acid
6:2 FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid
NaDONA	Sodium dodecafluoro-3H-4,8-dioxanonoate
4:2 FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid
PFEESA	Potassium perfluoro(2-ethoxyethane)sulfonate
HFPO-DA	Hexafluoropropylene oxide dimer acid
PF5OHxA	Perfluoro-5-oxahexanoic acid
PF4OPeA	Perfluoro-4-oxapentanoic acid
3.6-OFHpA	Perfluoro-3,6-dioxaheptanoic acid
FBSA	Perfluorobutane sulfonamide

Table C.2: Average and min-max ranges of bioaccumulated PFAS concentrations (ng/g ww) in Asian clams per sampling site. The Limit Of Quantification (LOQ) is shown below each compound.

		PFBA	PFHxA	PFOA	PFDA	PFDoDA	PFTTrDA	PFTeDA	NaDONA
site	LOQ:	0.30	0.45	0.38	0.74	0.64	0.55	1.1	0.38
Temse	average	<LOQ	<LOQ	4.2	1.7	3.9	1.3	4.5	12
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.7
	max	<LOQ	<LOQ	19	4.4	9.9	4.9	21	29
Rupel	average	<LOQ	<LOQ	0.74	<LOQ	0.33	<LOQ	0.50	1.9
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.1
	max	<LOQ	<LOQ	1.3	<LOQ	0.82	<LOQ	1.2	2.8
Hemiksem	average	<LOQ	<LOQ	1.7	<LOQ	3.7	1.8	5.8	11
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.1
	max	<LOQ	<LOQ	3.5	<LOQ	8.0	4.7	16	34
Steenplein	average	<LOQ	<LOQ	2.6	0.64	1.3	<LOQ	1.1	4.7
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.5

	max	<LOQ	<LOQ	7.9	2.2	6.1	<LOQ	3.5	7.3
Zwijndrecht	average	<LOQ	<LOQ	2.0	<LOQ	2.6	2.7	3.4	6.9
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.3
	max	<LOQ	<LOQ	3.3	<LOQ	9.5	8.1	14	18
Lillo	average	2.0	0.30	1.1	<LOQ	2.0	1.5	2.3	2.1
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.81
	max	8.3	0.84	2.1	<LOQ	3.6	3.1	6.9	2.8

Table C.3: Average and min-max ranges of PFAS concentrations (ng/L) detected in water samples per sampling site. The Limit Of Quantification (LOQ) is shown below each compound.

		PFBA	PFPeA	PFHxA	PFOA	PFOS	9CI-PF3ONS
site	LOQ:	18	4.11	5.33	11.7	7.52	6.21
Temse	average	<LOQ	2.5	9	<LOQ	<LOQ	119
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	max	<LOQ	7.1	20	<LOQ	<LOQ	614
Rupel	average	<LOQ	3.7	10	<LOQ	<LOQ	132
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	max	<LOQ	6.3	17	<LOQ	<LOQ	283
Hemiksem	average	<LOQ	5.1	6.9	<LOQ	<LOQ	<LOQ
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	max	<LOQ	8.3	19	<LOQ	<LOQ	<LOQ
Steenplein	average	<LOQ	5.0	10	16	5.9	37
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	max	<LOQ	7.2	17	23	12	124
Zwijndrecht	average	31	3.5	12	6.8	10	<LOQ
	min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	max	72	6.3	18	18	24	
Lillo	average	53	2.9	12	10	7.7	<LOQ
	min	32	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	max	116	7.0	16	24	17	<LOQ

Table C.4: Average and min-max ranges of PFAS concentrations (ng/L) detected in sediment samples per sampling site. The Limit Of Quantification (LOQ) is shown below each compound.

site		PFOA	PFDoDA	PFTTrDA	PFOS
	LOQ:	0.78	1.5	0.61	1.2
Temse	average	0.75	3.4	0.90	<LOQ

	min	<LOQ	2.6	<LOQ	<LOQ
	max	1.4	3.9	1.7	<LOQ
Hemiksem	average	<LOQ	3.1	0.93	1.7
	min	<LOQ	2.1	0.75	<LOQ
	max	<LOQ	3.6	1.1	3.7
Steenplein	average	0.79	<LOQ	<LOQ	<LOQ
	min	<LOQ	<LOQ	<LOQ	<LOQ
	max	1.5	<LOQ	<LOQ	<LOQ
Zwijndrecht	average	2.1	<LOQ	<LOQ	<LOQ
	min	1.7	<LOQ	<LOQ	<LOQ
	max	2.4	<LOQ	<LOQ	<LOQ

Table C.5: Dilution series of 100 µg/ml Gallic acid stock solution in 80% ethanol to generate standard curve.

Concentration (µg/ml)	Stock of 100µg/ml Gallic acid (µl)	80%ethanol (µl)
0	0	1000
5	50	950
10	100	900
20	200	800
30	300	700
40	400	600
50	500	500
60	600	400
70	700	300
80	800	200
90	900	100
100	1000	0

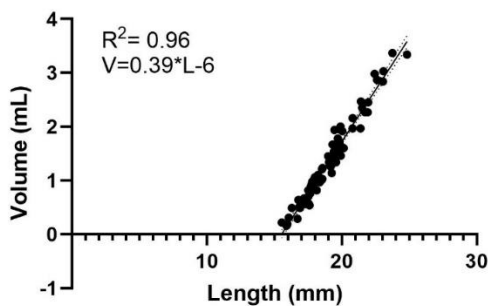
Table C.6: averages and min – max ranges of FRAP (µmol Trolox/g), Polyphenol content (mg GA/gFW) and MDA (nmol/g), condition index and respiration (µgO₂/h*g).

		FRAP	Polyphenols	MDA	Condition index	respiration
Temse	average	29	11	8.4	1.5	22
	min.	22	9.4	5.9	1.1	9.0
	max.	34	13	12	2.1	30
Schelle	average	18	9.4	4.1	1.9	18
	min.	16	7.8	3.6	1.8	5.2
	max.	21	11	4.4	2.1	38
Hemiksem	average	28	13	7.0	1.5	17
	min.	16	11	5.0	1.2	5.1
	max.	48	17	12	2.2	27

Steenplein	average	22	12	5.1	1.5	6.7
	min.	20	10	3.8	1.0	3.0
	max.	26	15	6.8	2.0	10
Zwijndrecht	average	24	9.6	6.0	1.4	13
	min.	20	8.4	4.3	1.1	4.7
	max.	29	10	8.6	1.8	16
Lillo	average	27	13	5.3	1.6	12
	min.	24	11	4.3	1.1	6.1
	max.	33	15	5.9	2.6	21

Table C.7: Correlation coefficients (*r*) and *p*-values of the correlation analysis

		PFBA	PFHxA	PFOA	PFDA	PFDODA	PFTTrDA	PFTeDA	NaDONA	SUM
MDA	<i>r</i>	-0.40	0.61	0.28	0.34	0.46	0.20	0.54	0.41	0.47
	<i>p</i>	0.52	0.27	0.15	0.33	0.01	0.39	0.002	0.03	0.01
Polyphenols	<i>r</i>	-0.70	-0.13	-0.02	-0.22	0.12	-0.20	0.17	0.12	0.18
	<i>p</i>	0.23	0.83	0.92	0.54	0.54	0.40	0.69	0.53	0.36
FRAP	<i>r</i>	-0.60	0.07	0.05	0.31	0.27	-0.04	0.25	0.20	0.24
	<i>p</i>	0.35	0.90	0.80	0.39	0.16	0.87	0.20	0.30	0.22
Condition index	<i>r</i>	-0.16	-0.15	-0.41	-0.30	-0.11	-0.18	0.07	-0.33	-0.33
	<i>p</i>	0.66	0.67	0.002	0.20	0.44	0.27	0.61	0.01	0.02
respiration	<i>r</i>	-0.50	0.71	0.37	0.43	0.35	0.06	0.20	0.19	0.24
	<i>p</i>	0.45	0.18	0.05	0.21	0.07	0.81	0.31	0.32	0.22

Figures:Figure C.1: Linear regression analysis between clam shell length (*L*, mm) and clam volume (*V*, mL)

Appendix D: Chapter V

Details on PFAS extraction method:

- After homogenisation, the samples were 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).
- 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 hours.
- Samples were centrifuged (Eppendorf 5804R) for 10 min at 1037 x 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 one minute.
- After centrifugation (10 min, 9279 x 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; diluted with ACN).
- A filtration step was added in order to remove remaining solids from the final extract. The samples were vortexed and filtered into polypropylene injector vials by transferring them into a 10 mL syringe (Braun) to which an Ion Chromatography Acrodisc 13mm Syringe Filter with 0.2 µm Supor polyethersulfone (PES) Membrane was attached.

Tables:

Table D.1: Overview of all sampling locations including (x,y)-coordinates

Map nr.	Location ID	waterstream	City/Town	Coordinates	
				x	y
1	L1	Vlietbosbeek	Antwerpen	51.22332	4.34211
2	L2	Bruchtse Scheibeek	Antwerpen	51.21331	4.33996
3	L3	Laakbeek	Lille	51.2309	4.84782
4	L4	Bankloop	Olen	51.18981	4.87442
5	L5	Bergebeek	Heist-op-den-berg	51.06614	4.76019
6	L6	Hoeikensloop	Willebroek	51.07694	4.36092
7	L9	Leibeek	Bertem	50.83589	4.64731

8	L11	Demer	Diest	50.97637	5.07187
9	L14	Echelwater	Bilzen	50.89341	5.49807
10	L15	witbeek	kinrooi	51.15265	5.83087
11	L16	Dommelloop	Pelt	51.26789	5.42837
12	L18	Vondelbeek	Lebbeke	51.00878	4.13356
13	L19	Molenbeek	Denderleeuw	50.87239	4.06159
14	L20	Melsenbeek	Merelbeke	50.99933	3.74101
15	L21	Zwarte beek	Wachtebeke	51.16603	3.88618
16	L29	Kattebeek	Deinze	50.96889	3.52484
17	L33	Hoofdsloot	Brugge	51.17984	3.27187
18	L35	Roobeek	Izegem	50.94078	3.18011
19	L36	Tappelbeek L1	Massenhoven	51.208533	4.629002
20	L37	Tappelbeek L2	Massenhoven	51.223138	4.646681
21	L38	Molenbeek	Zandhoven	51.186152	4.659610
22	L40	Struisbeek L1	Antwerpen	51.147520	4.380311
23	L41	Struisbeek L2	Antwerpen	51.147462	4.384779
24	L43	Fabriksloop	Willebroek	51.072106	4.374234
25	L44	Palingbeek	Zwijndrecht	51.22884	4.3316
26	KN1	Kleine nete L1	Retie	51.244	5.05256
27	KN4	Kleine nete L2	Kasterlee	51.21669	4.96709
28	L45	Pulder beek	Lille	51.245989	4.771684
29	L46	Zwarte beek	Willebroek	51.066312	4.372174
30	L47	Molenbeek	Ronse	50.751122	3.607005

Table D.2: MMIF calculation based on 5 individual metrics (TAX= Taxonomic Richness, EPT = number of Ephemeroptera, Plecoptera & Trichoptera taxa, NST= Number of other Sensitive Taxa (except EPT), SWD = Shannon-Wiener Diversity index and MTS = Mean Tolerance Score).

location ID	MMIF	Metric values				
		TAX	EPT	NST	SWD	MTS
L2	0.5	20	1	4	1.1	4.3
L3	0.7	23	3	5	2.5	5.0
L4	0.25	4	1	0	0.23	4.8
L5	0.4	12	2	4	0.68	5.1
L9	0.5	20	0	3	1.9	4.2
L11	0.55	21	4	3	2.3	4.5
L14	0.55	17	1	4	1.9	4.7
L15	0.7	23	2	5	2.2	4.7

L16	0.45	12	4	1	0.72	5.0
L18	0.35	11	1	1	1.3	3.8
L19	0.4	21	0	1	1.5	3.7
L20	0.35	13	0	1	1.3	3.9
L21	0.3	8	0	1	1.6	3.6
L29	0.3	14	0	2	0.95	4.0
L33	0.55	19	3	4	1.4	4.7
L35	0.15	4	0	0	0.86	3.8
L36	0.5	17	3	1	1.6	4.7
L37	0.75	25	6	2	2.0	5.4
L38	0.55	17	1	5	1.7	5.1
L40	0.4	17	0	3	1.6	4.2
L41	0.45	14	1	2	1.4	4.4
L45	0.3	9	1	0	1.6	4.0
L46	0.35	9	1	1	1.6	4.2
L47	0.5	14	1	4	1.7	4.8
KN1	0.9	22	8	7	2.3	6.1
KN4	0.8	19	5	7	2.1	5.7

Table D.3: List of PFAS target analytes

Abbreviation	Compound
PFBA	Perfluorobutanoic acid
PFPeA	Perfluoropentanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PFDoDA	Perfluorododecanoic acid
PFTriDA	Perfluorotridecanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFBS	Perfluorobutanesulfonic acid
PFPeS	Perfluoropentanesulfonic acid
PFHxS	Perfluorohexanesulfonic acid
PFHpS	Perfluoroheptanesulfonic acid
PFOS	Perfluorooctanesulfonic acid
PFDS	Perfluorodecanesulfonic acid
11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid

9CL-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane--sulfonic acid
8:2 FTS	1H,1H,2H,2H-Perfluorodecane sulfonic acid
6:2 FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid
NaDONA	Sodium dodecafluoro-3H-4,8-dioxanonanoate
4:2 FTS	1H,1H,2H,2H-Perfluorohexane sulfonic acid
PFEESA	Potassium perfluoro(2-ethoxyethane)sulfonate
HFPO-DA	Hexafluoropropylene oxide dimer acid
PF5OHxA	Perfluoro-5-oxahexanoic acid
PF4OPeA	Perfluoro-4-oxapentanoic acid
3.6-OFHpA	Perfluoro-3,6-dioxaheptanoic acid
FBSA	Perfluorobutane sulfonamide

Table D.4: Average Limits Of Quantification (LOQ) in ng/g ww combined for all three macroinvertebrate species and for zebra mussels per compound.

Compound	LOQ	LOQ
	Macroinvertebrates	zebra mussels
PFBA	0.26	0.13
PFPeA	0.24	0.98
PFHpA	-	0.87
PFHxA	0.54	2.2
PFOA	0.36	1.3
PFNA	0.172	0.28
PFDA	0.40	0.51
PFUnDA	0.40	0.43
PFDoDA	1.2	0.73
PFTTrDA	0.58	0.30
PFTeDA	1.5	1.4
PFBS	69	2.9
PFPeS	1.0	0.64
PFHxS	0.24	0.72
PFHpS	-	1.9
PFOS	0.43	1.3
PFDS	0.42	1.3
6:2 FTS	1.6	-
3.6-OFHpA	3.0	-
FBSA	0.15	0.39

Table D.5: PFAS concentrations in ng/g ww measured in Asellus sp. per sampling site (N=1). 'ND' indicates that the compound was not detected at this specific site and '<LOQ' indicates that the measurements were below the Limit Of Quantification.

ID	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFND	PFDA	PFUnDA	PFDaDA
L2	0.80	<LOQ	0.37	<LOQ	0.59	<LOQ	<LOQ	<LOQ	4.5
L3	ND	<LOQ	0.96	<LOQ	1.6	0.34	1.30	<LOQ	6.9
L5	ND	<LOQ	0.80	<LOQ	0.37	ND	0.73	<LOQ	6.3
L11	ND	<LOQ	<LOQ	<LOQ	0.97	0.40	1.06	0.97	19
L14	ND	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.52	<LOQ	7.6
L15	<LOQ	<LOQ	<LOQ	<LOQ	0.22	<LOQ	0.34	0.24	7.6
L18	<LOQ	<LOQ	ND	<LOQ	<LOQ	ND	<LOQ	<LOQ	17
L19	<LOQ	<LOQ	<LOQ	<LOQ	0.60	ND	<LOQ	ND	6.9
L20	<LOQ	<LOQ	<LOQ	<LOQ	0.50	ND	0.50	<LOQ	14
L21	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	<LOQ	<LOQ	1.7
L29	<LOQ	<LOQ	<LOQ	<LOQ	0.56	ND	ND	1.02	16
L33	<LOQ	<LOQ	<LOQ	<LOQ	0.77	ND	<LOQ	0.63	18
L36	<LOQ	<LOQ	<LOQ	<LOQ	4.2	ND	1.4	0.88	5.5
L37	1.2	0.43	<LOQ	<LOQ	2.1	<LOQ	<LOQ	<LOQ	7.1
L38	<LOQ	<LOQ	<LOQ	<LOQ	0.59	ND	0.63	<LOQ	6.0
L40	0.83	<LOQ	<LOQ	<LOQ	<LOQ	ND	<LOQ	<LOQ	6.9
L41	0.80	<LOQ	<LOQ	<LOQ	0.40	ND	0.43	ND	8.3
KN1	<LOQ	0.44	0.83	<LOQ	2.3	0.33	1.3	0.58	8.4
L44	94	36	137	30	520	5.9	18	1.0	12
ID	PFTTrDA	PFTTeDA	PFBS	PFPeS	PFHxS	PFHpS	PFOS	PFDS	FBSA
L2	3.8	13	<LOQ	ND	ND	<LOQ	0.33	ND	ND
L3	2.9	4.3	ND	ND	ND	<LOQ	<LOQ	ND	ND
L5	2.6	7.3	ND	ND	ND	<LOQ	ND	ND	ND
L11	4.8	8.1	ND	ND	ND	<LOQ	2.3	ND	ND
L14	4.8	8.9	ND	ND	ND	<LOQ	ND	ND	ND
L15	3.2	6.1	ND	ND	ND	<LOQ	<LOQ	ND	ND
L18	3.5	5.5	<LOQ	ND	ND	<LOQ	<LOQ	ND	ND
L19	2.4	5.0	7.0	ND	ND	<LOQ	ND	ND	ND
L20	5.2	5.1	<LOQ	ND	ND	<LOQ	ND	ND	ND
L21	0.89	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND
L29	5.5	8.9	ND	ND	ND	<LOQ	ND	ND	ND
L33	16	13	7.9	ND	ND	<LOQ	<LOQ	ND	ND

L36	1.6	3.6	42	ND	ND	<LOQ	<LOQ	ND	ND
L37	1.6	1.6	17	4.6	ND	1.0	<LOQ	ND	ND
L38	1.5	3.7	<LOQ	ND	ND	<LOQ	ND	ND	ND
L40	4.2	11.	54	ND	ND	<LOQ	<LOQ	ND	ND
L41	4.1	9.3	13	ND	ND	<LOQ	<LOQ	ND	ND
KN1	3.0	3.6	<LOQ	ND	ND	<LOQ	2.0	ND	ND
L44	5.7	4.5	63	14	312	97	5114	5.7	17

Table D.6: PFAS concentrations in ng/g ww measured in Gammarus sp. per sampling site. 'ND' indicates that the compound was not detected at this specific site and '<LOQ' indicates that the measurements were below the Limit Of Quantification.

ID	PFBA	PFPeA	PFHxA	PFOA	PFND	PFDA	PFUnDA
L3	<LOQ	0.48	ND	1.9	0.54	1.5	0.56
L11	<LOQ	<LOQ	<LOQ	1.3	1.1	3.7	2.2
L14	<LOQ	1.3	ND	1.4	0.72	1.9	1.3
L15	<LOQ	<LOQ	<LOQ	0.65	0.22	1.1	0.98
L16	0.56	1.6	ND	3.8	1.2	1.9	0.76
L36	0.95	0.42	<LOQ	2.2	0.27	1.2	0.70
L38	<LOQ	ND	<LOQ	1.7	ND	0.76	0.49
L40	<LOQ	ND	ND	1.0	0.31	1.1	0.50
L41	<LOQ	ND	<LOQ	0.92	ND	1.3	0.80
KN1	<LOQ	ND	<LOQ	1.1	1.2	3.5	1.2
KN4	0.48	0.33	0.82	3.2	0.68	1.8	1.6
L45	<LOQ	<LOQ	<LOQ	2.90	<LOQ	0.42	0.54
ID	PFDoDA	PFTTrDA	PFTeDA	PFBS	PFPeS	PFOS	6:2 FTS
L3	3.3	1.2	0.77	281	0.51	2.8	<LOQ
L11	16	3.8	7.0	1931	1.1	9.5	<LOQ
L14	27	6.7	11	ND	2.8	1.1	<LOQ
L15	9.0	2.3	4.2	504	5.5	<LOQ	<LOQ
L16	8.7	3.4	3.0	ND	0.51	5.4	ND
L36	2.3	0.67	<LOQ	1034	ND	1.2	<LOQ
L38	5.4	1.5	<LOQ	2355	ND	0.85	<LOQ
L40	17	4.8	11	5367	ND	2.2	<LOQ
L41	16	4.8	9.3	4064	ND	2.1	<LOQ
KN1	9.2	2.0	1.7	<LOQ	ND	7.5	2.5
KN4	5.3	2.2	0.77	<LOQ	ND	2.6	ND
L45	7.4	3.3	<LOQ	1061	ND	1.1	ND

Table D.7: PFAS concentrations in ng/g ww measured in Chironomus sp. per sampling site. 'ND' indicates that the compound was not detected at this specific site and '<LOQ' indicates that the measurements were below the Limit Of Quantification.

ID	PFPeA	PFHxA	PFOA	PFDA	PFUnDA	PFDoDA
L2	ND	<LOQ	0.17	0.14	<LOQ	0.55
L4	ND	ND	0.49	0.19	<LOQ	<LOQ
L5	<LOQ	<LOQ	0.32	0.28	<LOQ	<LOQ
L6	<LOQ	<LOQ	0.14	0.13	<LOQ	<LOQ
L9	0.05	<LOQ	0.15	<LOQ	<LOQ	<LOQ
L18	ND	<LOQ	<LOQ	<LOQ	ND	<LOQ
L19	ND	<LOQ	<LOQ	0.42	<LOQ	ND
L20	ND	ND	0.58	0.43	ND	<LOQ
L29	ND	<LOQ	1.6	0.53	<LOQ	<LOQ
L35	ND	ND	ND	ND	ND	<LOQ
L43	ND	5.4	20	3.4	2.7	6.8
L46	<LOQ	ND	<LOQ	<LOQ	<LOQ	<LOQ
ID	PFTTrDA	PFTTeDA	PFBS	PFPeS	PFOS	6:2 FTS
L2	0.46	2.0	ND	3.6	0.72	<LOQ
L4	<LOQ	<LOQ	<LOQ	4.3	0.91	0.82
L5	<LOQ	<LOQ	ND	1.9	0.66	<LOQ
L6	<LOQ	<LOQ	ND	ND	0.79	2.7
L9	ND	<LOQ	ND	ND	<LOQ	<LOQ
L18	ND	<LOQ	9.4	ND	ND	<LOQ
L19	<LOQ	<LOQ	ND	ND	ND	<LOQ
L20	ND	<LOQ	ND	ND	<LOQ	<LOQ
L29	<LOQ	<LOQ	ND	ND	ND	<LOQ
L35	ND	<LOQ	ND	ND	ND	<LOQ
L43	1.3	<LOQ	<LOQ	<LOQ	72	0.97
L46	<LOQ	ND	ND	ND	54	ND

Table D.8: Mean, median (med.) and min.- max. range of PFAS concentrations in ng/g ww measured in zebra mussels per sampling site with 5-10 replicates per site. '-' indicates that the compound was not detected or that the measurements were below the Limit Of Quantification.

ID		PFBA	PFPeA	PFHpA	PFHxA	PFBS	FBSA	PFHxS	PFOA	PFNA
L15 N=5	min.	-	-	-	0.97	-	-	-	0.14	-
	max.	-	-	-	-	-	-	-	6.0	-
	mean	-	-	-	-	-	-	-	1.7	-
	med.	-	-	-	-	-	-	-	0.68	-
L18 N=5	min.	-	-	-	1.2	-	-	-	0.5	-
	max.	-	-	-	-	-	-	-	22	-
	mean	-	-	-	-	-	-	-	6.4	-
	med.	-	-	-	-	-	-	-	3.7	-
L19	min.	-	-	-	1.0	-	-	-	0.68	-

<i>N=5</i>	max.	-	-	-	-	-	-	-	6.3	-
	mean	-	-	-	-	-	-	-	2.6	-
	med.	-	-	-	-	-	-	-	1.4	-
L29	min.	-	-	-	1.0	-	-	-	0.52	-
<i>N=5</i>	max.	-	-	-	1.4	-	-	-	3.3	-
	mean	-	-	-	1.2	-	-	-	1.6	-
	med.	-	-	-	1.2	-	-	-	1.4	-
L16	min.	-	-	-	1.2	-	-	-	0.83	-
<i>N=5</i>	max.	-	-	-	1.5	-	-	-	3.5	-
	mean	-	-	-	1.3	-	-	-	1.5	-
	med.	-	-	-	1.3	-	-	-	1.2	-
L9	min.	-	-	-	1.0	-	-	-	1.4	-
<i>N=5</i>	max.	-	-	-	2.0	-	-	-	3.1	-
	mean	-	-	-	1.5	-	-	-	2.2	-
	med.	-	-	-	1.5	-	-	-	2.0	-
L14	min.	-	-	-	1.2	-	-	-	1.5	-
<i>N=5</i>	max.	-	-	-	2.8	-	-	-	4.7	-
	mean	-	-	-	2.0	-	-	-	3.0	-
	med.	-	-	-	2.0	-	-	-	2.2	-
L5	min.	-	-	-	1.9	-	-	-	0.82	-
<i>N=5</i>	max.	-	-	-	-	-	-	-	3.3	-
	mean	-	-	-	-	-	-	-	2.0	-
	med.	-	-	-	-	-	-	-	2.1	-
L38	min.	-	-	-	1.0	-	-	-	0.54	-
<i>N=5</i>	max.	-	-	-	-	-	-	-	2.3	-
	mean	-	-	-	-	-	-	-	1.5	-
	med.	-	-	-	-	-	-	-	1.4	-
L37	min.	-	-	-	0.92	-	-	-	1.3	-
<i>N=5</i>	max.	-	-	-	1.9	-	-	-	3	-
	mean	-	-	-	1.4	-	-	-	2.1	-
	med.	-	-	-	1.4	-	-	-	2.1	-
L3	min.	-	-	-	1.2	-	-	-	1.0	-
<i>N=5</i>	max.	-	-	-	1.9	-	-	-	2.6	-
	mean	-	-	-	1.7	-	-	-	1.8	-
	med.	-	-	-	1.9	-	-	-	1.6	-
KN1	min.	-	-	-	1.18	-	-	-	0.92	-
<i>N=5</i>	max.	-	-	-	-	-	-	-	2.8	-
	mean	-	-	-	-	-	-	-	1.5	-
	med.	-	-	-	-	-	-	-	1.2	-
L44	min.	0.57	2.2	2.6	3.8	25	19	18	26	0.35
<i>N=5</i>	max.	1.1	13	4.0	11	-	40	39	42	0.52
	mean	0.86	6.2	3.1	5.9	-	27	30	33	0.45
	med.	0.86	4.6	2.7	5.1	-	24	31	29	0.49
L47	min.	-	-	-	-	3.2	2.7	-	0.65	0.25
<i>N=10</i>	max.	-	-	-	-	17	398	-	1.6	1.9
	mean	-	-	-	-	9.4	64	-	1.1	0.68
	med.	-	-	-	-	8.5	5.9	-	1.1	0.56
L46	min.	-	-	-	-	3.3	-	-	0.37	0.23
<i>N=10</i>	max.	-	-	-	-	7.4	-	-	1.1	0.55
	mean	-	-	-	-	4.9	-	-	0.88	0.41
	med.	-	-	-	-	4.2	-	-	0.93	0.38

ID		PFOS	PFDA	PFUnDA	PFDoDA	PFTTrDA	PFTeDA	PFHpS	PFPeS
L15	min.	-	0.68	0.43	0.79	0.35	0.97	-	-
	<i>N</i> =5 max.	-	1.7	1.2	2.4	0.51	2.4	-	-
	mean	-	1.2	0.7425	1.3	0.41	1.6	-	-
	med.	-	1.2	0.67	1.1	0.37	1.3	-	-
L18	min.	-	0.89	0.33	0.98	0.45	1.9	-	-
	<i>N</i> =5 max.	-	6.7	0.54	5	0.61	2.7	-	-
	mean	-	2.4	0.40	2.1	0.53	2.3	-	-
	med.	-	1.2	0.34	1.3	0.53	2.4	-	-
L19	min.	0.28	1.1	0.28	0.85	0.39	1.6	-	-
	<i>N</i> =5 max.	1.2	2.9	0.66	1.7	0.51	3.1	-	-
	mean	0.74	1.5	0.49	1.2	0.46	2.1	-	-
	med.	0.74	1.2	0.52	1.1	0.49	2.0	-	-
L29	min.	0.18	0.26	0.32	0.67	0.25	1.6	-	-
	<i>N</i> =5 max.	0.31	1.2	0.84	1.6	0.58	2.0	-	-
	mean	0.25	0.82	0.51	1.1	0.44	1.8	-	-
	med.	0.25	0.91	0.45	1.1	0.48	1.7	-	-
L16	min.	-	0.75	0.25	0.99	-	1.3	-	-
	<i>N</i> =5 max.	-	1.9	0.64	1.7	-	1.9	-	-
	mean	-	1.2	0.43	1.2	-	1.7	-	-
	med.	-	1.1	0.42	1.1	-	1.7	-	-
L9	min.	0.24	0.75	0.33	0.76	0.40	1.2	-	-
	<i>N</i> =5 max.	-	1.4	0.41	1.9	-	2.1	-	-
	mean	-	1.1	0.36	1.1	-	1.6	-	-
	med.	-	1.1	0.35	0.9	-	1.5	-	-
L14	min.	-	0.57	0.40	0.62	0.38	1.3	-	-
	<i>N</i> =5 max.	-	2.4	0.90	2.4	0.47	2.5	-	-
	mean	-	1.5	0.58	1.7	0.42	1.9	-	-
	med.	-	1.7	0.43	1.9	0.40	1.9	-	-
L5	min.	-	1.0	0.50	1.1	0.37	0.76	-	-
	<i>N</i> =5 max.	-	1.5	1.3	1.5	0.67	2.3	-	-
	mean	-	1.3	0.81	1.4	0.53	1.7	-	-
	med.	-	1.4	0.83	1.4	0.56	1.6	-	-
L38	min.	-	0.63	0.31	0.81	0.25	1.2	-	-
	<i>N</i> =5 max.	-	1.2	0.86	1.4	0.35	2.1	-	-
	mean	-	0.9	0.51	1.1	0.30	1.6	-	-
	med.	-	0.96	0.42	0.96	0.30	1.6	-	-
L37	min.	-	0.68	0.28	0.67	0.50	1.6	-	-
	<i>N</i> =5 max.	-	1.8	0.85	1.3	-	2.1	-	-
	mean	-	1.3	0.55	1.0	-	2.0	-	-
	med.	-	1.4	0.56	0.99	-	2.1	-	-
L3	min.	-	0.87	0.41	0.59	0.22	1.7	-	-
	<i>N</i> =5 max.	-	1.8	0.63	1.3	0.49	2.1	-	-
	mean	-	1.4	0.53	1.0	0.36	1.9	-	-
	med.	-	1.5	0.55	1.1	0.37	1.9	-	-
KN1	min.	-	0.73	0.34	0.55	0.29	1.1	-	-
	<i>N</i> =5 max.	-	1.3	0.72	1.2	0.34	1.8	-	-

	mean	-	1.1	0.48	0.91	0.32	1.4	-	-
	med.	-	1.1	0.43	0.97	0.32	1.4	-	-
L44	min.	1004	2.9	0.22	1.2	0.50	1.4	3.9	0.32
<i>N=5</i>	max.	1277	4.6	0.75	2.6	0.85	2.3	8.6	1.1
	mean	1137	3.7	0.50	1.9	0.68	1.9	6.0	0.56
	med.	1091	3.7	0.51	1.9	0.68	1.9	5.5	0.32
L47	min.	0.35	0.53	-	2.2	1.5	3.2	-	-
<i>N=10</i>	max.	1.3	1.6	-	4.3	2.4	4.8	-	-
	mean	0.80	1.23	-	3.3	1.9	4.2	-	-
	med.	0.69	1.33	-	3.1	2.0	4.2	-	-
L46	min.	1.5	0.72	-	2.37	1	3.2	-	-
<i>N=10</i>	max.	2.5	1.4	-	4.3	1.5	4.8	-	-
	mean	2.0	1.0	-	3.4	1.3	3.8	-	-
	med.	2.2	1.1	-	3.4	1.4	3.6	-	-

Figures:

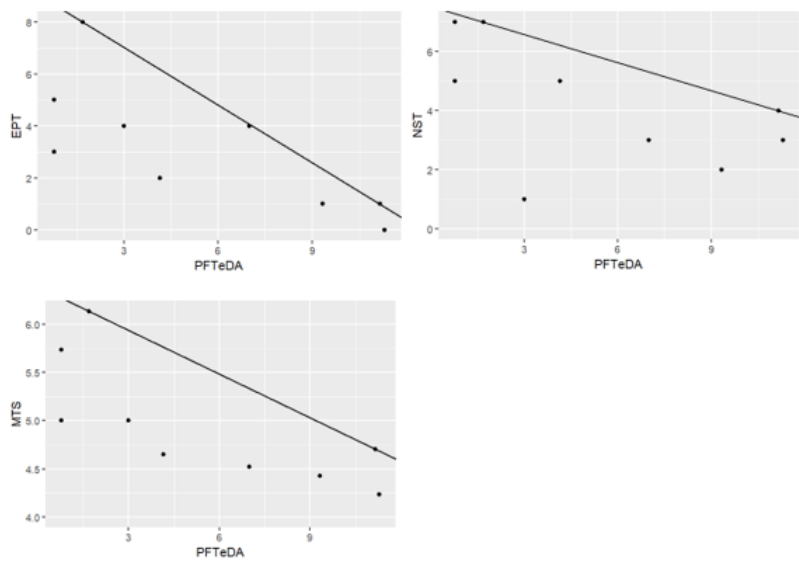


Figure D.1: Scatter plots of the significant Quantile regressions between accumulated PFTeDA concentrations in *Gammarus* sp. (N=9) and the number of EPT- taxa (EPT), number of other sensitive taxa (NST) and the Mean Tolerance Score (MTS).

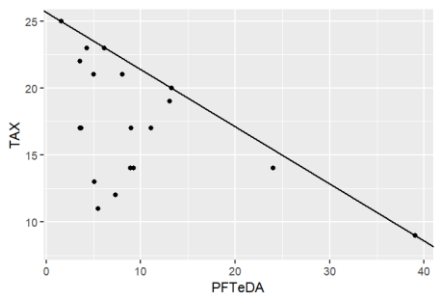


Figure D.2: Scatter plots of the significant Quantile regression between accumulated PFTeDA concentrations in *Asselus* sp. (N=16) and the Taxonomic Richness (TAX).

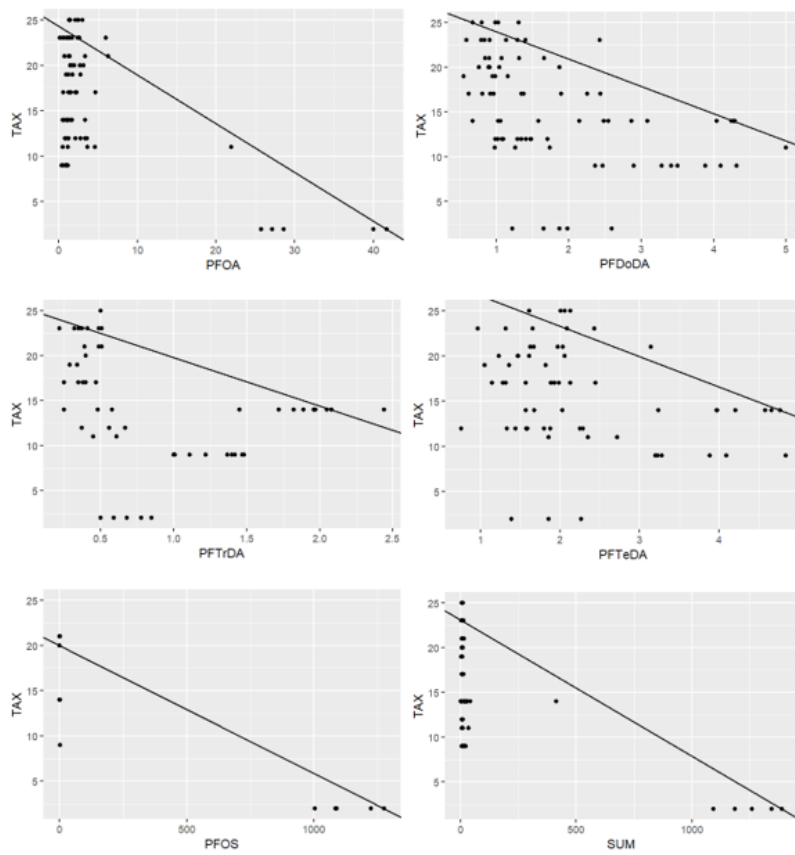


Figure D.3: Scatter plots of the significant Quantile regression between accumulated PFOA (N=83), PFDoDA (N=80), PFTrDA (N=50), PFTeDA (N=62) and Σ PFAS (N=84) concentrations in zebra mussels and the Taxonomic Richness (TAX).

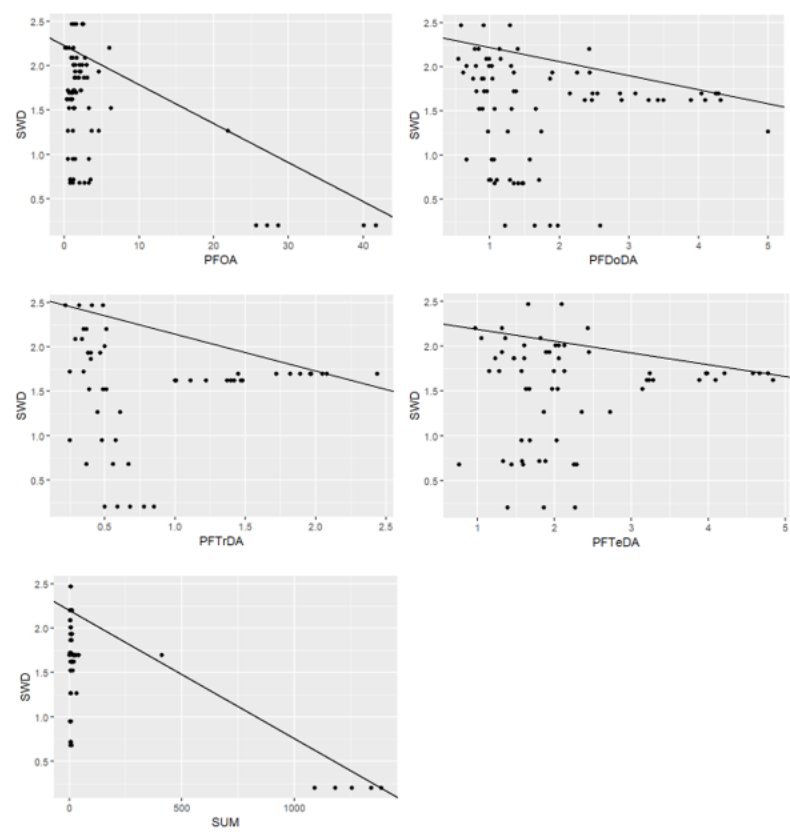
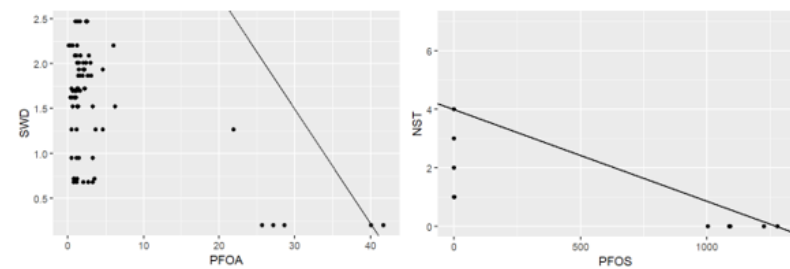


Figure D.4: Scatter plots of the significant Quantile regression between accumulated PFOA (N=83), PFDoDA (N=80), PFTrDA (N=50), PFTeDA (N=62) and Σ PFAS (N=84) concentrations in zebra mussels and the Shannon Wiener Diversity Index (SWD).



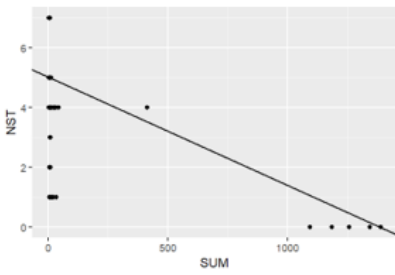


Figure D.5: Scatter plots of the significant Quantile regression between accumulated PFOA (N=83), PFOS (N=39), Σ PFAS (N=84) concentrations in zebra mussels and the number of other sensitive taxa (NST).

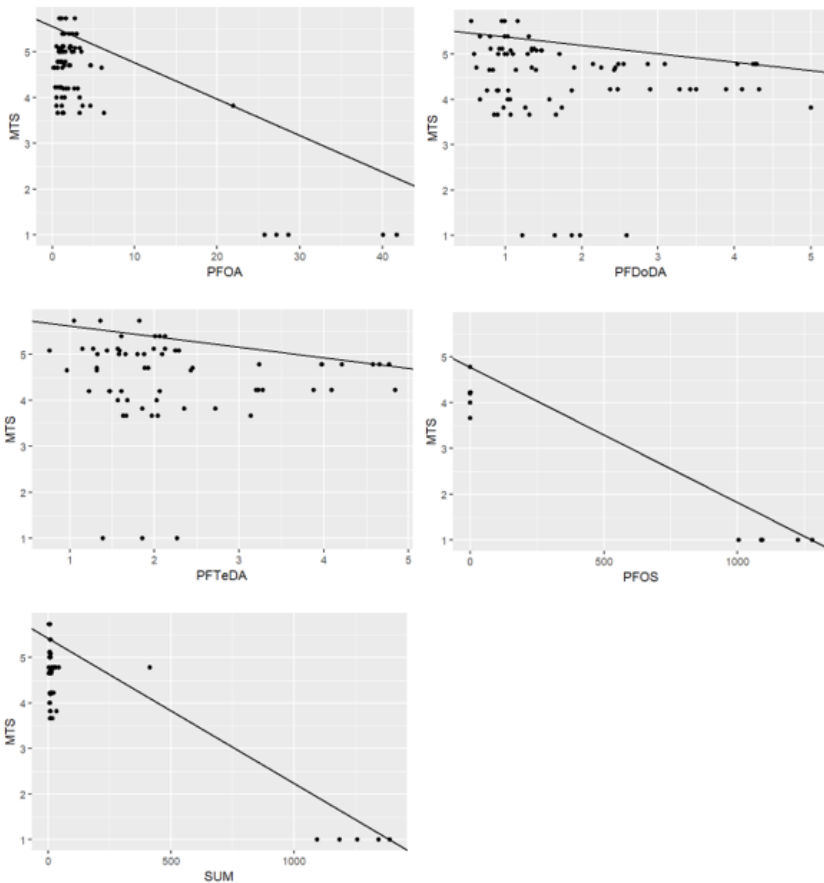


Figure D.6: Scatter plots of the significant Quantile regression between accumulated PFOA (N=83), PFDoDA (N=80), PFTeDA (N=62), PFOS (N=29) and Σ PFAS (N=84) concentrations in zebra mussels and the Mean Tolerance Score (MTS).

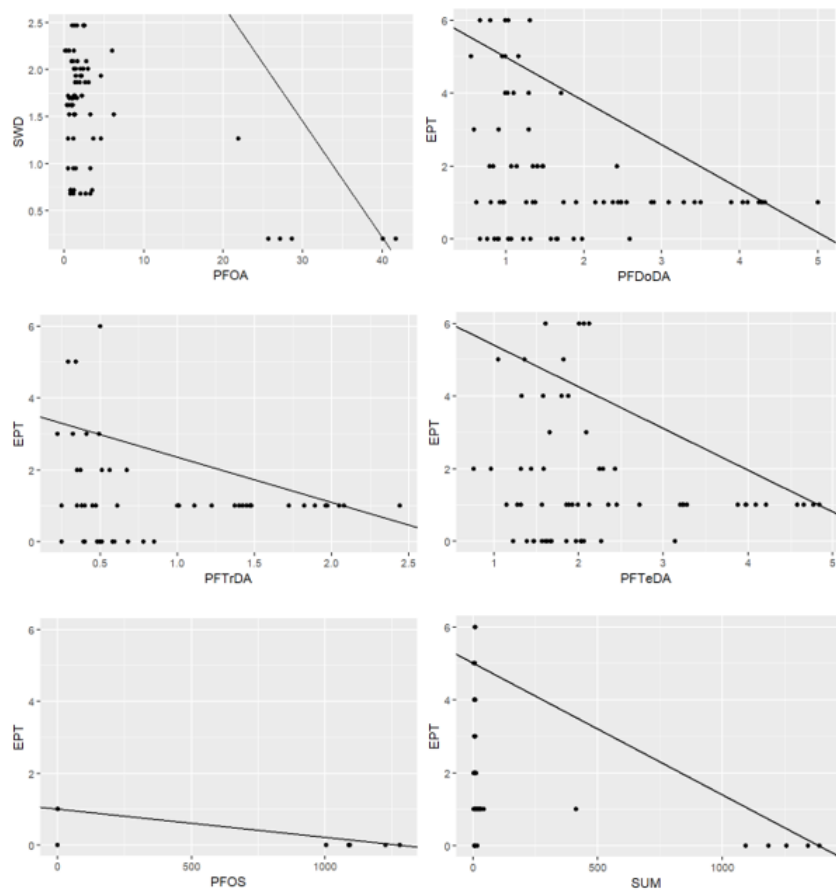


Figure D.7: Scatter plots of the significant Quantile regression between accumulated PFOA (N=83), PFDODA (N=80), PFTrDA (N=50), PFTeDA (N=62), PFOS (N=29) and Σ PFAS (N=84) concentrations in zebra mussels and the number of Ephemeroptera, Plecoptera and Trichoptera taxa (EPT).

Appendix E: Chapter VI

Tables:

Table E.1: Overview of Maximum contaminant Levels (MLs) in µg/kg ww for the three fish categories and the considered species per category.

	Maximum contaminant Levels (MLs) in µg/kg ww				
	PFOS	PFOA	PFNA	PFHxS	SUM
Category 1					
Muscle meat of fish, excluding muscle meat as listed under Category 2 and 3. Meat from fish listed in Category 2 and 3, if intended for the production of food for infants and young children.	2.0	0.20	0.50	0.20	2.0
Category 2					
Meat of the following fish species, if not intended for the production of food for infants and young children: Baltic Sea herring (<i>Clupea harengusmembras</i>) Bonito (<i>Sarda & Orcynopsis spp.</i>) Bubbot (<i>Lota lota</i>) European sprat (<i>Sprattus sprattus</i>) Flathead grey mullet (<i>Mugil cepahlus</i>) European flounder (<i>Platichthys flesus and Glyptocephalus cynoglossus</i>) Pike (<i>Esox spp.</i>) Plaice (<i>Pleuronectes and Lepidopsetta spp.</i>) Sardine (<i>Sardina spp.</i>) Seabass (<i>Dicentrarchus spp.</i>) Catfish (<i>Silurus and Pangasius spp.</i>) Sea lamprey (<i>Petromyzon marinus</i>) Tench (<i>Tinca tinca</i>) Little Maren (<i>Coregonus albula and Corgonus vandesius</i>) Silver lightfish (<i>Phosichthys argenteus</i>) Wolffish (<i>Anarhichas spp.</i>)	7.0	1.0	2.5	0.20	8.0
Category 3					
Meat of the following fish species, if not intended for the production of food for infants and young children: Anchovies (<i>Engraulis spp.</i>) Barbel (<i>Barbus barbus</i>) Bream (<i>Ambramis spp.</i>) Arctic char (<i>Salvelinus spp.</i>) Eel (<i>Anguilla spp.</i>) Pikeperch (<i>Sander spp.</i>) European Perch (<i>Percha fluviatilis</i>)	35	8	8	1.5	45

European smelt (<i>Osmerus spp.</i>)					
Common whitefish (<i>Coregonus spp.</i>)					

Table E.2: Overview of target analytes considered per chapter

Compound	Chapter II	Chapter III	Chapter IV	Chapter V
PFBA	x	x	x	x
PFPeA	x	x	x	x
PFHpA	x	x	x	x
PFHxA	x	x	x	x
PFOA	x	x	x	x
PFNA	x	x	x	x
PFDA	x	x	x	x
PFUnDA	x	x	x	x
PFDoDA	x	x	x	x
PFTTrDA	x	x	x	x
PFTeDA	x	x	x	x
PFBS	x	x	x	x
PFPeS		x	x	x
PFHxS	x	x	x	x
PFHpS		x	x	x
PFOS	x	x	x	x
PFDS	x	x	x	x
11Cl-PF3OUdS		x	x	x
9Cl-PF3ONS		x	x	x
8:2 FTS		x	x	x
6:2 FTS		x	x	x
NaDONA		x	x	x
4:2 FTS		x	x	x
PFEESA		x	x	x
HFPO-DA		x	x	x
PF5OHxA		x	x	x
PF4OPeA		x	x	x
3.6-OFHpA		x	x	x
FBSA		x	x	x

