Faculteit Wetenschappen
Departement Biologie

The relevance of environmental quality standards for biota in the evaluation of the ecological quality of aquatic ecosystems

De relevantie van milieukwaliteitsnormen voor biota in de evaluatie van de ecologische kwaliteit van aquatische ecosystemen

Proefschrift voorgelegd tot het behalen van de graad van doctor in de Wetenschappen: Biologie aan de Universiteit Antwerpen te verdedigen door Lies TEUNEN

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Antwerpen, 2022

This thesis was partially funded by the Flemish Environment Agency.
Cover Photo: Lieven Bervoets
Cover design: Nieuwe Media Dienst, Universiteit Antwerpen

Dankwoord/Acknowledgements

Na zes jaar is dan eindelijk het einde in zicht! Iets langer dan dat oorspronkelijk de bedoeling was, heb ik het geluk gehad om aan dit onderzoek te kunnen werken. Het is met gemengde gevoelens dat ik naar dit punt heb uitgekeken. Aan de ene kan opgelucht dat het eindelijk allemaal op papier staat, aan de andere kant vind ik het toch ook jammer dat ik dit fantastische hoofdstuk moet afsluiten. Ik heb oprecht een prachtige tijd beleefd bij SPHERE (ondertussen ECOSPHERE). Aangezien een doctoraat typisch gekenmerkt wordt door een aantal dingen die mislopen, ging het ook wel gepaard met wat frustraties. Maar algemeen houd ik hier toch een overwegend positief gevoel aan over. Dat heb ik voornamelijk te danken aan de mensen die ik mocht ontmoeten, met wie ik mocht samenwerken en op wie ik kon terugvallen tijdens mijn doctoraat. In de hoop dat ik niemand vergeet, wil ik dan ook heel wat mensen individueel bedanken.

In de eerste plaats wil ik graag mijn promotoren, Lieven en Ronny, bedanken om mij de kans en het vertrouwen te geven om aan dit project te mogen werken. Lieven wil ik daarnaast nog in het bijzonder bedanken om altijd beschikbaar te zijn voor ‘enkele kleine vragen’ en al op mails te antwoorden alvorens ze verstuurd waren (of zo leek het toch). Een extra woordje van dank gaat ook uit naar de voorzitter en jury: prof. dr. Patrick Meire, prof. dr. Adrian Covaci, dr. Michiel Kraak, dr. Krishna Das en dr. Carmen Casado-Martinez. Jullie kritische blik en daaruit volgende suggesties hebben dit doctoraat naar een hoger niveau getild.

Verder wil ik ook Maarten De Jonge en Annelies Rogge van de VMM bedanken voor de aangename samenwerking en de transparante communicatie over analysemethoden en –resultaten van de VMM die een belangrijk onderdeel vormden van dit doctoraat. Ook Claude Belpaire, Isabel Lambeens en de visploegen van het INBO wil ik bedanken voor het verzamelen van baars en paling.

Daarnaast wil ik ook al mijn collega's van de SPHERE-groep bedanken. De groep heeft de laatste jaren een sterk fluctuerende samenstelling gekend, en recent heb ik ook weer een groot aantal nieuwe collega's mogen verwelkomen in het ECOSPHERE-verhaal.

Hierin wil ik in de eerste plaats graag onze technische ploeg bedanken: Steven, Karin, Valentine en Femke. Jullie waren altijd bereid om te helpen met analyses, het uitwerken van protocols en het zoeken naar verdwenen voorwerpen. Bedankt ook aan Lia en Bieke om op het secretariaat altijd antwoord te bieden op nuttige en soms minder nuttige vragen.

Daarnaast wil ik ook alle doctoraatscollega's Bart, Thimo, Robin, Cara, Gunther en Vyshal bedanken om altijd klaar te staan voor een babbel of een kleine wetenschappelijke discussie. Mijn speciale dank gaat ook uit naar iedereen die in al die jaren met mij mee op veldwerk is geweest en mij telkens veilig op locatie hebben gebracht, aangezien ik zelf stellig probeerde te vermijden om met onze toch wel groot uitgevallen labo-auto's te moeten rijden.

Het nadeel van lang te 'blijven plakken', is dat veel mensen die vooral een belangrijk deel hebben uitgemaakt van die eerste jaren van mijn doctoraat ondertussen elders hun gading hebben gevonden. Daardoor is het contact jammer genoeg ook wat verwaterd. Toch wil ik ook voor hen nog even de tijd nemen: Sofie, Marjolein, Hanne, Kristine, Kristien, Camilla, Johnny, Elvio en Bossissi. Jullie zorgden mee voor de warme werkomgeving waarin ik tijdens mijn eerste jaren terechtkwam en waar ik mij onmiddellijk thuis voelde. Bedankt daarvoor!

Daarnaast wil ik uiteraard ook mijn vrienden bedanken om op tijd en stond voor ontspanning te zorgen en een luisterend oor te bieden. Ook al is de frequentie van onze ontmoetingen wat verminderd, toch wil ik zowel de hele tekenschool groep (Julie & Ruben, Freya & Lennart, Manu & Paulien en Ilona) als de Diepenbeek crew (Gitte, Lina, Kathleen, Niels, Jordy, Yves & Alexia en Stan & Binke) bedanken. Mijn speciale dank gaat ook uit naar Lynse voor de gezellige avonden.

Verder wil ik mijn familie bedanken voor hun warmte en aanmoedigingen. Extra dank gaat uit naar mijn ouders voor het doorgeven van de wetenschapsgenen en de vrijheid die ze mij gegeven hebben om mijn dromen na te jagen.

Last but not least, wil ik natuurlijk ook mijn verloofde Mats, mijn rots in de branding, mijn klankbord, bedanken. Jij stond altijd klaar voor een korte bespreking over hoe ik nu weer mijn resultaten moest interpreteren of weergeven en wat de correcte Engelse vertaling was, ook al leed je eigen werk daar soms onder (sorry daarvoor). Daarnaast zorgde je dan ook nog voor overheerlijke maaltijden die mij er ook altijd weer bovenop kregen. Ik hoop dat ik dezelfde steun kan bieden wanneer jij je doctoraat afwerkt en kijk al enorm uit naar alles wat we daarna nog samen gaan beleven.

List of Abbreviations

Abbreviation	Meaning	Abbreviation	Meaning
ABM	Active biomonitoring	HQ	Hazard Quotient
ACN	Acetonitrile	HR-ICP-MS	High-resolution Inductively Coupled Plasma Mass Spectrometer
ASE	Accelerated Solvent Extraction	HRMS	High Resolution Mass Spectrometer
ATSDR	Agency for Toxic Substances and Disease Registry	IBI	Index of Biotic Integrity
B(a)p	Benzo(a)pyrene	INBO	Research Institute for Nature and Forest
CI	Condition Index	IRMS	Infrared Mass Spectrometry
HBCD	Hexabromocyclododecane	ISTD(s)	Internal Standard(s)
HCB	Hexachlorobenzene	K_{ow}	Octanol-1-water partition coefficient
HCBD	Hexachlorobutadiene	LOQ	Limit of Quantification
Hg	Mercury	lw	Lipid weight
DCM	Dichloromethane	MADC	Maximum Amount Daily Consumed
DDT	Dichloro-diphenyl-trichloroethane	MeHg	Methylmercury
DOC	Dissolved Organic Carbon	ML	Maximum Levels
dw	Dry weight	MMIF	Multimetric Macroinvertebrate Index Flanders
EC20	Electrical conductivity at 20°C	MQ	Milli-Q
ECNI/MS	Electron capture negative ion mass spectrometer	MRL(s)	Minimal risk level(s)
EDI	Estimated Daily Intake	MRM	Multiple Reaction Monitoring
EFSA	European Food Safety Authority	MS	Mass Spectrometry
EI/MS	Mass spectrometer in electron ionization mode	NOAEL	No Observed Adverse Effect Level
EPT	Ephemeroptera, Plecoptera and Trichoptera	OCP(s)	Organochlorine pesticide(s)
EQR	Ecological Quality Ratio	PAH(s)	Polyaromatic hydrocarbon(s)
EQS	Environmental Quality Standard	(P)BDE(s)	(Pol)ybrominated diphenylether(s)
ES	Electrospray	PBM	Passive biomonitoring
Flu	Fluoranthene	PCB(s)	Polychlorinated biphenyl(s)
FWO	Research Foundation Flanders	PCB-DL	Dioxin-like polychlorinated biphenyls
GC	Gas Chromatography	PCDDs	Polychlorinated dibenzo-p-dioxins
HOC	Hydrophobic Organic Compounds	PCDFs	Polychlorinated dibenzofurans
HpC	Heptachlor	PFAS	Perfluoroalkyl substances
HpCepx	Heptachlor epoxide	PFBA	Perfluorobutanoic acid
HPLC	High Performance Liquid Chromatography	PFBS	Perfluorobutane sulfonate

Abbreviation	Meaning	Abbreviation	Meaning
PFCA(s)	Perfluorocarboxylic acid(s)	S/N	Signal-to-noise
PFDA	Perfluorodecanoic acid	SEA	Standard Ellipse Area
PFDoDA	Perfluorododecanoic acid	SIBER	Stable Isotope Bayesian Ellipse in R
PFDS	Perfluorodecane sulfonate	SPMD	Semi-Permeable Membrane Devices
PFHpA	Perfluoroheptanoic acid	SPME	Solid Phase Micro-extraction
PFHxA	Perfluorohexanoic acid	TBI	Trent Biotic Index
PFHxS	Perfluorohexane sulfonate	TDI	Tolerable Daily Intake
PFNA	Perfluorononanoic acid	TL	Trophic Level
PFOA	Perfluorooctanoic acid	TMF(s)	Trophic Magnification Factor(s)
PFOS	Perfluorooctane sulfonate	TOC	Total Organic Carbon
PFPeA	Perfluoropentanoic acid	TQD	Triple Quadrupole
PFSA(s)	Perfluorosulfonic acid(s)	TWT	Tissue Wet Weight
PFTeDA	Perfluorotetradecanoic acid	UA	University of Antwerp
PFTrDA	Perfluorotridecanoic acid	UPLC	Ultra-Performance Liquid Chromatography
PFUnDA	Perfluoroundecanoic acid	US EPA	U.S. Environmental Protection Agency
POCIS	Polar Organic Chemical Integrative Samplers	VMM	Flanders Environment Agency
POP(s)	Persistent Organic Pollutant(s)	WFD	Water Framework Directive
PP	Polypropylene	WHO	World Health Organisation
PTWI	Provisional Tolerable Weekly Intake	ww	Wet weight
RfD	Reference Dose		

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Chapter 1

General Introduction

1.1 Global pollution of freshwater ecosystems

Surface waters and aquatic ecosystems on a global scale are under constant pressure of chemical pollution, mainly of anthropogenic origin (Bernhardt et al., 2017; Häder et al., 2020). High concentrations of chemical pollutants in the environment may be harmful to aquatic ecosystems, causing a decrease in biodiversity (EC, 2008b; Malaj et al., 2014). Persistent organic pollutants (POPs) and metals can cause long-term detrimental effects, even decades after they have been banned (Schwarzenbach et al., 2006). Micropollutants can be either organic or inorganic (mostly man-made) active compounds which can be toxic even at very low concentrations (even in ng L^{-1} range). Furthermore, their specific physio-chemical characteristics will lead to bioaccumulation or even biomagnification (Deribe et al., 2011; Lavoie et al., 2013). The process of biomagnification refers to higher accumulated concentrations of pollutants being reached at higher trophic levels, through consumption of contaminated individuals (Mackay and Fraser, 2000). Consequently, besides exposure via drinking water, top predators and humans can be exposed to high pollution levels in the aquatic environment through their diet, i.e. consumption of fish and crustaceans (Lavoie et al., 2013; Mackay and Fraser, 2000).

1.2 The history of the European Water Framework Directive and the importance of EQS_{biota}

In 2000, the Water Framework Directive (WFD) was derived by the European Commission as a coherent water policy action framework among its member states aiming to ensure at least an overall 'good water quality' status (2000/60/EC). Originally, this goal should have been reached by 2015, but currently this deadline has been postponed to 2027 (EU, 2013). For this purpose, monitoring priority hazardous substances and reporting the chemical status of European water bodies was requested (EC, 2000). Furthermore, pollution sources should be identified and appropriate control measures implemented accordingly.

Consequently, in 2008 (2008/105/EC) Environmental Quality Standards (EQS) for surface water were published for 33 priority substances in order to protect the aquatic

environment and human health against the adverse effects of chemical pollution. These priority substances were selected based on their persistence, bioaccumulation tendency and toxicity (EC, 2000). For mercury, hexachlorobenzene and hexachlorobutadiene Environmental Quality Standards for biota were introduced, because of their hydrophobicity and biomagnification potential.

In the 2013 Directive (2013/39/EU), Environmental Quality Standards were added for 8 priority substances and their derivatives (*Table 1.1*) to be measured in biota (tissue of living organisms; EQS_{biota}) instead of in water or sediment samples. As can be seen, the EQS_{biota} for PBDEs, dioxins and heptachlor (epoxide) are very low, making it difficult to even measure these concentrations (i.e. they will often be below LOQ). This already highlights the ambiguous relevance of these standards.

Table 1.1: Priority compounds included in the European EQS_{biota} and their respective standards (EU, 2013).

Compound	Abbreviation	EQS_{biota} (µg kg^{-1} ww)
Hexachlorobenzene	HCB	10
Hexachlorobutadiene	HCBD	50
Brominated diphenyl ethers	PBDE	0.0085
Perfluorooctane sulfonate and its derivatives	PFOS	9.1
Mercury	Hg	20
Hexabromocyclododecane	HBCD	167
Dicofol	Dicofol	33
Dioxins and dioxin-like compounds	Dioxines	0.0065[a]
Heptachlor and heptachlor epoxide	HpC and HpCepx	0.0067
Benzo(a)pyrene	B(a)p	5
Fluoranthene	Flu	30

[a] concentration in µg $WHO\text{-}TEQ_{2005}$ kg^{-1} ww.

Due to their hydrophobic qualities, these compounds are difficult to detect in the water column (Belpaire & Goemans, 2007b; EU, 2013; Jürgens et al., 2013). Previous studies showed that, even though concentrations in the water column were low, high concentrations were still reached in biota (Belpaire et al., 2008; Weltens et al., 2002). Furthermore, most of these pollutants show a strong lipophilic character and will easily accumulate and magnify in biota and thus reach high concentrations in individuals at higher trophic levels (Deribe et al., 2011; Lavoie et al., 2013). Analysing water samples might therefore underestimate the concentrations in biota, especially at higher trophic levels. In order to assess the real-time pollution pressure on the ecosystem and to determine the risk of secondary poisoning of top predators (specifically fish-eating

birds, mammals and humans), it is therefore necessary to monitor these compounds in biota, preferably from higher trophic levels (EU, 2013; EC, 2014). Although for most compounds fish of higher trophic levels are the most relevant monitoring species, an exception was made for polyaromatic hydrocarbons (PAHs), including benzo(a)pyrene and fluoranthene. Due to the fast metabolization and elimination of these compounds in fish, they are to be measured in bivalves or crustaceans (EC, 2014; Van der Oost et al., 1994).

The octanol-1-water partition coefficient (K_{ow}) is often used to express the lipophilicity of chemical compounds. The $logK_{ow}$ typically ranges between -3 (extremely hydrophilic) and 10 (extremely lipophilic). All compounds included in this thesis therefore show a $logK_{OW}$ reflecting their lipophilic characteristics (*Table 1.2*). A $logK_{ow}$ > 5 reveals the potential to bioconcentrate (Gobas et al., 1999). For PBDEs and dioxins a range of values was given since they consist of multiple congeners with varying chemical characteristics based on the molecular size and composition. In general, their lipophilicity increases with halogenation. Compounds with a $logK_{ow} \geq 7$ are considered superhydrophobic (Mackay et al., 2015). On the other hand, it should be stated that the $logK_{OW}$ for PFOS is rather arbitrary, since it cannot be measured accurately due to the surface-active properties of PFOS (i.e. it forms multiple layers in an octanol-water solution; ATSDR, 2015). Furthermore, the $logK_{ow}$ for Hg (i.e. methylmercury as the most common species in biota) rather low and thus reflects that this compound has a less pronounced lipophilic character than the other compounds in the EQS_{biota} list.

Table 1.2: $logK_{ow}$ values of EQS_{biota} compounds and their literature sources.

Compound	$LogK_{ow}$	Literature
HCB	5.5	Mackay et al., 1992
HCBD	4.78	Hansch et al., 1995
Hg	0.41	Halbach, 1985
PBDEs	5.9-7.9	Braekevelt et al., 2003
PFOS	4.49	https://pubchem/ncbi.nlm.nih.gow/
HBCD	5.6	Macgregor and Nixon, 1997
Dioxins[a]	4.75-8.60	Van Noort, 2010
Dicofol	5.02	Saito et al., 1993
HpC	6.10	Simpson et al., 1995
B(a)p	6.13	De Maagd et al., 1998
Flu	5.16	Hansch et al., 1995

[a] $LogK_{ow}$ range for individual dl-PCBs, PCDDs and PCDFs.

Mercury and PFOS are considered the odd ones out. Instead of a distinct lipophilic character, they are known to show a high affinity to proteins, also leading to high accumulated concentrations in fish tissues. Methylmercury specifically binds to sulphur-based amino-acids and thiol groups in proteins, largely present in muscle tissue (Amlund et al., 2007; Bradley et al., 2017). Perfluoroalkyl substances, on the other hand, are known to bind to blood serum albumin, fatty acid binding proteins and organic anion transporters in mammals and fish (Forsthuber et al., 2020; Ng and Hungerbühler, 2013), resulting in high levels in blood and liver tissue (Martin et al., 2003; Valsecchi et al., 2020).

In addition to the compounds in the current EQS_{biota} list, polychlorinated biphenyls (PCBs) were also included in this PhD. Currently there is no EQS_{biota} available for this group of pollutants (i.e. with the exclusion of dioxin-like PCBs, already included in the dioxins). However, they show very comparable characteristics to the other compounds and might be expected to be added in the future. First of all, $logK_{ow}$ values range between 5.6 and 6.6 and increase with chlorination of the congeners (Larsen et al., 1992). This shows the lipophilic properties and tendency to biomagnify. On the other hand, maximum human health risk levels that can be present in foodstuff do exist (EC 1259/2011). These are often exceeded in fish, indicating high concentrations are being reached in the higher trophic levels (Belpaire et al., 2011).

Technical guidelines for the implementation of the EQS_{biota} were published (EC, 2014). As stated before, the EQS_{biota} have a double protection goal. Firstly, they protect against the risk of secondary poisoning of top predators ($EQS_{biota, secpois}$). Secondly, also human health risk through consumption of aquatic organisms (e.g. fish, crustaceans, molluscs) is accounted for ($EQS_{biota, hh}$). For each goal a corresponding threshold value was determined based on multiple studies. The effective EQS_{biota}, however, was determined as the most stringent standard, resulting in the automatic achievement of the other goal. Which one of the two threshold values was used, differed between compounds. Further requirements for biomonitoring (e.g. species, characteristics, tissue, standardisation) are explained in section 1.3.

For the following compounds the current EQS_{biota} was based on a threshold value for secondary poisoning. For **mercury** (i.e. methylmercury) the current standard was based on a single study on the growth effect of spiked pellets fed to five groups of five rhesus monkeys (*Macaca* sp.) for 365 days, using a safety factor of 10 (EC, 2014; Kawasaki et al., 1986). The standard for **HCBD** was driven by chronic toxicity tests in rats and mice for 2 years by the WHO (WHO/IPCS, 1994). They derived a NOAEL (No Observed Adverse Effect Level) of 0.2 mg kg^{-1} body weight per day. A conversion factor of 8.3 was then used to determine the EQS_{biota} for what can be present in tissue for consumption, also including a safety factor of 30 (EC, 2014). The **HBCD** standard was based on the NOEC (No Observed Effect Concentration) from a 6-week reproduction study on Japanese quail (*Coturnix japonica*), using a safety factor of 30 (EC, 2014; MOEJ, 2009). Next, the chosen threshold for the **dicofol** standard was the NOEC from a study where they detected reduced shell thickness in the eggs of American kestrel (*Falco sparverius*) after dietary exposure, with a safety factor 30 (EC, 2014).

For the other compounds, the current EQS_{biota} was based on a human health risk threshold. The standard for **HCB** was based on the guidance level for neoplastic (i.e. tumor inducing) effects (WHO/UNEP, 1997). The EQS_{biota} was then for an average person of 70 kg and including the average European consumption rate of 115 g day^{-1} (EC, 2014). Furthermore, the current standard for **PBDEs** was driven by neurobehavioral toxicity data of 5 groups of 4 female mice after dietary exposure to BDE-99 for 1442 days during the gestational and post-natal period, with a safety factor of 30 (Branchi et al., 2002, 2005; EC, 2014). For **PFOS**, the current standard was based on the NOAEL of 44 rhesus monkeys exposed to orally administered PFOS during 183 days, using a safety factor of 90 (EC, 2014; Seacat et al., 2002). The current standard for **heptachlor** was derived as a virtually safe dose for a 10^{-6} carcinogenic risk determined on mice after oral exposure for two years. No safety factor was implemented (Davis, 1965; EC, 2014). Next, the **dioxins** standard was based on the sum of the European Maximum Levels that can be present in foodstuff (excluding eel) for polychlorinated dibenzodioxins, polychlorinated dibenzofurans and dioxin-like PCBs (EC 2006; EC, 2014). For PAHs, specifically **benzo(a)pyrene**, the existing Maximum Levels (EC, 2006) were used as well. In this case, those for crustaceans and bivalves

(EC, 2014). Finally, the EQS_{biota} for **fluoranthene** was derived as virtually safe dose for a 10^{-6} carcinogenic risk determined on rats after a chronic oral exposure for two years (EC, 2014; Kroese et al., 2001).

1.3 Biomonitoring

The most extensive definition of monitoring was given by Hellawell (1991): 'intermittent (regular or irregular) surveillance undertaken to determine the extent of compliance with a predetermined standard or the degree of deviation from an expected norm'. Biomonitoring includes 'the systematic use of living organisms or their responses to determine the condition or changes of the environment' (Li et al., 2010). To allow for a complete and representable monitoring of a specific waterbody, monitoring species should have a high bioaccumulation capacity and tolerance for a wide range of chemicals (Belpaire & Goemans 2007a; EC, 2014). Preferably, it should be a non-migratory, sedentary species in order to reflect the local pollution pressure.

Specific requirements described in the EQS_{biota} guidance document include the use of the same species, which is representative of the local population, in (almost) all sample sites over several years (EC, 2014). To guarantee this, the use of multiple species is recommended. When selecting the appropriate species, it is important to take into account general traits possibly affecting bioaccumulation (e.g. trophic level, habitat use).

Under the terminology of biomonitoring, two different methods can be distinguished, namely passive and active biomonitoring.

1.3.1 Passive biomonitoring

Passive biomonitoring refers to the collection and monitoring of indigenous species, reflecting local conditions (Lacroix et al., 2015). Depending on the research objective, different trophic levels might be of interest, including macroinvertebrates and/or fish. Important requirements for indicator species used in large passive monitoring campaigns, include them being widespread and abundant through the study area, resident and of sufficient size to perform analyses efficiently (Belpaire & Goemans,

2007a; EC, 2014). Furthermore, they should be eurytopic (i.e. able to adapt and survive in a wide variety of environments). In the specific case of this thesis, with a focus on EQS_{biota}, two fish species, European perch (*Perca fluviatilis*; Linnaeus, 1758) and eel (*Anguilla anguilla*; Linnaeus, 1758) in its 'yellow eel' stage were used.

Eel and perch are frequently used indicator species for water quality assessment in Flanders (Belpaire & Goemans 2007b; De Jonge et al. 2014; Maes et al. 2005; Maes et al. 2007) and Europe (Durrieu et al. 2005; Jürgens et al., 2013; Sures et al., 1999; Wyn et al. 2010). In contrast to mussels, a smaller number of fish is needed to collect sufficient tissue for analysing different contaminants. Furthermore, fish occupy a higher trophic level and therefore generally ingest higher concentrations through food consumption (biomagnification). Eels are catadromous fish, spending their juvenile life stage in freshwater rivers and lakes and then migrate as adult silver eels to the oceans to mate (Tesch, 1977). Their early life stages (leptocephalus) will then transform to glass eels and elvers respectively, migrating back to fresh water to complete the cycle. As a result, in their sedentary yellow eel stage they can realistically reflect the local pollution (Laffaille et al., 2005; Maes et al., 2005; Tesch, 1977). Belpaire et al. (2008) found that yellow eels along a river showed different pollution profiles even at distances <5 km. Eels are widespread through Europe and can be found along the Atlantic coast from Scandinavia to Morocco (Deelder, 1984). They are a benthic, bottom dwelling species (Vezza et al., 2020). Their omnivorous life style exposes them to high concentrations of pollutants through biomagnification and exposure to the sediment (Deelder, 1984). Finally, the high consumption frequency of eels by local Flemish fishermen makes it an important species with regards to anthropogenic intake of micropollutants (Bilau et al., 2007; Maes et al. 2008). Perch, on the other is a species widely distributed through Europe with a limited home range throughout its lifetime (Ahlbeck Bergendahl et al., 2017). In addition, this fish is an opportunistic diurnal feeder and will switch during its lifetime from plankton over benthic macroinvertebrates to fish (Allen, 1935). Comparable to eel, perch lives in close vicinity of the sediment (Westrelin et al., 2018). In addition to the fish species used in this thesis, other freshwater monitoring fish species are often used in literature, such as bream (*Abramis brama*), roach (*Rutilus rutilus*) and gudgeon (*Gobio gobio*), also fulfilling the requirements for indicator

species (Foekema et al., 2016; Lappalainen et al., 2001; Nastova et al., 2017; Valsecchi et al., 2020; Van Campenhout et al., 2003).

General factors, possibly inducing a large inter and intra-species variation in accumulation should be taken into account and minimized or standardized if possible (e.g. size/age, sex, seasonality). Age, or more specifically residence time at a certain location (exposure time), is expected to show a direct link with bioaccumulated pollutant concentrations. For many fish species, a positive relationship was found between age and accumulation of POPs or mercury with older individuals typically exhibiting higher concentrations (Choo et al., 2020; Durrieu et al. 2005; Ion et al. 1997; Weis and Ashley, 2007). Often, length or weight are used as a proxy for age. Furthermore, due to their high lipid content, eels reach high accumulated concentrations of lipophilic compounds compared to lower-fat fish (Belpaire and Goemans 2007a; Maes et al., 2008; Weltens et al. 2002). For this reason, standardization of fish concentrations on a 5% lipid content is recommended for lipophilic compounds (EC, 2014). Since mercury and PFOS bind to proteins, they should be standardized for 26% dry weight residue instead. Due to the high affinity of lipophilic compounds to gonads and eggs, an effect of sex can be found, especially during the reproductive period, with females showing lower accumulated concentrations compared to males (Choo et al., 2020; Weis and Ashley, 2007). However, the measured concentrations in yellow eel are not influenced by reproduction, since they are analysed in their juvenile stage.

Finally, also to the target tissue can play an important role. Physical characteristics of different compounds might result in better accumulation in different tissues (Munschy et al., 2020). As stated before, for PFOS, the liver might accumulate higher concentrations and therefore be a more relevant tissue (Martin et al., 2003; Valsecchi et al., 2020). Furthermore, the exposure route influences which tissues are mostly associated with specific compounds (Section 1.5). When specifically investigating human health risk, the muscle tissue or ‘fillet’ is the most often consumed. The risk of secondary poisoning, on the other hand, can best be calculated using whole animal concentrations.

1.3.2 Active Biomonitoring

Active biomonitoring refers to the exposure of caged individuals to monitor a specific sample location (Besse et al., 2012). Individuals are usually collected from a reference site or a culture with low known background concentrations. After a fixed exposure time, the biota reach an equilibrium and will have accumulated a pollutant profile representative of the local environment. Animal groups often used for active biomonitoring of the aquatic environment include crustaceans, bivalves and fish (Alric et al., 2019; Andral et al., 2004; Schoenaers et al., 2016; Vermeirssen et al., 2005; Verschoor et al., 2012), although this method is not recommended for fish due to restricted mobility and confinement stress (Besse et al., 2012). In the context of this thesis, freshwater bivalves were used as active monitoring species.

In Europe, zebra mussels (*Dreissena polymorpha* Pallas, 1771) are a frequently used monitoring species (Bashnin et al., 2019; Bervoets et al. 2005b; Hendriks et al., 1998; Poma et al., 2014; Sures et al., 1999). This freshwater bivalve is native to eastern Europe (Bidwell, 2010) and shows a relatively high uptake efficiency for various metals and POPs and can therefore even be used for the detection of trace elements (Riva et al., 2008; Roditi and Fisher, 1999). Translocated individuals already accumulated pollutant concentrations comparable to indigenous individuals after six weeks of exposure (Bervoets et al. 2004). Low inter-individual differences allow even smaller samples to give reliable results (Bervoets et al. 2004).

Globally, trends have been observed of quagga mussels (*Dreissena bugensis* Andrusov, 1897) outcompeting zebra mussels, their close relatives (Heiler et al., 2013; Karatayev et al. 2014; Matthews et al., 2014). Even though zebra mussels have a higher initial invasion rate, they will eventually be largely replaced by quagga mussel in locations were they both are present (Karatayev et al., 2011). This was also the case in our Flemish reference sites. Quagga mussels are prone to survive deeper, colder waters, while zebra mussels will retreat to more shallow waters (Karatayev et al. 2015; Karatayev et al., 2021). For the purpose of this thesis, both fresh and brackish waters were studied. However, neither *Dreissena* spp. can survive salinity levels above 5 ppt (Spidle et al., 1995). Therefore, a third mussel species was exposed in the brackish water bodies.

The Asian clam (*Corbicula fluminea* Müller, 1774) is a freshwater bivalve endemic to Southeast Asia, Africa, and Australia (McMahon, 1982), but currently also widespread in Europe, North and South America (McMahon 1999). They are able to withstand a much broader salinity range and are even used as monitoring species in estuaries (Verbrugge et al., 2011). In contrast to *Dreissena* spp. who attach to hard substrates using their byssus threads (Kobak, 2013), Asian clams burrow themselves in soft, sandy sediments (Vaughn and Hakenkamp, 2008).

1.3.3 Active vs. passive biomonitoring (EC, 2014)

While active biomonitoring allows for a more standardized method (e.g. selection of size range and species, controlled background concentrations), only a relative short exposure time is used. The use of indigenous individuals, on the other hand, provides a long-term measurement, although it is more difficult to assess the exact exposure time. Furthermore, caging experiments are usually performed on smaller species, resulting in a higher number of individuals needed in order to reach the sufficient amount of tissue for analyses. It might not always be possible to create a specific habitat use (e.g. exposure to sediment).

Since both methods have their advantages and disadvantages, an integrated combination might provide the most complete image.

1.4 Alternative: Passive samplers?

With the development of passive samplers, a non-invasive alternative method of monitoring water quality has emerged. Passive samplers rely on the use of a diffusive layer (diffusion sampler) or a filter membrane (permeation sampler) and a receiving phase, analysing bioavailability and mimicking the accumulation of pollutants from the water in biota (Vrana et al. 2005). Specific characteristics of the receiving phase and sampler designs facilitate the binding of certain organic and inorganic target compounds. Frequently used designs are Semi-Permeable Membrane Devices (SPMD) (Vrana et al. 2005), Polar Organic Chemical Integrative Samplers (POCIS) (Alvarez et al., 2004), Chemcatchers (Kingston et al., 2000) and Solid Phase Micro-extraction (SPME) (Pawliszyn, 1997).

Although passive sampling is considered a promising novel technique, some intermediate links are missing to appropriately extrapolate to effective accumulation in biota (e.g. metabolization, active elimination, avoidance behaviour and detoxification) (Morrone et al., 2021). Therefore, additional research investigating the relationship with bioaccumulation is needed.

1.5 Anthropogenic applications, exposure routes and persistence of POPs and mercury

The restriction or ban on the use and production of all EQS_{biota} priority compounds (mainly in the Stockholm Convention) has led to a decrease in environmental concentrations and improvement of the overall Flemish water quality over the last decades (De Jonge et al., 2014; Maes et al., 2008). Nonetheless, high (background) concentrations remain present in the aquatic environment. POPs can persist in the food cycle because they are stored in sediments and bound to small particles (Weis and Ashley, 2007). Furthermore, the effects of biomagnification will result in high concentrations in higher trophic levels. General exposure routes of POPs to the aquatic environment are through runoff of pesticides in agriculture, industrial waste water, long-range atmospheric transport and deposition through emission and volatilization after (incomplete) combustion and municipal waste and waste processing of household products. Long-range transport of POPs and mercury via atmosphere or ocean currents has led to their presence even in the most remote areas, such as the Arctic and Antarctic (Balmer et al., 2019; de Wit et al., 2010; Vecchiato et al., 2015).

Below, the applications, exposure routes and half-life times of all compounds of interest in this thesis are listed. **Hexachlorobenzene** was originally introduced as a fungicide (Mukesh Kumar et al., 2013). In addition, high amounts of this substance are released via the chemical industry or via atmospheric deposition after combustion processes including chlorine (Bailey, 2001). However, its use and production have been banned since 2004 (Stockholm Convention, 2004; EC 850/2004). Like HCB, **Hexachlorobutadiene** is mainly emitted as an unintentional by-product of industry, mainly in the production of chlorinated compounds (Wang et al., 2021). Since 2015, however, both use and production of this pollutant have been banned (Stockholm

Convention, 2015). **Mercury** has multiple applications in, among others, agriculture (e.g. fungicide, fertilizer), households (e.g. cosmetics, batteries, lamps), medicine (thermometers, dental amalgam), industry (e.g. production of car components, munition, chlor-alkali plants) and Hg amalgamation in gold mining (Horowitz et al., 2014; Kidd and Batchelar, 2012), but can also be transported atmospherically over large distances. European mercury legislation has banned the transport and production of mercury-containing products since 2018 (EU 2017/852). Bacteria (mainly sulphate-reducing) in anaerobic sediments will transform inorganic mercury to the highly toxic methylmercury as part of their basic carbon metabolism (Macalady et al., 2000). Both **Polybrominated diphenyl ethers (PBDEs)** and **hexabromocyclododecane (HBCD)** are used as flame retardants, mainly in textile industry (de Wit, 2002; Rahman et al., 2001). In Directive 2003/11/EC, the sale of pentaBDE and octaBDE mixtures were banned from the European market. Furthermore, from 2006 under Directive 2002/95/EC, all new electrical and electronic equipment should be free from these mixtures. In July 2008 decaBDE was added to the list. The permitted production and use of HBCD, on the other hand, has been restricted since 2013 (Stockholm Convention, 2013; EU 2016/293). **Perfluoroalkyl substances (PFAS)** are widely used in technical applications and consumer products and mainly serves as a repellent against dirt, water and oil (Glüge et al., 2020). In addition, they are an important ingredient in the foam of fire extinguishers. It is also known that atmospheric transport (and deposition) of the volatile precursors of these pollutants can occur (Yamashita et al., 2012). The use of PFOS, one of the main PFAS compounds, has been restricted since 2009 (Stockholm Convention, 2008; EU 757/2010) and was already phased out by certain manufactories before (USEPA, 2000). **Dicofol** is a miticide, often used on fruits and vegetables, with DDT (Dichlorodiphenyltrichloroethane) being an important component in its production (Qiu et al., 2005). The amount of DDT that can be present in dicofol, has already been strongly reduced since 1978 and should be less than 0.1% (EC, 2004; Rasenberg et al., 2003). The use and production of dicofol, however, have only been officially banned in Europe since 2019 (Stockholm Convention, 2019). **Dioxins** are atmospherically released during incomplete combustion of plastics and as part of the iron industry (iron ore sintering) (Cardellicchio, 2020). Since 2004, unintentional

releases must be limited (Stockholm Convention, 2004; EC 850/2004). **Heptachlor** had an important implication as an insecticide but has been banned since 2004 (Stockholm Convention, 2004; EC 850/2004). Abiotic processes in the environment (e.g. photoconversion) can transform heptachlor to the more persistent heptachlor epoxide metabolite (Buser and Müller, 1993). Heptachlor epoxide has a known half-life time in the water of at least 4 years (https://www.atsdr.cdc.gov/toxguides/toxguide-12.pdf). **Polychlorinated biphenyls (PCBs)** were mainly used as cooling liquids or flame retardant of transformers and capacitors (Erickson and Kaley II, 2010). The production of PCBs was banned in 2004, in Europe even from the 1980s, while existing devices containing PCBs could continue to be used (Stockholm Convention, 2004; EC 850/2004). However, the most polluting devices were destroyed in 2010. Nowadays, unintentional release during thermal processes (e.g. steel production) still largely contributes to atmospheric distribution of PCBs to the environment, with PCB 28 being the dominant indicator PCB congener (Shen et al., 2021). **Polycyclic aromatic hydrocarbons**, including benzo(a)pyrene and fluoranthene, are released during incomplete combustion of fossil fuels. Therefore, atmospheric deposition and transport is considered an important exposure route. General sources are rubber industry, steel works, diesel exhaust and wood preservation plants (Cirla et al., 2007; Covino et al., 2010; Khalili et al., 1995; Yang et al., 2002). The emission of these pollutants must be limited (EC 850/2004).

The persistence of POPs is mainly caused by their chemical stability and resistance to degradation. Half-life times of these compounds are listed for different media (atmosphere, water and sediment) in *Table 1.3*. They will generally persist in the environment for months to years. In aquatic ecosystems biodegradation will play an important role. Furthermore, abiotic degradation (e.g. hydrolysis or photolysis) can take place (Brooke et al., 2004). PFOS is considered not biodegradable in water or sediment and can only be degraded abiotically (OECD, 2002). In general, POPs will persist longer in soil or sediment. Since most of the compounds are considered strongly hydrophobic, they tend to bind to organic particles and accumulate in sediment (Weis and Ashley, 2007). For compounds consisting of several congeners, a large variation in persistence can be observed due to the resistance of different congeners, with larger molecules

taking a longer time to be degraded. Mercury in the atmosphere, will mainly be available as inorganic mercury, while in the aquatic ecosystems, methylmercury is the most toxic and bioaccumulated form (Jackson, 1998). However, it was found that methylmercury in water and sediment had a very fast turnover (i.e. demethylation), compared to inorganic mercury in the atmosphere and so a constant input is required (Hintelmann et al., 2009; Zhang et al., 2019). Finally, it is important to state that the half-life values below should be interpreted as relative rather than exact values. Degradation efficiency is strongly subjected to environmental conditions (e.g. oxygen, temperature, light intensity) (Nadal et al., 2015; Varjani et al., 2017).

Table 1.3: Half-life time of EQS_{biota} compounds in different media (atmosphere, water and soil) and literature.

Compound	atmosphere	Water	sediment	Literature
HCB	2.7-6 yr	2.7-6 yr	>6 yr	Mackay et al., 1992
HCBD	2 mo-3 yr	3 mo	6 mo	ATSDR, 1994; Howard, 1991; Vulykh et al., 2005
Hg	1 yr (Hg^o)	1.1 d (CH_3Hg)	1.7 d (CH_3Hg)	Hall, 1995; Hintelmann et al., 2009; Zhang et al., 2019
PBDEs	7.5 d-1.5 mo	1.4 mo- >3.4 yr	1.1- >3.4 yr	Gouin and Harner, 2003[c]
PFOS	3 mo	>3.7 yr[a] >41 yr[b]	NA	Brooke et al., 2004; OECD, 2002
HBCD	2 d	2 mo	8 mo	Wania, 2003
Dioxins	8 d-1.1 yr	5.5 mo-22 yr	17-148 yr	Sinkkonen and Paasivirta, 2000
Dicofol	3-11 d	2 mo[b]	6 mo	Stockholm Convention – Decision POPRC-10/3
HpC	6.3 h	1-3.5 d	6-9 mo	Reed and Koshlukova, 2014
PCBs	3 d-1.4 yr	2 mo-27 yr	3-38 yr	Sinkkonen and Paasivirta, 2000
B(a)p	7 d	2.3 mo	2-6 yr	Mackay et al., 1992
Flu	2 d	12.5-42 d	>3.4 yr	Mackay et al., 1992; Wild et al., 1991

h: hours; d: days; mo: months; yr: years. [a] photolysis; [b] hydrolysis. [c] no data available for BDE100 and BDE154.

1.6 Human health effects

Long-term exposure to pollutants through consumption can cause adverse health effects in humans. Besides the EQS_{biota}, specific threshold values for human health risk assessment were derived. These provide an allowable provisional intake of a certain pollutant on a daily or weekly basis, without posing a health risk and taking into account the weight of the consumer. Calculated allowable consumption rates (based on the pollution load) can then be compared directly to known local consumption rates. Thus, this method is most often used to assess human health risks.

In literature, a multitude of possible human health risks is reported for POPs and mercury. An increase in the prevalence of neurodevelopmental disabilities (e.g. autism, ADHD, dyslexia and others) in children has been detected after exposure to known neurotoxicants such as methylmercury, PFOS, PCBs and PBDEs (as reviewed by Grandjean and Landrigan, 2014). Furthermore, POPs can induce reproductive and developmental disorders or they can interfere with the metabolism, the immune system and endocrine system or even cause cancer (Li et al., 2015a; Reed et al., 2007; Shen et al., 2021; Sousa et al., 2021). Besides neurotoxicity, PFOS has also been associated with hepatotoxicity, immunotoxicity and disruption of reproduction and thyroid functioning in humans, or ultimately death (as reviewed by Zeng et al., 2019).

Metabolic disruption can alter the general homeostasis, contributing to the development of metabolic disorders such as obesity or diabetes (Han et al., 2020; Heindel et al., 2017). PFAS can disrupt the lipid and weight regulation, even at low concentrations (Gorrochategui et al., 2014). Long-term PCB exposure of detrivorous fish in Brazil declined their ability to control temporal lipid variation (Speranza et al., 2021).

During pregnancy and the early life stages, humans (and other organisms) are extremely sensitive to exposure to toxicants. Maternal transfer of lipophilic compounds through breastfeeding occur mainly during the first month of lactation (Witczak et al., 2021). Furthermore, the underdeveloped immune and nervous system of infants, makes them more vulnerable to the adverse effects of pollutants (Rice and Barone, 2000). Maternal PCB exposure has been shown to negatively affect growth (pre and postnatal), general metabolism and thyroid functioning of the foetus (as reviewed by Kiess et al., 2021) and – as is the case for dioxins - result in a decrease in cognitive ability in young children (Caspersen et al., 2016).

Methylmercury, specifically, can affect the perceptive systems (i.e. vision, hearing) and mobility (i.e. immobility, uncontrollable movements) even at low concentrations (Clarkson, 1992; Karagas et al., 2012). PAHs are known to form DNA-adducts, inducing mutations and possibly causing cancer (Ali and Wang, 2021). Furthermore, they have been associated with premature delivery or delayed child development (Ali and Wang, 2021). Benzo(a)pyrene is known to cause skin irritations (Rand and

Petrocelli, 1985) and is considered one of the most toxic PAHs. Fluoranthene can cause neurobehavioral toxicity, including ataxia, decreased grip strengths (Saunders, 2003).

1.7 Ecological quality based on biotic indices

Besides chemical pollution, also the ecological quality is an important assessment tool for the general water quality of an aquatic ecosystem. Generally ecological quality is determined using biotic indices, mainly focussing on macroinvertebrate or fish communities. Furthermore, multiple studies have shown direct relationships between ecological and chemical water quality (Bashnin et al., 2019; Bervoets et al., 2005a; Bervoets et al., 2016; Dyer et al., 2000; Hartwell, 1997; Van Ael et al., 2014).

The Trent Biotic Index (TBI) was developed in 1964 by Woodiwiss as a scoring tool for ecological quality exclusively based on macroinvertebrates for the Trent River in the UK (Woodiwiss, 1964). This type of indices has been widely applied and adapted to local conditions and macroinvertebrate communities ever since (Moya et al., 2011; Pond et al., 2013). A specific method was adapted for Belgium (Belgian Biotic Index; BBI) by De Pauw and Vanhooren (1983), merging the TBI and the French Biotic Index of Tuffery and Verneaux (1968). All of these biotic indices are based on presence/absence of specific taxa and their sensitivity. Eventually this index was updated to the Multimetric Macroinvertebrate Index for Flanders, the northern part of Belgium (MMIF), by Gabriels et al. (2010) conform the WFD guidelines and taking the typology of the sampling sites into account. A multimetric index combines multiple characteristics of the community, rather than just presence (e.g. abundance, species composition). The MMIF gives a water quality score based on 5 metrics: taxa richness, number of Ephemeroptera, Plecoptera and Trichoptera taxa (EPT), number of other (non-EPT) sensitive taxa (e.g. Plathyhelminthes, Mollusca, Coleptera and Hemiptera) the Shannon-Wiener Diversity index and the mean tolerance score (of all sampled taxa).

A first fish based index was introduced by Karr in 1981 as the Index of Biotic Integrity (IBI) for Midwestern rivers in the USA (Karr, 1981) and was based on multiple metrics based on species composition and richness, and ecological factors. Globally, indices based on fish communities have since been adapted to local conditions (Harris and

Silveira, 1999; Kamdem Toham and Teugels, 1999; Lyons et al., 1995; Zhu and Chang, 2008). The European Fish Index (EFI) was developed for specific environmental conditions found throughout Europe (Schmutz et al., 2007). In Flanders, the IBI was adjusted for the different water types occurring in freshwater and brackish environments (Belpaire et al., 2000; Breine et al., 2004, 2007). The 8 metrics included in this index are based on species richness and composition, fish condition and abundance, and trophic composition.

1.8 Aim and outline of the thesis

The general aim of this thesis was to investigate the relevance of the European Biota Quality Standards (EQS_{biota}) - included in the Water Framework Directive (WFD) - in evaluating the ecological quality of aquatic freshwater and brackish ecosystems. The main motivation for this study was that although there can be observed frequent exceedances of the current EQS_{biota} (often with a large factor) for multiple pollutants, no apparent effects on the aquatic ecosystem (including secondary poisoning) are reported. Furthermore, bioaccumulation trends between multiple fish species were studied for compounds included in the EQS_{biota} and a comparison between active and passive biomonitoring was made. Finally, the efficiency of using biomonitoring instead of environmental (water/sediment) samples was evaluated for these compounds.

All chapters of this thesis were field-based. Overall, active biomonitoring was performed using transplanted caged freshwater mussels (*Dreissena polymorpha, Dreissena bugensis* and *Corbicula fluminea*). For one salty location, indigenous blue mussel (*Mytilus edulis*) was used. However, unless stated otherwise, this sample was removed from statistical analyses. For passive biomonitoring, European perch (*Perca fluviatilis*) and eel (*Anguilla anguilla*) in its 'yellow eel' stage were used.

Chapter 2 contains the summary of a 4-year field campaign (2015-2018) on biota monitoring in the context of the WFD performed in Flanders (Belgium) commissioned by the Flanders Environment Agency. This chapter mainly serves as an additional introduction on the general status of Flemish water bodies. A total of 44 locations were sampled, using the abovementioned active and passive biomonitoring techniques. In

general, all priority compounds included in the current EQS_{biota} (*Table 1.1*) were analysed in fish, with the exception of the PAHs fluoranthene and benzo(a)pyrene. These were measured in mussels due to their high metabolization in fish. Finally, also PCBs were measured in fish due to their similar characteristic to the priority compounds. Compliance with the EQS_{biota} was discussed as well as general trends and findings (i.e. comparison between species, pollution distribution throughout Flanders and possible sources). Finally, biomonitoring was compared with passive sampling for a subset of compounds. The extensive dataset collected from this campaign served as a base for many of the following chapters.

In the EQS_{biota} guidelines neither the species to be used, nor the desired method of biomonitoring are explicitly specified. Therefore, accumulation data could provide additional information on the importance of species (and tissue) selection and interpretation of results in the context of EQS_{biota}. Bioaccumulation differences were compared between perch and eel, two common monitoring species, for both mercury (**Chapter 3**) and PFAS (**Chapter 4**). Both of these compounds show a divergent character compared to the other compounds included in the biota standards. Their high affinity for proteins results in a different expected accumulation pattern between species. Finally, for both compounds, the human health risk for consumption of these fish was evaluated.

In **Chapter 3,** mercury accumulation was compared for both muscle and liver tissue in both fish species. Fish were collected from 26 sampling locations, reflecting a variety of environmental situations. Furthermore, the relationships between the accumulated mercury concentrations and both weight and length were investigated. The use of mixed models, allowed to determine if these findings were generally present or location-dependent and took into account different environmental background effects. Additionally, seasonal variation in lipid and thus total weight of tissues was accounted for by correcting concentrations based on the lipid content. Since mercury is not accumulated in lipids, but rather proteins, whole weights (including the lipid portion) could create a larger variation and therefore a distorted image of the stored mercury concentrations.

Accumulated PFAS concentrations (4 perfluoroalkyl sulfonic acids and 11 perfluoroalkyl carboxylic acids) were investigated in **Chapter 4**. PFAS profiles were compared between passive biomonitoring (fish) and active biomonitoring (mussels), thus drafting a comparison between trophic levels. Furthermore, trophic levels (based on stable isotopes) and isotopic niches were determined to add to the trophic position of the monitoring species.

In **Chapter 5**, we studied to what extent bioaccumulated concentrations of priority compounds (POPs and Hg) could be predicted by environmental concentrations (water and sediment) and characteristics (pH, oxygen, conductivity, nitrate, nitrite, clay content, TOC and DOC). Furthermore, detection frequency for all compounds were compared between the different biotic and abiotic matrices, as well as the PBDE and PCB profiles. Finally, we investigated the extrapolation potential of accumulated concentrations in perch and eel.

The main objective was then addressed in **Chapter 6**, where we investigated whether the EQS_{biota} is protective of the ecological quality, based on the macroinvertebrate community (MMIF). For this purpose, threshold values (concentrations in biota) were calculated above which the ecological quality was never good. This was done using a 90^{th} quantile model and a 95^{th} percentile calculation. Based on these results, the efficiency of the current EQS_{biota} could be assessed.

Finally, the general findings of all chapters are summarized and discussed in **Chapter 7**. Overall conclusions and perspectives for future research were included. Additionally, general exceedances of the current EQS_{biota} were standardised as in Chapter 6 and human health risks were determined for the remaining priority compounds (apart from Hg and PFOS, since this had been done in previous chapters). Overall, results of exceedances, human health risk and ecological relevance were combined to interpret the overall relevance and protection level of the current EQS_{biota}. These results might serve as a primary indication for the potential need for revision or fine-tuning of the standards for specific compounds.

Chapter 2

Biota Quality Standards monitoring in Flanders: Findings and trends

Based on:

Lies Teunen, Claude Belpaire, Freddy Dardenne, Ronny Blust, Adrian Covaci, Lieven Bervoets, 2020. Veldstudies naar monitoring van biota in het kader van de rapportage van de chemische toestand voor de Kaderrichtlijn Water 2015-2018 (algemene trends en relaties). Universiteit Antwerpen (UA) in samenwerking met het Instituut voor Natuur- en Bosonderzoek (INBO), in opdracht van de Vlaamse Milieumaatschappij (VMM). Antwerpen, België, 99 blz.

Abstract

The present chapter is a summary of the biota monitoring conducted between 2015 and 2018. A set of 11 priority compounds and their derivatives (Water Framework Directive, EU), with distinct hydrophobic/lipophilic and bioaccumulative characteristics, were measured in biota (fish or fresh water bivalves). Accumulated concentrations of hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), mercury (Hg), brominated diphenyl ethers (PBDE), perfluorooctane sulfonate (PFOS), hexabromocyclododecane (HBCD), dicofol, dioxins and dioxin-like compounds, and heptachlor and heptachlor epoxide were measured in muscle tissue of European perch (*Perca fluviatilis*) and yellow eel (*Anguilla anguilla*), collected at 44 sampling locations. PCBs were measured in these samples as well. The polycyclic aromatic hydrocarbons benzo(a)pyrene and fluoranthene were measured in exposed bivalves using active biomonitoring. Accumulated concentrations in biota were checked for compliance with the EQS_{biota} and compared with accumulated concentrations in passive samplers. Mercury (100%) and ∑PBDE (≥ 85%) exceeded their respective EQS_{biota} in almost all sample locations. Furthermore, many exceedances were recorded for PFOS (≥ 58%), dioxins in eel (69%) and cis-heptachlor epoxide in eel (90%). Exceedances with the highest factor were measured for ∑PBDE and cis-heptachlor epoxide in both species. For most compounds, the highest pollutant concentrations were measured in eel. For PFOS the opposite was true (especially in dry weight concentrations), possibly caused by their high protein affinity. A correction based on lipid content in both fish species resulted in comparable concentrations (for ∑PBDE and ∑PCB), a smaller concentration difference (for HCB and HBCD) or significant higher concentrations in perch than eel (for Hg and PFOS). Passive samples were a good predictor for accumulation of benzo(a)pyrene in mussels, HCB in perch and ∑PCB in eel. The Zenne, Demer and different parts of the Scheldt showed high pollution levels for several compounds. The most important sources of pollution were atmospheric deposition and leaching of historically polluted sediment or point pollutions of industry (and households). Our findings underline the importance of bioaccumulation monitoring of these hydrophobic priority compounds in order to assess the local pollution pressure and risk of secondary poisoning. However, the combination with passive sampling can provide additional information on bioavailability and – accumulation of pollutants and might in time serve as a less invasive alternative method for biota monitoring.

2.1 Introduction

In order to protect the aquatic environment against detrimental effects of pollution, the European Commission initiated the Water Framework Directive (WFD), which encourages member states to monitor pollutants in surface waters (EC, 2008b). In general, chemical pollutants can be measured in water, suspended matter or in sediment. A set of strong hydrophobic/lipophilic components are, however, difficult to measure in water due to their poor solubility. Additionally, they are strongly bioaccumulative and show a high biomagnification potential. Hence, the European Commission derived Environmental Quality Standards for biota (EQS_{biota}) for 11 priority compounds and their derivatives (Directive 2013/39/EC). The main objective of these standards is to protect top predators, such as mammals and birds, and humans against effects of secondary poisoning through consumption. Depending on the compound they need to be measured in fish and/or invertebrates. Member states are free to select adequate biomonitor species (EC, 2014).

The present chapter is a summary and interpretation of four Flemish biota monitoring field studies conducted between 2015 and 2018 (Teunen et al., 2017, 2018, 2019 and 2020a). The priority compounds and their derivatives are stated in the Directive (2013/39/EC) for EQS_{biota} were hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), mercury (Hg), brominated diphenyl ethers (PBDEs), perfluorooctane sulfonate (PFOS), hexabromocyclododecane (HBCD), dicofol, dioxins and dioxin-like compounds, heptachlor and heptachlor epoxide, benzo(a)pyrene and fluoranthene. These compounds are characterised by their distinct hydrophobic/lipophilic and bioaccumulative properties. Accumulated concentrations were measured in muscle tissue of European perch (*Perca fluviatilis*) and eel (*Anguilla anguilla*), collected at 44 sampling locations. Additionally, polychlorinated biphenyls (PCBs) were measured in the fish samples as well, due to their hydrophobic and bioaccumulative characteristics. Up to date no EQS_{biota} exists for PCBs, but maximal allowable concentrations for human consumption are available (EU, 2011a). The polycyclic aromatic hydrocarbons (PAHs) fluoranthene and benzo(a)pyrene were measured in exposed bivalves (zebra mussels

Dreissena polymorpha, quagga mussels *Dreissena bugensis,* Asian clams *Corbicula fluminea*, swan mussels *Anodonta cygnea* and blue mussels *Mytilus edulis*) using active biomonitoring because of their fast metabolization in fish (Van der Oost et al., 1994; EU, 2013).

A promising, less invasive method that could be used as an alternative to biota monitoring, is the deployment of passive samplers (Figueiredo et al., 2017; Smedes et al., 2010). Passive samplers consist of absorbant membranes that bind specific compounds available from the water column and enable a long-time exposure. For lipophilic compounds, typically silicone or polyethylene membranes are used (Mayer et al., 2003). However, specific biological processes, such as elimination or metabolization of compounds, are usually not taken into account using passive samplers and should be further investigated (Figueiredo et al., 2017; Miège et al., 2015). The present project served as a preliminary study to investigate to what extent passive samplers can be used to predict bioavailability and accumulation of certain hydrophobic compounds in biota from Flemish waterbodies.

The main aim of the study was to check accumulated concentrations in different biota for compliance with existing standards. The study was designed to fulfil the monitoring requirements for the WFD, specifically for the priority compounds included in Directive 2013/39/EC that need to be measured in biota. Concentrations were also compared with accumulated concentrations in passive samplers. Finally, we tried to identify possible (known) pollution sources that could explain high concentrations in biota.

This chapter serves as an additional introduction of the current situation in the Flemish waterbodies regarding monitoring of hydrophobic/lipophilic compounds in biota according to the EQS_{biota} of the European WFD. The elaborate network and dataset gained from this project was the baseline for further analyses and research questions addressed in this PhD thesis.

2.2 Materials and methods

2.2.1 General

Sampling locations ($N = 44$) were selected from the Flemish monitoring network under the WFD of the Flanders Environment Agency (VMM). Originally, the network was strategically established (i.e. optimal geographical distribution, accessibility of sampling locations, representative for specific ecological regions and sufficiently distant from local pollution sources). A second important criterion was the agreement with the freshwater fish network of the Research Institute for Nature and Forest (INBO) and previous observations of perch and eel. A detailed map with sampling locations and years can be found in *Appendix A1*.

A more detailed description of sampling methods and analysis of pollutants is given in the following chapters. General methods used for analysis and their accuracy were listed in *Appendix D1*.

Brominated diphenyl ethers (PBDEs) were included as the sum of 6 congeners (BDE 28, 47, 99, 100, 153 and 154), hereafter referred to as **∑PBDE**. **Hexabromocyclododecane** (HBCD) was measured as the sum of the α-, β-, and γ-congeners. The **∑PCB** was calculated as the sum of 7 indicator PCB congeners (PCB ICES 7): i.e. PCB 28, 52, 101, 118, 138, 153 and 180. Finally, dioxins were represented as the sum of polychlor-dibenzo-p-dioxins (PCDDs), polychlordibenzofurans (PCDFs) and dioxin-like PCBs (PCB-DL), calculated in µg WHO-TEQ_{2005} kg^{-1} ww. For all of the above, in case at least one of the congeners was below LOQ, ½ LOQ was used in the calculations (Bervoets et al., 2004; Custer et al., 2000). Significant outliers were identified using Grubbs' test in Graphpad and removed for further analysis and presentation of the results.

2.2.2 Passive biomonitoring: Fish collection

Applied fishing techniques were depending on the physical properties of the waterbody (e.g. depth, width). Fish were caught by electrofishing and/or fykes (*Pictures 2.1-2.6*). An attempt was made to collect 20 perches and 3 eels at every sampling location. Per location, perches were divided into 2-3 pools based on their weight. All eels from the same location were added to a single pool. Unfortunately, not at every location both species could be collected in sufficient numbers. In the case that insufficient perch was collected, multiple eel pools were created. A general overview of the numbers of fish collected and pools created per location can be found in *Appendix A2*.

Analyses of the selected pollutants were exclusively performed on muscle tissue, being the most relevant tissue for human consumption and for monitoring the risk of secondary poisoning due to high accumulated concentrations of multiple compounds in muscle tissue compared to other organs. Interpreting the results, lipid content and dry weight were taken into account, as a standardisation for working with lipophilic compounds and aquatic organisms respectively.

Picture 2.1: Electrofishing in shallow water, wading. (From Teunen et al., 2020a)

Picture 2.2: Electrofishing in shallow water, wading and from boat. (From Teunen et al., 2020a)

Picture 2.3: Electrofishing in deeper water, from boat. (From Teunen et al., 2020a)

Picture 2.4: Fyke fishing in deeper water. (From Teunen et al., 2020a)

*Picture 2.5: European perch (*Perca fluviatilis); ©Rollin Verlinden.

*Picture 2.6: European 'yellow' eel (*Anguilla anguilla); ©Rollin Verlinden.

2.2.3 Active biomonitoring: Bivalves

As stated before, PAHs needed to be measured in crustaceans or bivalves, due to their fast metabolization in fish (Van der Oost et al., 1994; EU, 2013). A pilot study revealed that insufficient crustaceans could be collected at all sampling locations (De Jonge et al. 2014). Therefore, active biomonitoring (ABM) was used, with organisms from a reference location or culture being exposed at the locations of interest using cages (*Pictures 2.7-2.9*). This allows for a standardised comparison of the same species, with the same background (reference location) and in sufficient numbers, between locations.

In the current study, bivalves were used for this purpose. In freshwater locations zebra and quagga mussels were used (including swan mussels in the sampling year 2015). In brackish waters (N = 5), Asian clams were exposed, since *Dreissena sp.* would not survive these higher salinities (Spidle et al., 1995). At one location in the harbour channel of the IJzer resident blue mussels were used due to the high salinity similar to sea water. Although all exposed species already occur widespread throughout Flanders (Boets et al. 2014), mussels were exposed during autumn, outside their breeding season, in order to prevent spreading exotic species. Mussels were exposed for six weeks at every location in cages made of pond baskets. This exposure time was determined based on a previous study indicating that accumulated pollutant concentrations in translocated individuals were already comparable to those in indigenous individuals after six weeks of exposure (Bervoets et al. 2004). Species used, their survival per sampling location and sample sizes were listed in *Appendix A3*. Further interpretation of the results in the current chapter mainly focussed on zebra and quagga mussels.

Picture 2.7: Deploying cages. (From Teunen et al., 2020a)

Picture 2.8: Mussel cages. (From Teunen et al., 2020a)

*Picture 2.9: Zebra mussel (*Dreissena polymorpha*).*

*Picture 2.10: Quagga mussels (*Dreissena bugensis*; From Teunen et al., 2020a).*

*Picture 2.11: Asian clam (*Corbicula fluminea*).*

2.2.4 Trophic levels

Starting from the second sampling year (2016), the trophic levels of fish (pools) were determined as well. We believe this to be an important factor influencing accumulated concentrations. According to the biomagnification principle, individuals on higher trophic levels will accumulate higher pollutant concentrations.

Firstly, $\delta^{15}N$ was determined (the ratio between ^{15}N and ^{14}N stable isotopes), a relative measure for trophic position of a species within a local food web. The value of ^{15}N increases throughout the food chain, based on the diet. The higher this value, the higher the trophic level. In order to determine the ecosystem specific trophic level of a species, the following formula is then generally used (Cabana & Rasmussen 1994; Post et al. 2000).

$$\text{Trophic level} = \lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{primary producer}})/\Delta_n \quad (\mathbf{2.1})$$

This takes into account the $\delta^{15}N$ value of the primary producer ('baseline value'), with λ indicating the trophic level of the organism used to calculate the baseline value (i.e. 1 for primary producer, 2 for primary consumer). The value for Δ_n represents the enrichment of $\delta^{15}N$ per trophic level and is estimated on average at 3.4%. Due to the lack of this data for the locations of the current project, however, a modified formula was used (Post 2002; Post et al. 2000).

$$\text{Trophic level} = 2 + (\delta^{15}N_{\text{secundary consumer}} - \delta^{15}N_{\text{primary consumer}})/3.4 \quad (\mathbf{2.2})$$

Here the primary producer is replaced by the primary consumer, in this case mussels.

2.2.5 Passive samplers

Simultaneously with exposure of the mussels, passive samplers were deployed at all sampling locations by the VMM. Samplers consisted of a membrane with a polydimethylsiloxane sorbent (type silicon rubber samplers, Deltares) which absorbed specific dissolved pollutants from the flowing water. The organic compounds with a sufficiently low detection limit to be measured with these samplers in the water column include PAHs, HCB, HCBD, PBDEs and PCBs (Smedes et al., 2010). The passive samplers were surrounded by a stainless steel cage in order to protect them against damage by debris in the water column (*Picture 2.12*).

Picture 2.12: Passive sampler setup.

2.3 Results

Detailed tables with results of the target priority compounds can be found in *Appendix A4*; these were published in the individual reports (Teunen et al., 2017, 2018, 2019 and 2020a). In the present chapter only general trends and relationships will be discussed further. Firstly, the compliance of accumulated concentrations in biota with the EQS_{biota} and the general trends of exceedance were checked. Furthermore, accumulated concentrations in biota were compared to concentrations in passive samplers. Finally, a general, qualitative interpretation of the influences of known pollution sources was conducted.

2.3.1 EQS_{biota}

2.3.1.1 Exceedance of the standards

Compliance with the standards was checked for all target priority compounds. *Figures 2.1-2.16* visualise the exceedance of the EQS_{biota} of different pollutants on maps of Flanders. A red dot refers to an exceedance, a green dot to a concentration below the respective standard at certain sampling locations. The size of the red dots represent different categories of concentrations, starting with the respective EQS_{biota} and increasing with a factor 5 (EQS to EQS*5 - EQS*5 to EQS*10 - EQS*10 to EQS*15 - etc.). Separate maps were created for perch and eel per pollutant, in case both species were analysed. For ∑PBDE and cis-heptachlor epoxide larger ranges than factor 5 were used, since the respective maximal exceedance was a factor 860 and 280 in perch and a factor 10,000 and 2600 in eel.

Dicofol concentrations were below LOQ for both species at all sampling locations and thus below the EQS_{biota}. For HCBD, as well, the EQS_{biota} was never exceeded. Therefore, the maps of these pollutants were not included.

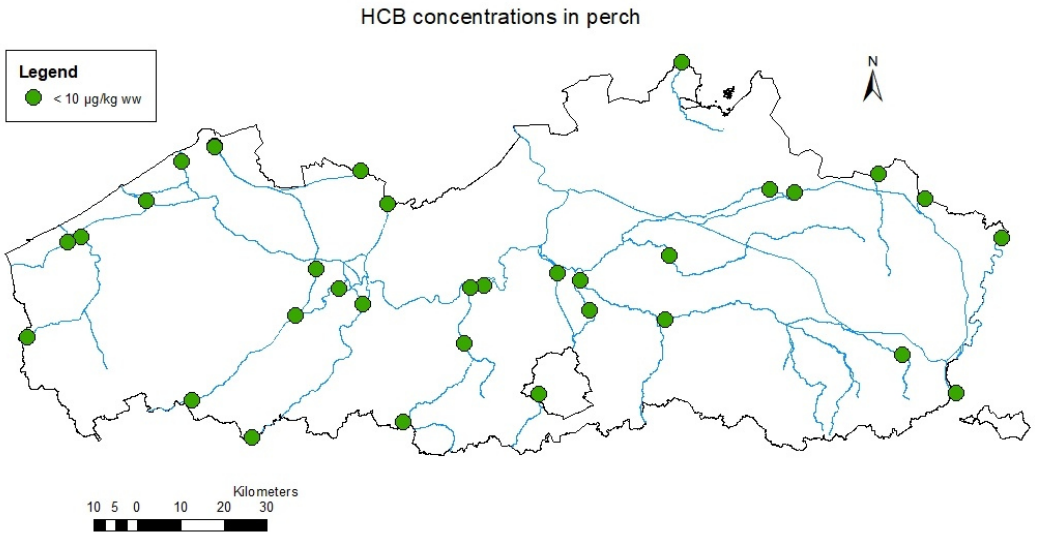


Figure 2.1: Map of Flanders with exceedance of the EQS_{biota} for HCB in perch at different sampling locations (N = 33). Green dots refer to locations with concentrations below the standard. The EQS_{biota} for HCB is 10 µg kg^{-1} ww. In the case of multiple perch pools per location, a mean concentration per location was calculated.

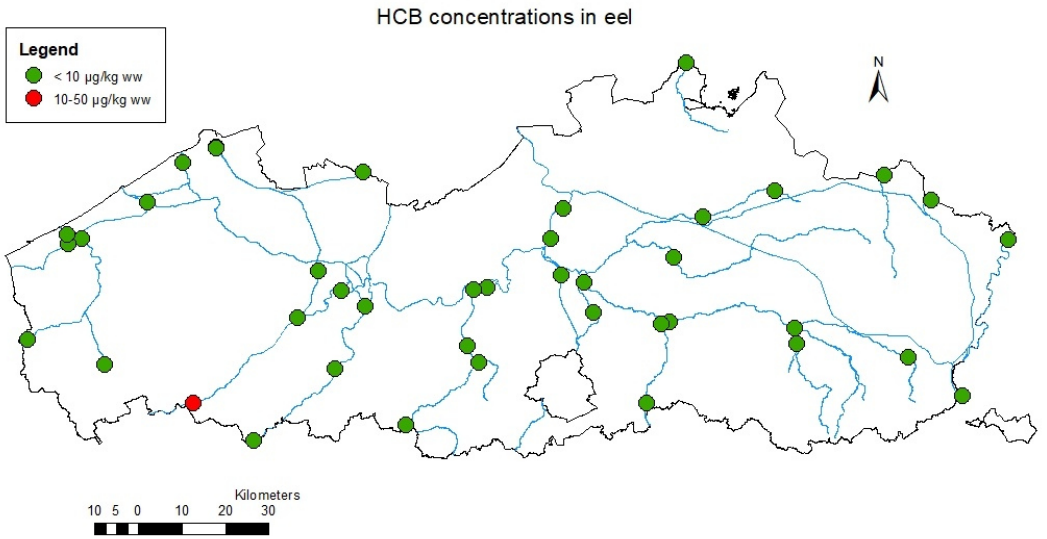


Figure 2.2: Map of Flanders with exceedance of the EQS_{biota} for HCB in eel at different sampling locations (N = 41). Green dots refer to locations with concentrations below the standard. The size of the red dot represents a range of concentrations, increasing with a factor 5. The EQS_{biota} for HCB is 10 µg kg^{-1} ww. In the case of multiple eel pools per location, a mean concentration per location was calculated.

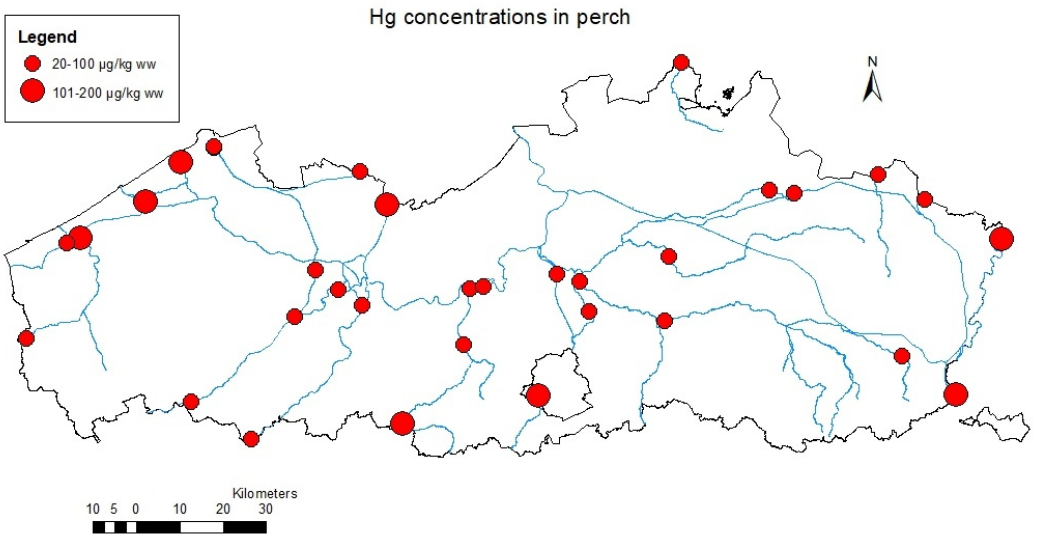


Figure 2.3: Map of Flanders with exceedance of the EQS_{biota} for Hg in perch at different sampling locations (N = 33). The size of the red dot represents a range of concentrations, increasing with a factor 5. The EQS_{biota} for Hg is 20 µg kg^{-1} ww. In the case of multiple perch pools per location, a mean concentration per location was calculated.

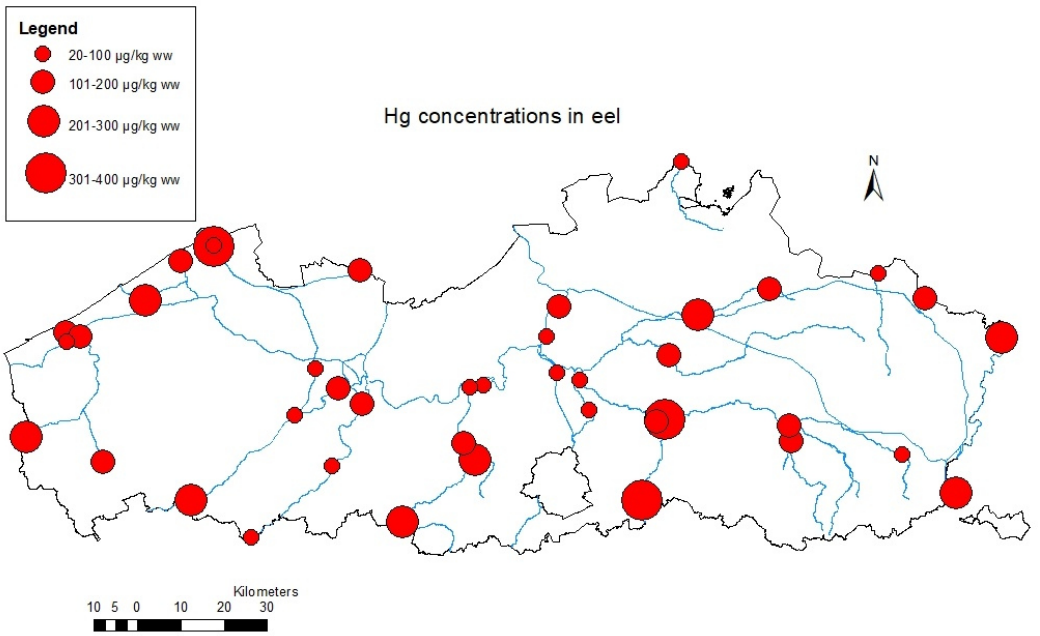


Figure 2.4: Map of Flanders with exceedance of the EQS_{biota} for Hg in eel at different sampling locations (N = 41). The size of the red dot represents a range of concentrations, increasing with a factor 5. The EQS_{biota} for Hg is 20 µg kg^{-1} ww. In the case of multiple eel pools per location, a mean concentration per location was calculated.

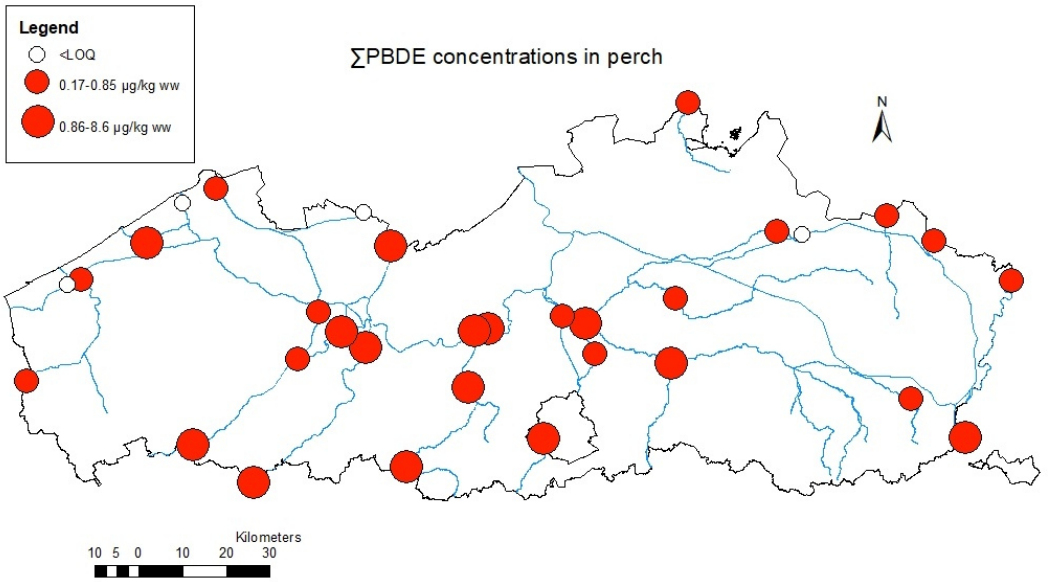


Figure 2.5: Map of Flanders with exceedance of the EQS_{biota} for ∑PBDE in perch at different sampling locations (N = 33). The size of the red dot represents a range of concentrations, increasing with a factor 10. The white dot represents a result below LOQ (0.3 µg kg^{-1} ww), for which there is no guarantee that the standard was exceeded. The EQS_{biota} for ∑PBDE is 0.0085 µg kg^{-1} ww. In the case of multiple perch pools per location, a mean concentration per location was calculated.

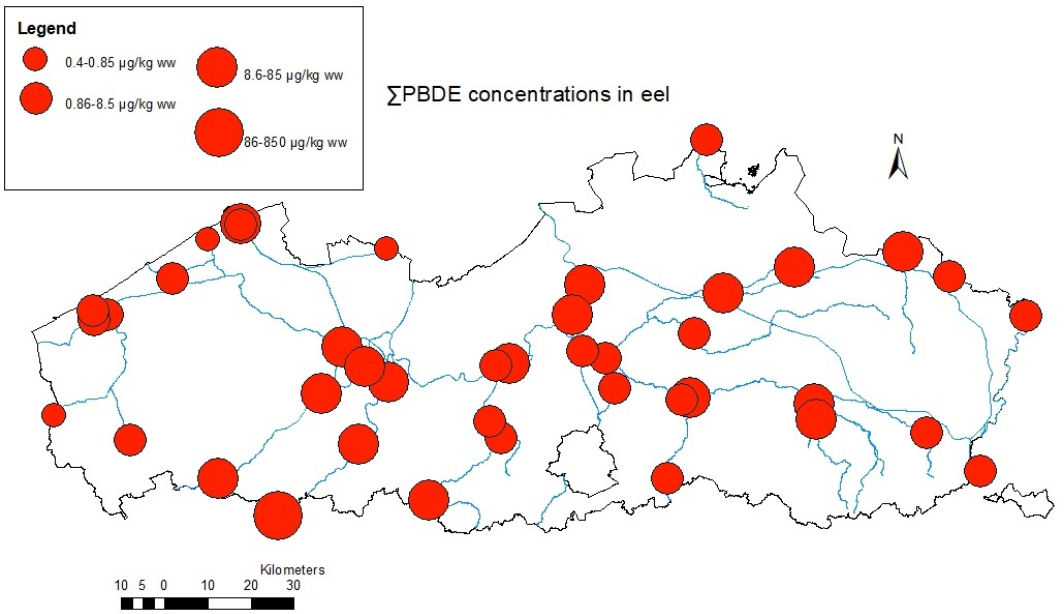


Figure 2.6: Map of Flanders with exceedance of the EQS_{biota} for ∑PBDE in eel at different sampling locations (N = 41). The size of the red dot represents a range of concentrations, increasing with a factor 10. The EQS_{biota} for ∑PBDE is 0.0085 µg kg^{-1} ww. In the case of multiple eel pools per location, a mean concentration per location was calculated.

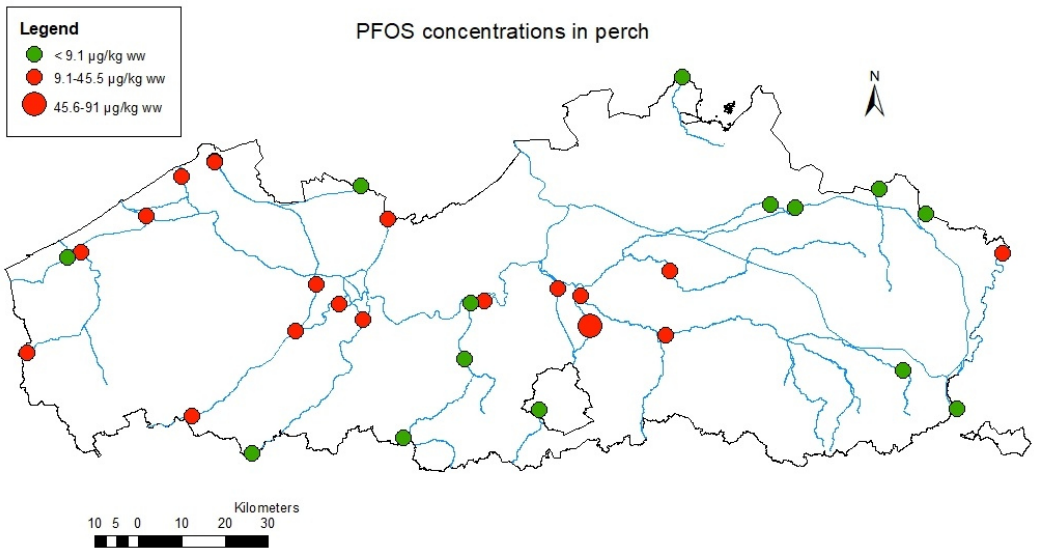


Figure 2.7: Map of Flanders with exceedance of the EQS_{biota} for PFOS in perch at different sampling locations (N = 33). Green dots refer to locations with concentrations below the standard. The size of the red dot represents a range of concentrations, increasing with a factor 5. The EQS_{biota} for PFOS is 9.1 µg kg^{-1} ww. In the case of multiple perch pools per location, a mean concentration per location was calculated.

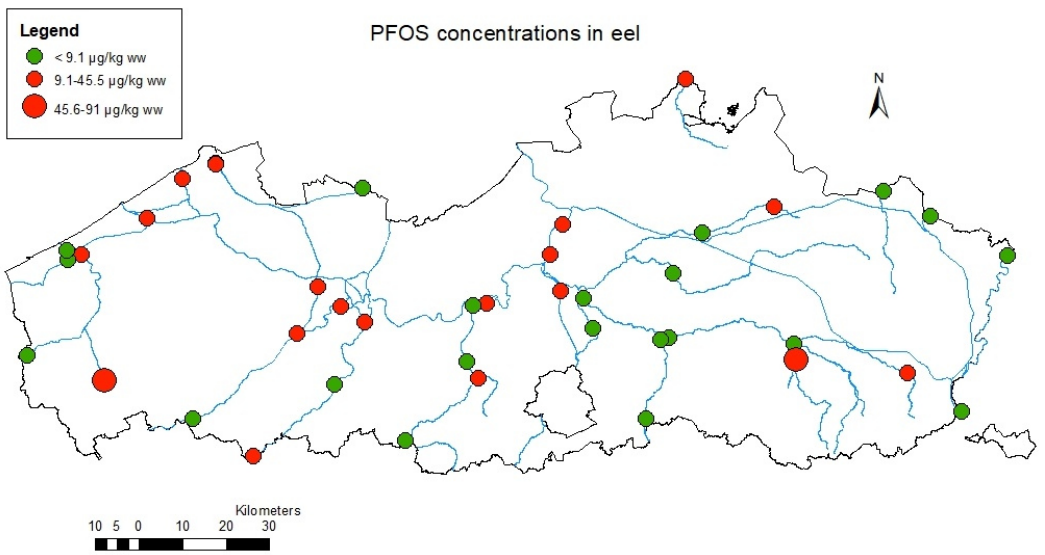


Figure 2.8: Map of Flanders with exceedance of the EQS_{biota} for PFOS in eel at different sampling locations (N = 41). Green dots refer to locations with concentrations below the standard. The size of the red dot represents a range of concentrations, increasing with a factor 5. The EQS_{biota} for PFOS is 9.1 µg kg^{-1} ww. In the case of multiple eel pools per location, a mean concentration per location was calculated.

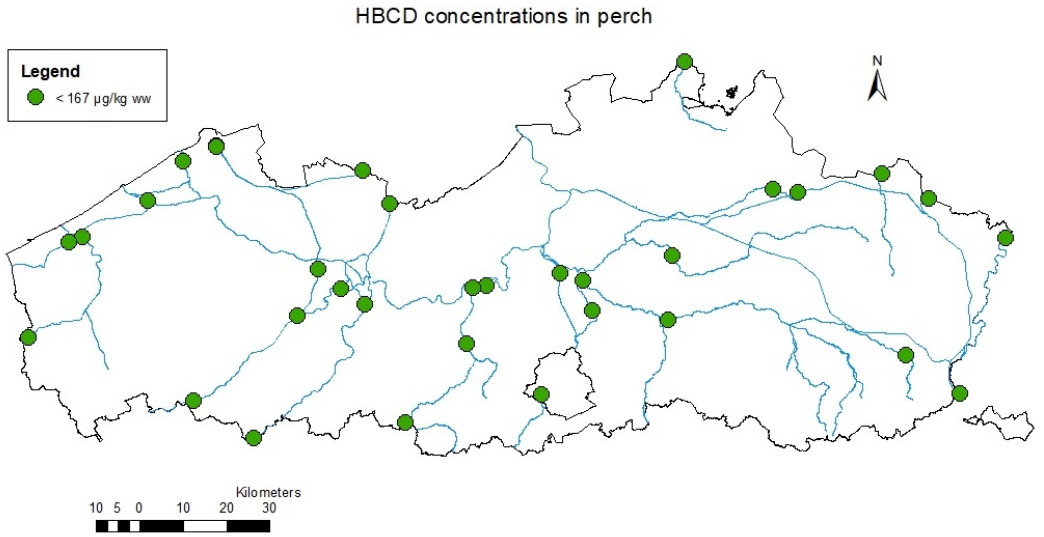


Figure 2.9: Map of Flanders with exceedance of the EQS_{biota} for HBCD in perch at different sampling locations (N = 33). Green dots refer to locations with concentrations below the standard. The EQS_{biota} for HBCD is 167 µg kg^{-1} ww. In the case of multiple perch pools per location, a mean concentration per location was calculated.

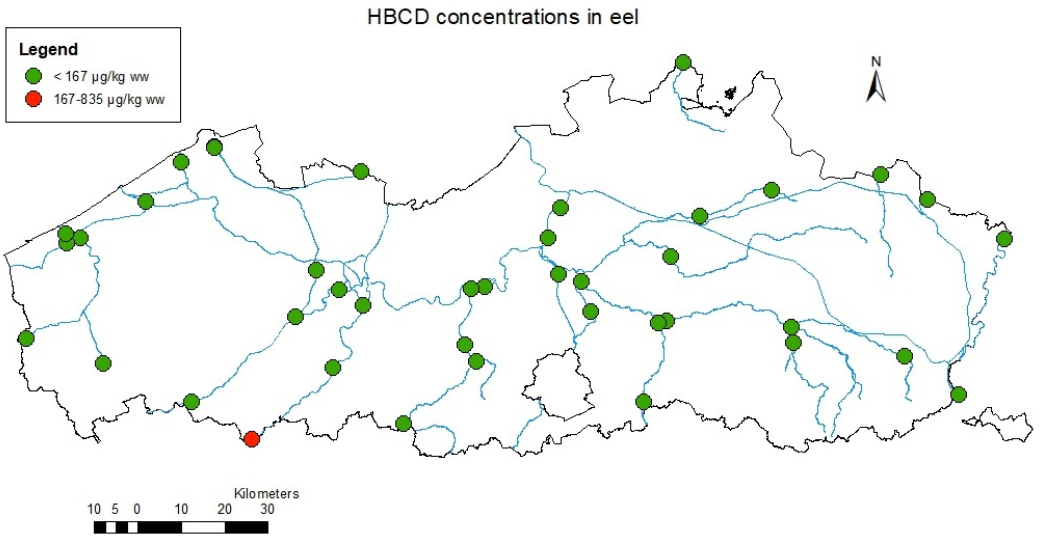


Figure 2.10: Map of Flanders with exceedance of the EQS_{biota} for HBCD in eel at different sampling locations (N = 41). Green dots refer to locations with concentrations below the standard. The size of the red dot represents a range of concentrations, increasing with a factor 5. The EQS_{biota} for HBCD is 167 µg kg^{-1} ww. In the case of multiple eel pools per location, a mean concentration per location was calculated.

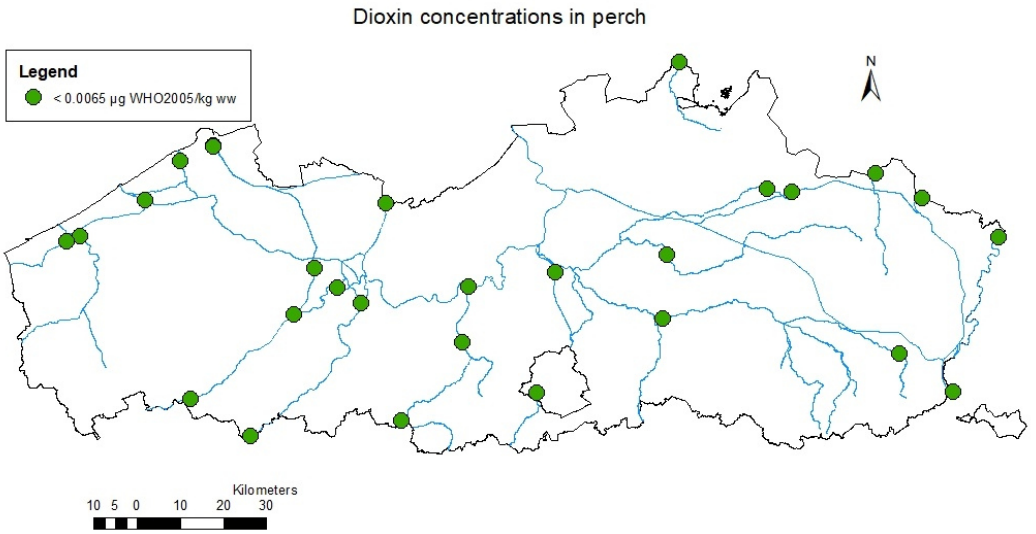


*Figure 2.11: Map of Flanders with exceedance of the EQS_{biota} for dioxins in perch at different sampling locations (*N = 27*). Green dots refer to locations with concentrations below the standard. The EQS_{biota} for dioxins is 0.0065 µg WHO-TEQ_{2005} kg^{-1} ww. In the case of multiple perch pools per location, a mean concentration per location was calculated.*

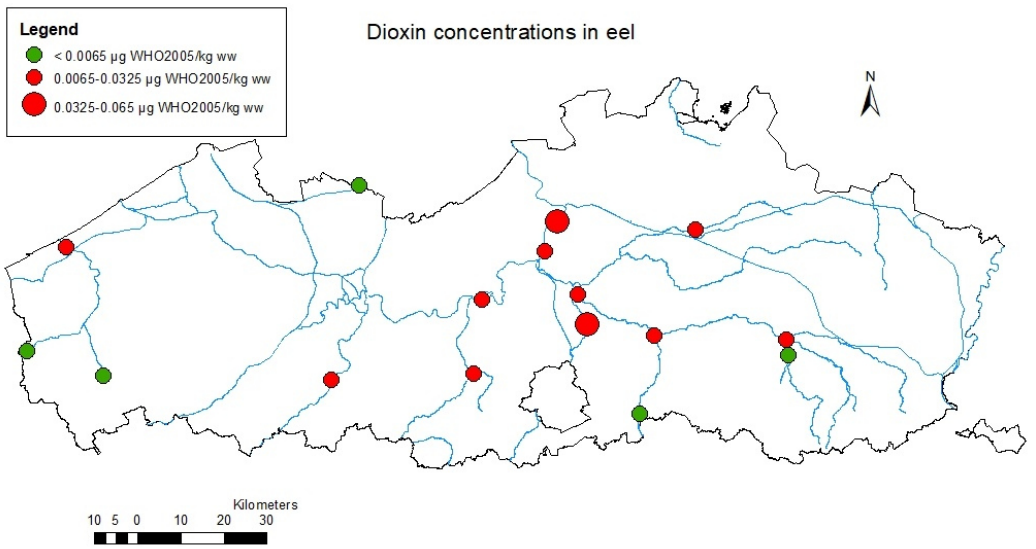


*Figure 2.12: Map of Flanders with exceedance of the EQS_{biota} for dioxins in eel at different sampling locations (*N = 16*). Green dots refer to locations with concentrations below the standard. The size of the red dot represents a range of concentrations, increasing with a factor 5. The EQS_{biota} for dioxins is 0.0065 µg WHO-TEQ_{2005} kg^{-1} ww. In the case of multiple eel pools per location, a mean concentration per location was calculated.*

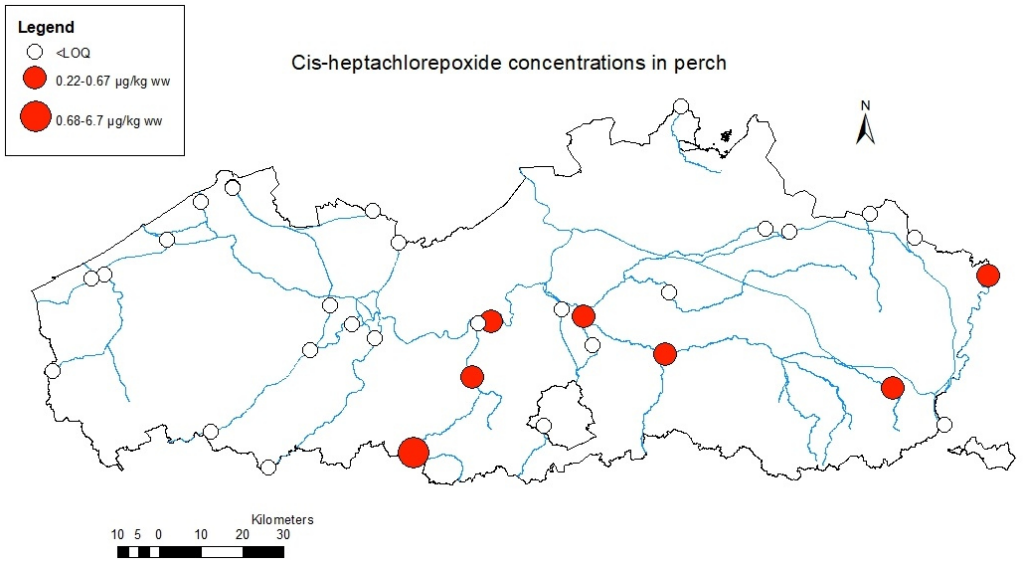


Figure 2.13: Map of Flanders with exceedance of the EQS_{biota} for cis-heptachlor epoxide in perch at different sampling locations (N = 33). The size of the red dot represents a range of concentrations, increasing with a factor 10. The white dot represents a result below LOQ (0.25 µg kg^{-1} ww), for which there is no guarantee that the standard was exceeded. The EQS_{biota} for cis-heptachlor epoxide is 0.0068 µg kg^{-1} ww. In the case of multiple perch pools per location, a mean concentration per location was calculated.

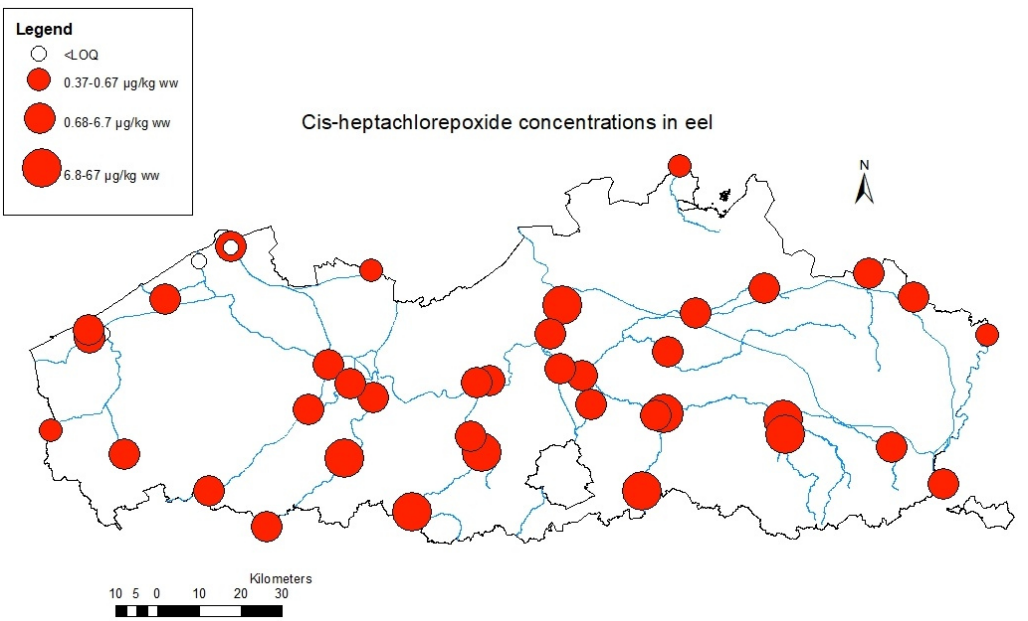


Figure 2.14: Map of Flanders with exceedance of the EQS_{biota} for cis-heptachlor epoxide in eel at different sampling locations (N = 41). The size of the red dot represents a range of concentrations, increasing with a factor 10. The white dot represents a result below LOQ (0.25 µg kg^{-1} ww), for which there is no guarantee that the standard was exceeded. The EQS_{biota} for cis-heptachlor epoxide is 0.0068 µg kg^{-1} ww. In the case of multiple eel pools per location, a mean concentration per location was calculated.

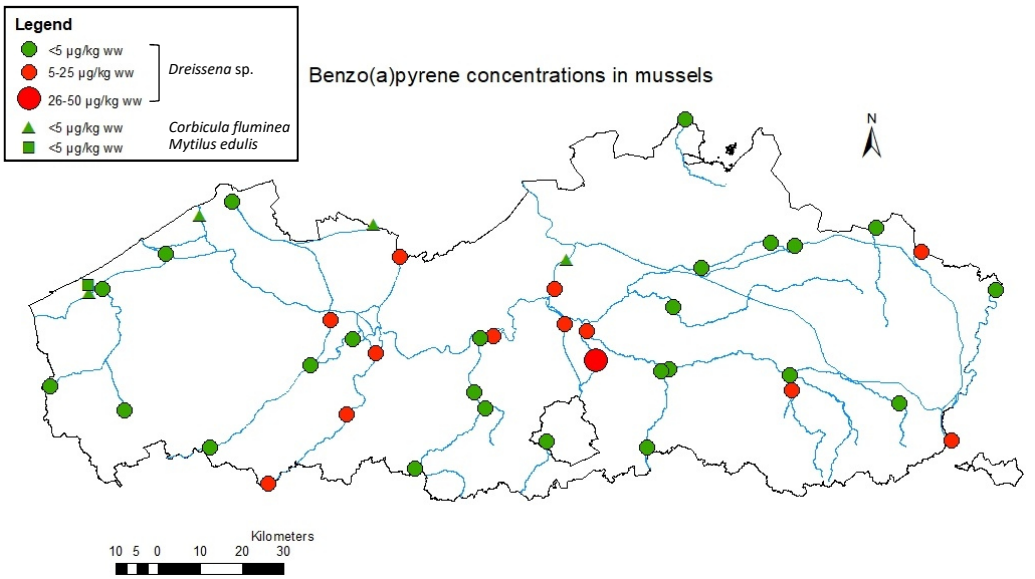


*Figure 2.15: Map of Flanders with exceedance of the EQS_{biota} for benzo(a)pyrene in mussels at different sampling locations (*N = 44). *Green dots refer to locations with concentrations below the standard. The size of the red dot represents a range of concentrations, increasing with a factor 5. Different symbols represent different species. The EQS_{biota} for benzo(a)pyrene is 5 µg kg^{-1} ww.*

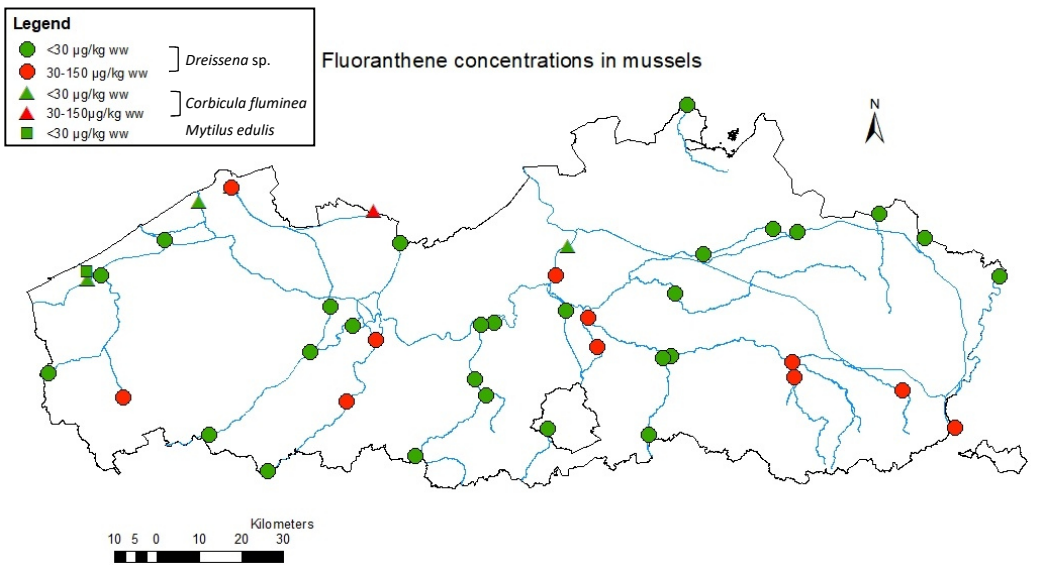


*Figure 2.16: Map of Flanders with exceedance of the EQS_{biota} for fluoranthene in mussels at different sampling locations (*N = 44). *Green dots refer to locations with concentrations below the standard. The size of the red dot represents a range of concentrations, increasing with a factor 5. Different symbols represent different species. The EQS_{biota} for fluoranthene is 30 µg kg^{-1} ww.*

2.3.1.2 General trends

In perch the highest percentage of locations with exceedances of the EQS_{biota} was measured for Hg (100%) and ∑PBDE (85%), followed by PFOS (58%) and cis-heptachlor epoxide (21%; cHpCepx) (*Figure 2.17*). For cis-heptachlor epoxide and ∑PBDE in perch, it should be taken into account that the LOQ was far above the EQS_{biota}. Therefore, for values below LOQ no exclusive statement on the exceedance of the standards could be made. The highest factor of exceedance in perch, however, was reached for ∑PBDE, followed by cis-heptachlor epoxide (*Figure 2.18*).

Furthermore, the highest number of pollutants with an exceedance in perch was measured in the Demer VII, Maas I+II+III, Zeeschelde II and Getijdedijle-Getijdezenne, (*Figure 2.23*). The lowest number of pollutants exceeding the respective standards was found in the Leopoldkanaal I, canal Duinkerke-Nieuwpoort and canal Bocholt-Herentals.

In eel the highest percentage of locations with an exceedance of the EQS_{biota} was again measured for Hg (100%) and ∑PBDE (100%), followed by cis-heptachlor epoxide (90%), dioxins (69%) and PFOS (46%) (*Figure 2.19*). The highest factor of exceedance in eel too was found for ∑PBDE, followed by cis-heptachlor epoxide (*Figure 2.20*).

The highest number of pollutants with an exceedance of the standards in eel was measured in Bovenschelde I, Zeeschelde IV, Zeeschelde II, Zeeschelde III + Rupel, and Bellebeek (*Figure 2.24*). The lowest number of exceedances was found in the Dijle I.

In general it can be stated that more exceedances of the EQS_{biota} were measured in the muscle tissue of eel compared to perch. However, for PFOS the opposite was true. Furthermore, it is apparent that for neither fish species there is a location without an exceedance of at least one of the compounds.

Finally, in mussels a slightly higher percentage of locations with an exceedance of the standard was found for benzo(a)pyrene (30%) compared to fluoranthene (27%) (*Figure 2.21*), with the highest factor of exceedance in benzo(a)pyrene (*Figure 2.22*).

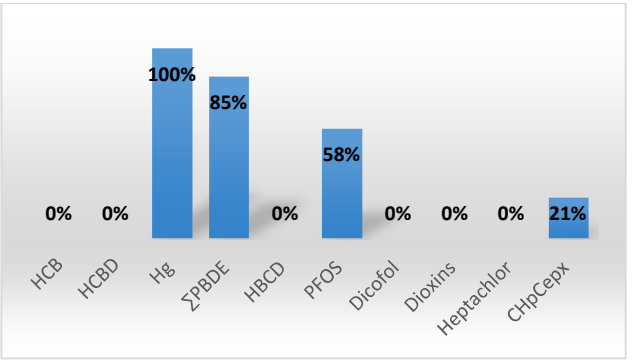


Figure 2.17: Percentage of locations (N = 33) with an exceedance of the EQS_{biota} in perch for the different priority compounds. In the case of multiple perch pools per location, mean concentrations were used to check for exceedance.

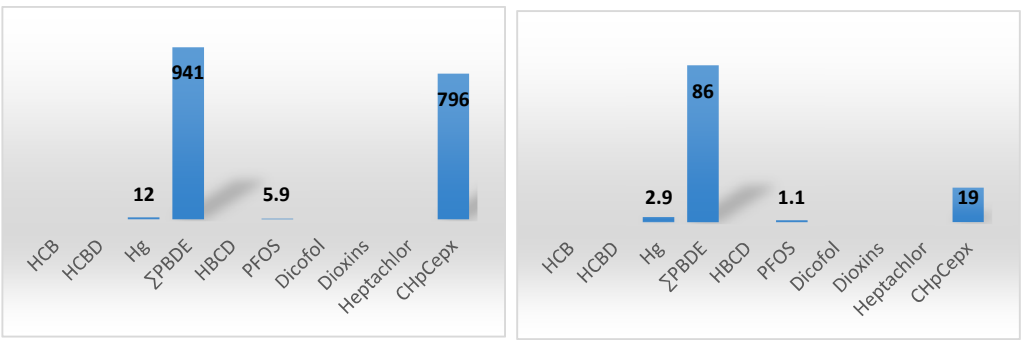


Figure 2.18: Factor of exceedance of the EQS_{biota} for different pollutants in perch. These factors were calculated on the maximal (left) and median (right) concentrations measured over all locations (N = 33). In the case of multiple perch pools per location, a mean concentration per location was calculated.

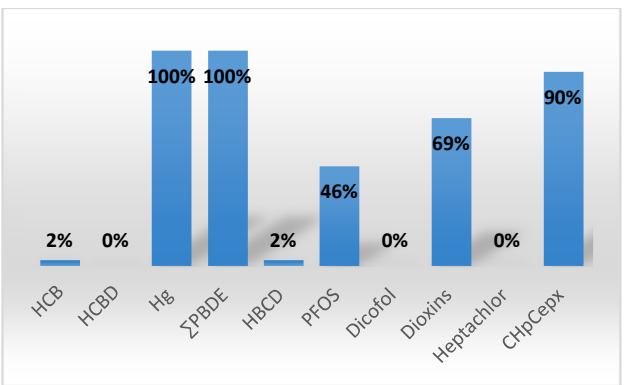


Figure 2.19: Percentage of locations (N = 41) with an exceedance of the EQS_{biota} in eel for the different priority compounds. In the case of multiple eel pools per location, mean concentrations were used to check for exceedance.

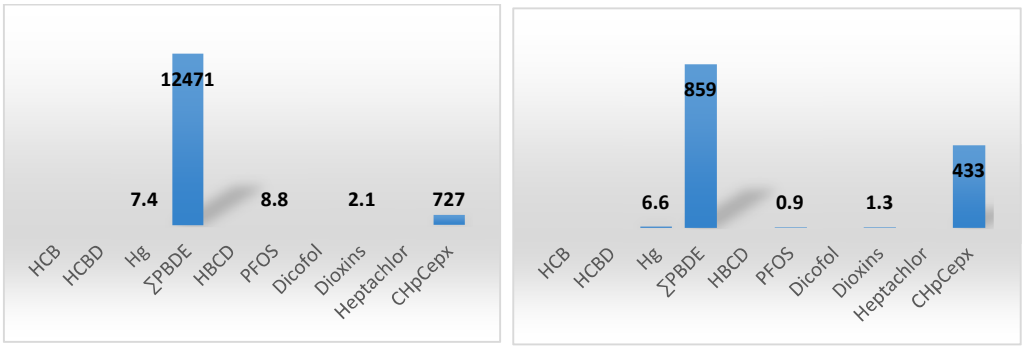


Figure 2.20: Factor of exceedance of the EQS_{biota} for different pollutants in eel. These factors were calculated on the maximal (left) and median (right) concentrations measured over all locations (N = 41)*. In the case of multiple perch pools per location, a mean concentration per location was calculated.*

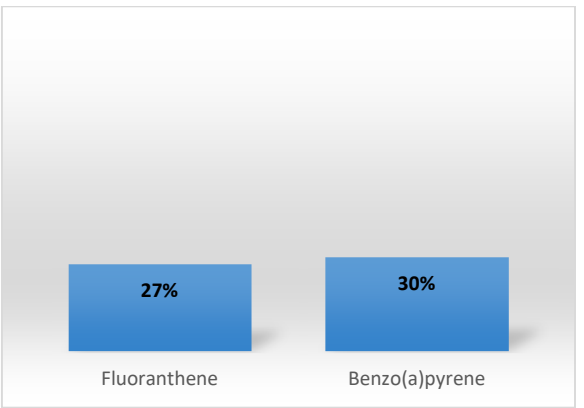


Figure 2.21: Percentage of locations (N = *44) with an exceedance of the EQS_{biota} in mussels for PAHs.*

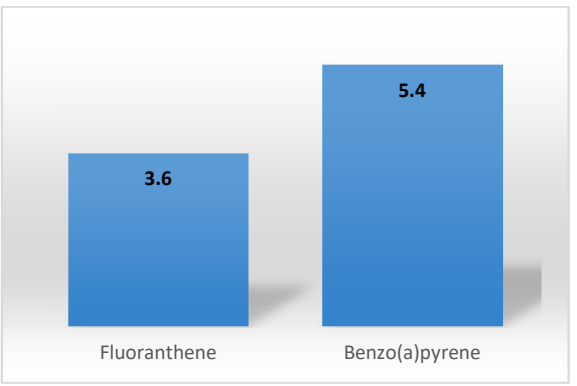


Figure 2.22: Factor of exceedance of the EQS_{biota} for maximal concentrations measured for PAHs in mussels over all locations (N = *44*)*. The median concentrations of both PAHs did not result in an exceedance of the standards.*

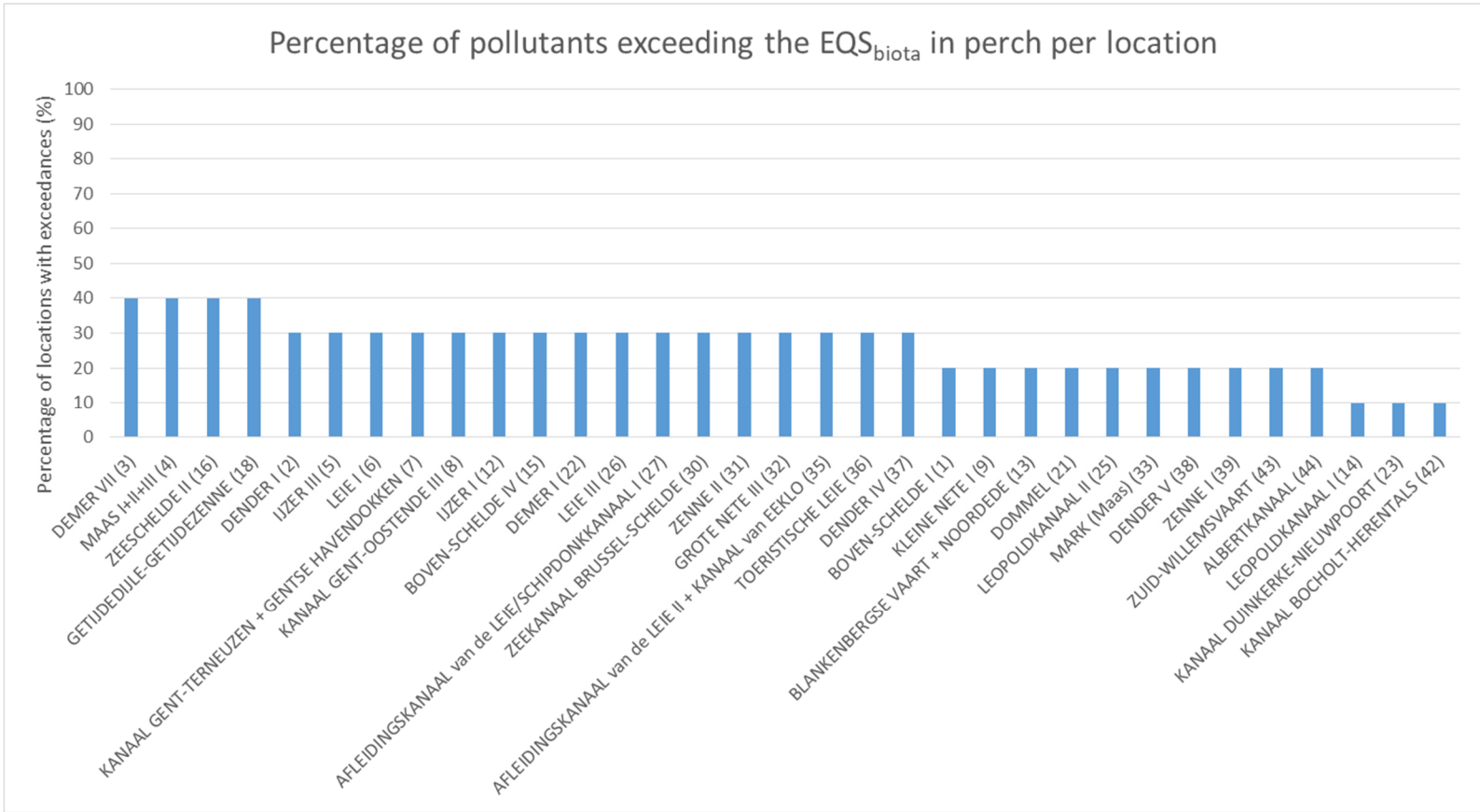


*Figure 2.23: Percentage of priority compounds (*N = *10) with an exceedance of the EQS_{biota} in perch, per location. For detailed information about the sampling locations, we refer to* Appendix A2. *The number between brackets after each location name refers to the location number as indicated in* Figure A1.1 (Appendix A1). *In the case of multiple perch pools per location, mean concentrations were used to check for exceedance.*

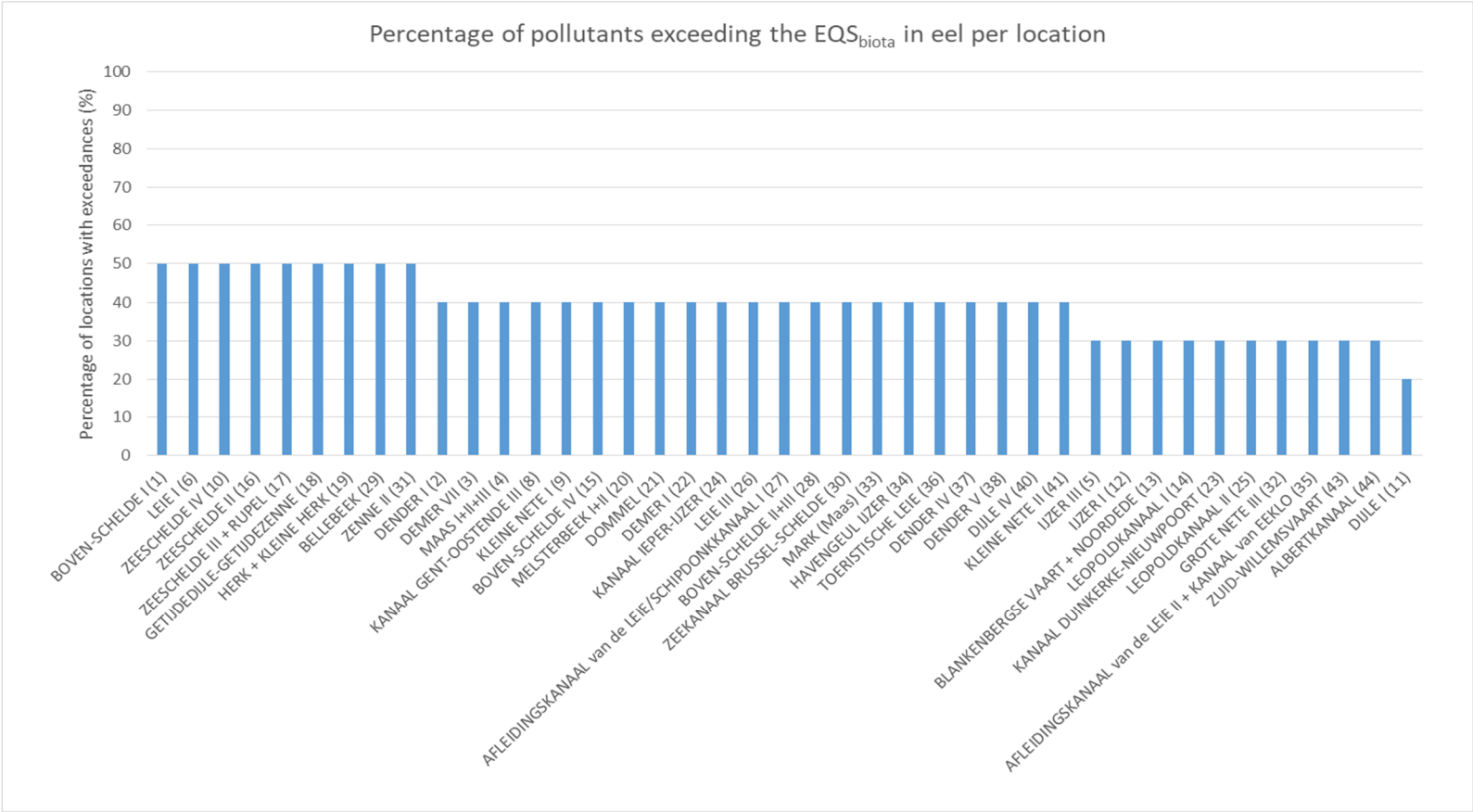


Figure 2.24*: Percentage of priority compounds (*N = 10*) with an exceedance of the EQS*$_{biota}$ *in eel, per location. For detailed information about the sampling locations, we refer to* Appendix A2*. The numbers between brackets after each location name refers to the location number as indicated in* Figure A1.1 (Appendix A1)*. In the case of multiple eel pools per location, mean concentrations were used to check for exceedance.*

2.3.2 Standardisation using lipid content and dry residue

Concentrations between perch and eel were compared. For this purpose a paired t-test (or non-parametrical Wilcoxon test) was used to determine if accumulated concentrations differed significantly ($p < 0.05$) between both species. For the extrapolation potential between both fish species, we refer to *Chapter 5*.

For the current analysis, only compounds were incorporated with at least 50% of the results above LOQ. This was not the case for HCBD, heptachlor, heptachlor epoxide and dicofol. Furthermore, for dioxins this comparison could not be performed, since at every location dioxins were only measured in one species.

Originally, with focus on the EQS_{biota} monitoring, concentrations were given in µg kg^{-1} wet weight (ww). However, as a measure of standardised comparison between species, concentrations were calculated in µg kg^{-1} lipid weight (lw) and µg kg^{-1} dry weight (dw) as well. Individually (pool based) results on lipid content and dry/wet weight ratio, as given in *Appendix A4*, were used.

2.3.2.1 Hexachlorobenzene (HCB)

Hexachlorobenzene concentrations in eel were significantly higher than those in perch, as calculated per ww ($p < 0.001$) and dw ($p < 0.001$) (*Figures 2.25 and 2.26*). Even with a standardisation per lipid content concentrations differed significantly ($p < 0.001$), although they were closer together (*Figure 2.26*).

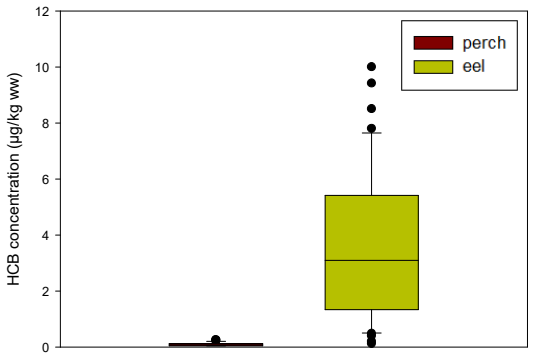


Figure 2.25: Boxplot HCB concentrations in muscle tissue of perch (N = 33) and eel (N = 41), calculated in µg kg^{-1} ww.

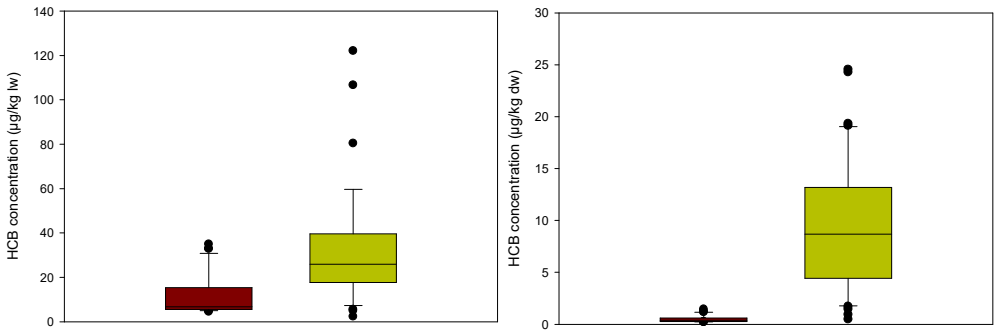


Figure 2.26: Boxplots HCB concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), calculated in µg kg^{-1}lw (left) and µg kg^{-1} dw (right).

2.3.2.2 Mercury (Hg)

For Hg, concentrations in eel were significantly higher than those in perch, as calculated per ww ($p < 0.001$) and dw ($p < 0.05$) (*Figures 2.27 and 2.28*). A correction based on lipid content, however, resulted in the opposite, with significantly higher concentrations in perch ($p < 0.001$) (*Figure 2.28*).

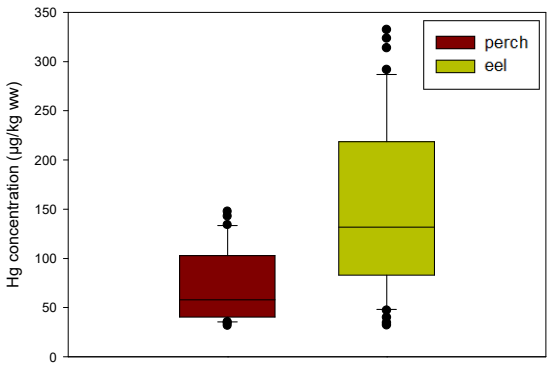


Figure 2.27: Boxplot Hg concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), as calculated in µg kg^{-1} ww.

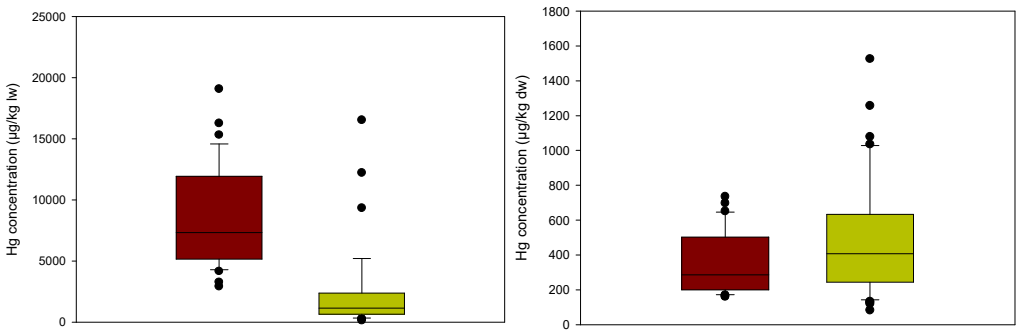


Figure 2.28: Boxplots Hg concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), as calculated in µg kg^{-1} lw (left) and µg kg^{-1} dw (right).

2.3.2.3 Polybrominated diphenyl ethers (PBDEs)

The ∑PBDE concentrations were significantly higher in eel compared to perch, as calculated per ww ($p < 0.001$) and dw ($p < 0.001$) (*Figures 2.29 and 2.30*). When recalculated based on lipid content, no significant difference in accumulated concentrations was found between perch and eel ($p = 0.34$) (*Figure 2.30*).

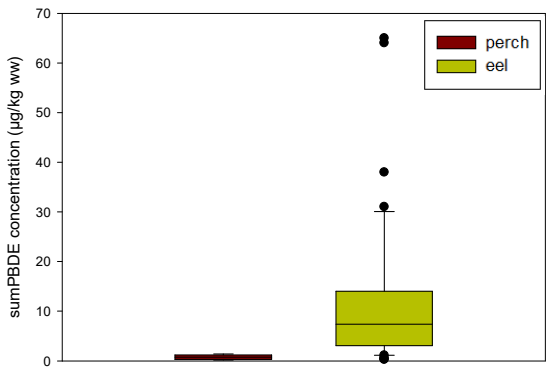


Figure 2.29: Boxplot ∑PBDE concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), as calculated in µg kg^{-1} ww.

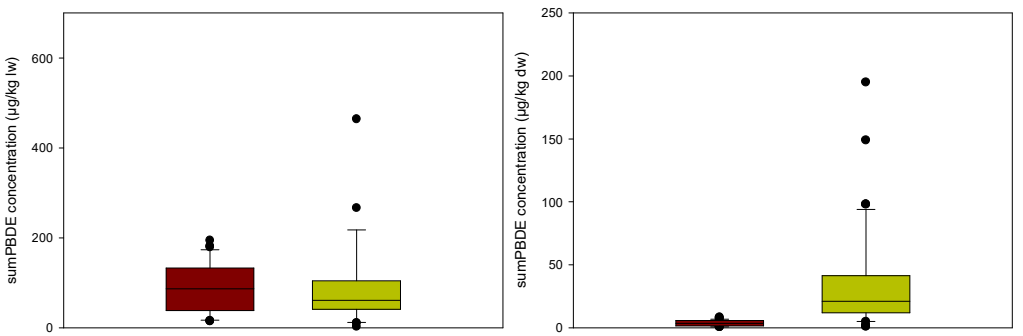


Figure 2.30: Boxplots ∑PBDE concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), as calculated in µg kg^{-1} lw (left) and µg kg^{-1} dw (right).

2.3.2.4 Hexabromocyclododecane (HBCD)

In eel, HBCD concentrations were significantly higher than compared to perch, as given per ww ($p < 0.001$) or dw ($p < 0.001$) (*Figures 2.31 and 2.32*). Concentrations per lipid weight still resulted in a significant difference between both species ($p = 0.004$). However, the difference was smaller than for ww or dw concentrations (*Figure 2.32*).

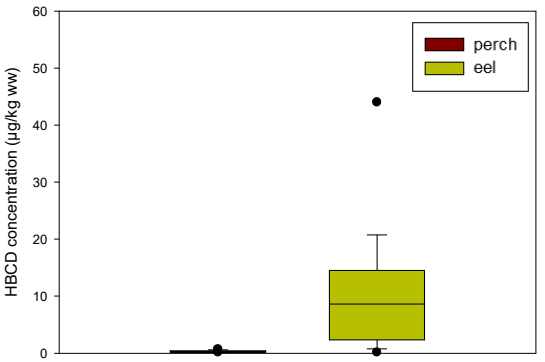


Figure 2.31: Boxplot HBCD concentrations in the muscle tissue of perch (N = 33) and eel (N = 40), as calculated in µg kg^{-1} ww.

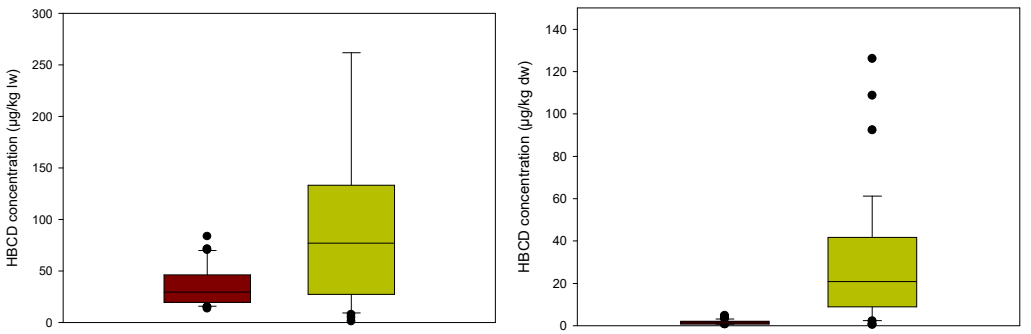


Figure 2.32: Boxplots HBCD concentrations in the muscle tissue of perch (N = 33) and eel (N = 40), as calculated in µg kg^{-1} lw (left) and µg kg^{-1} dw (right).

2.3.2.5 PFOS

In contrast to the other priority compounds, PFOS concentrations in eel were not significantly higher than those in perch (*Figure 2.33*), for concentrations per wet weight ($p = 0.35$). However, concentrations per dw ($p = 0.001$) and lw ($p < 0.001$) were higher in perch compared to eel (*Figure 2.34*).

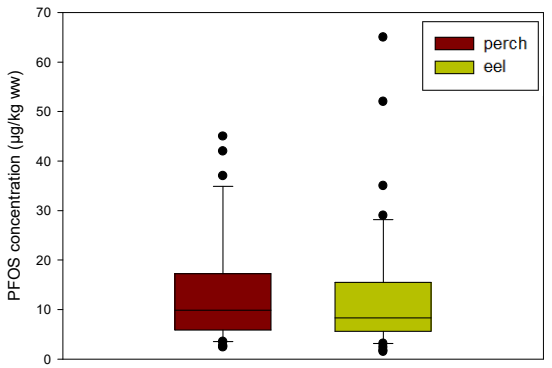


Figure 2.33: Boxplot PFOS concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), as calculated in µg kg^{-1} ww.

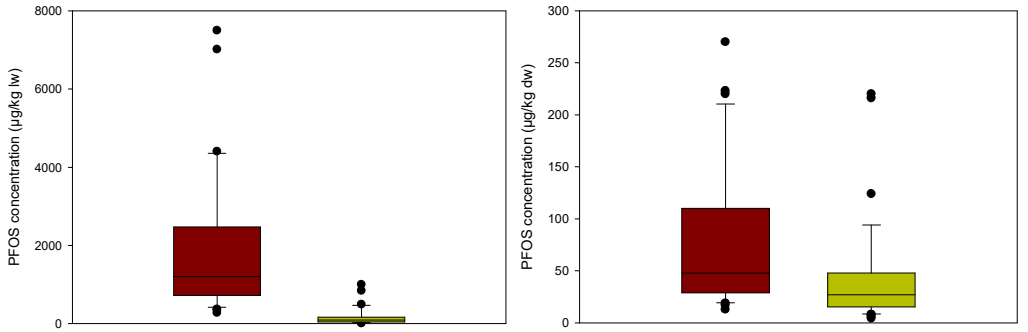


Figure 2.34: Boxplots PFOS concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), as calculated in µg kg^{-1} lw (left) and µg kg^{-1} dw (right).

2.3.2.6 Polychlorinated biphenyls (PCBs)

The ∑PCB concentrations in the muscle tissue of eel were significantly higher than those in perch, for concentrations per ww ($p < 0.001$) and dw ($p < 0.001$) (*Figures 2.35 and 2.36*). For concentrations per lw, no significant difference was found between perch and eel ($p = 0.13$) (*Figure 2.36*).

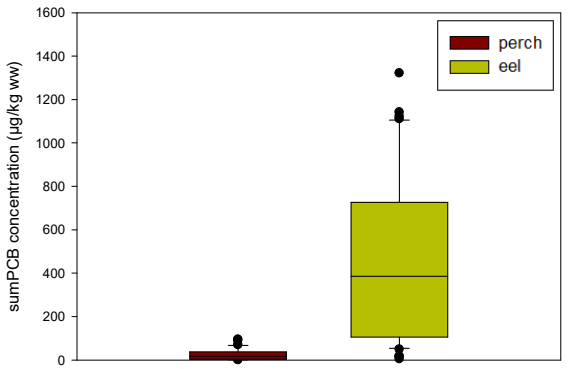


Figure 2.35: Boxplot ∑PCB concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), as calculated in µg kg^{-1} ww.

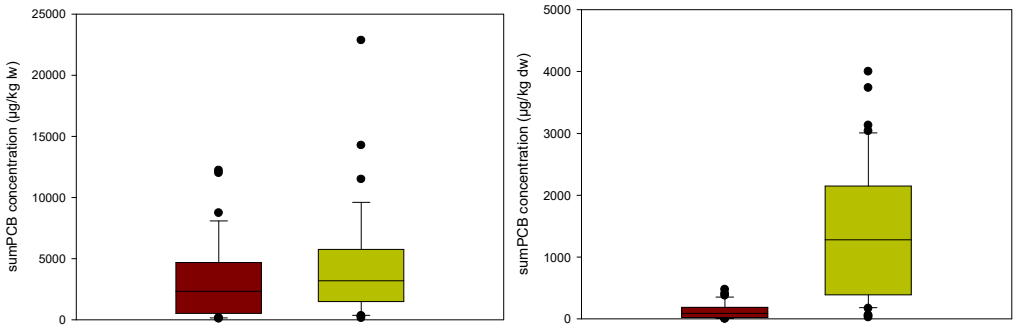


Figure 2.36: Boxplots ∑PCB concentrations in the muscle tissue of perch (N = 33) and eel (N = 41), as calculated in µg kg^{-1} lw (left) and µg kg^{-1} dw (right).

2.3.3 Comparison bioaccumulation vs. passive samplers

This comparison could only be performed for the pollutants that were monitored in both biota and passive samplers: HCB, HCB, PBDEs, benzo(a)pyrene, fluoranthene and PCBs. Analogous to the comparison in accumulation between perch and eel, a Pearson correlation test was performed between biota and passive samplers for this analysis. For significant correlations, a linear regression analysis was used in each case. In addition, a paired (non-parametric) Wilcoxon test was used to determine whether accumulated concentrations in passive samplers were significantly different between the two fish species respectively. For raw data of passive samplers, refer to *Appendix A5*.

2.3.3.1 Hexachlorobenzene (HCB)

Scatterplots were generated showing the relationship between concentrations measured in passive samplers and concentrations in biota (*Figure 2.37*). Accumulated concentrations in the passive samplers were correlated with perch ($R = 0.48$; $p < 0.05$) but not with eel ($R = 0.36$; $p = 0.05$). The equation obtained from linear regression is shown in *Table 2.1*.

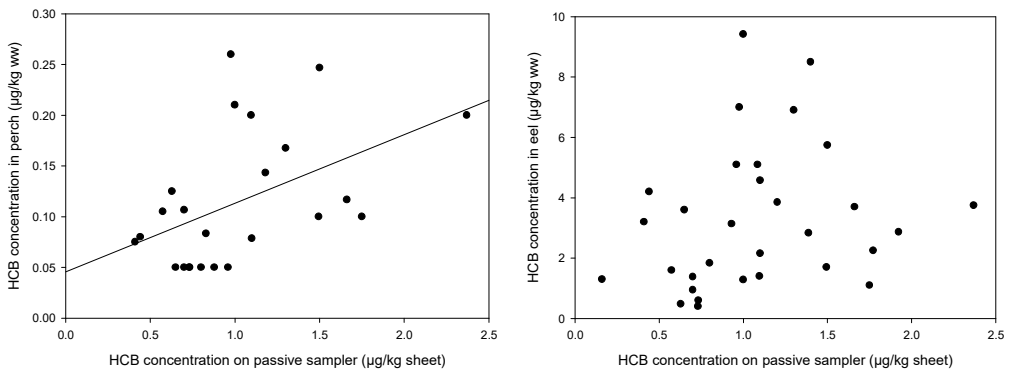


Figure 2.37: Relationship of accumulated HCB concentrations in perch (left, N = 28) and eel (right, N = 36), with concentrations in passive samplers.

Table 2.1: Equation from linear regression for HCB, comparison between biota and passive sampler.

Equation	R^2
$[HCB_{perch}] = 0.07*[HCB_{PS}] + 0.05$	0.23

Concentrations in biota are given in µg kg^{-1} ww and for passive samplers (PS) in µg kg^{-1} sheet.

2.3.3.2 Hexachlorobutadiene (HCBD)

For hexachlorobutadiene, the majority of measurements (95.5%) were below the LOQ of 0.5 µg kg^{-1} ww. Concentrations in passive samplers were between 0.07 and 214 µg kg^{-1} sampler (median: 0.26 µg kg^{-1} sampler). Hereby 20% of the measurements in passive samplers were above 0.5 µg kg^{-1} sampler. No further statistical tests could be performed on these data.

2.3.3.3 Polybrominated diphenyl ethers (PBDE)

The relationships between ∑PBDE concentrations measured in passive samplers and in fish tissue are shown in *Figure 2.38*. A correlation test showed no significant relationship between ∑PBDE concentrations in passive samplers and concentrations in eel (R = 0.32; $p = 0.07$) or in perch (R = 0.13; $p = 0.52$).

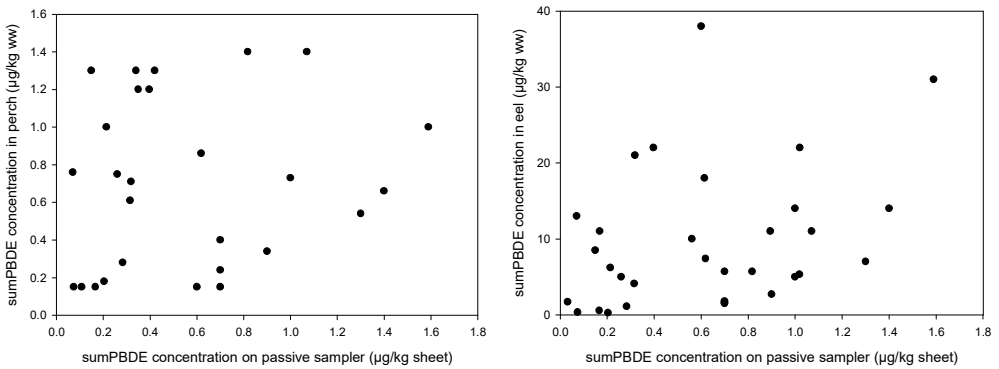


Figure 2.38: Relationship of accumulated ∑PBDE concentrations in perch (left, N *= 29) and eel (right,* N *= 37), with concentrations in passive samplers.*

2.3.3.4 Benzo(a)pyrene

The relationship between benzo(a)pyrene concentrations in passive samplers and in freshwater mussels is shown in *Figures 2.39 and 2.40*. Levels accumulated in passive samplers showed a correlation with accumulated concentrations in both zebra mussels (R = 0.58; $p < 0.05$) and quagga mussels (R = 0.58; $p < 0.01$) and mussels in general (R = 0.53; $p < 0.005$). The equation obtained from linear regression is shown in *Table 2.2*.

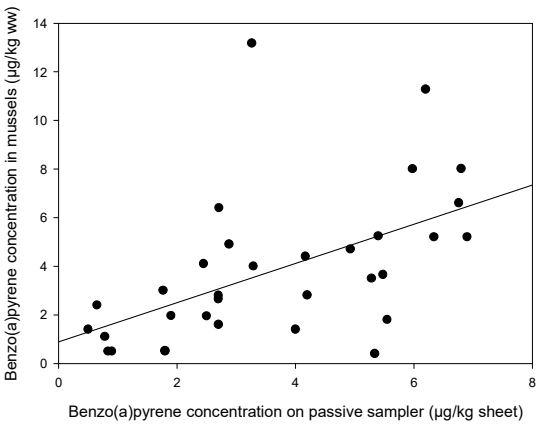


*Figure 2.39: Relationship of accumulated benzo(a)pyrene concentrations in mussels to concentrations in passive samplers (*N = *32*).

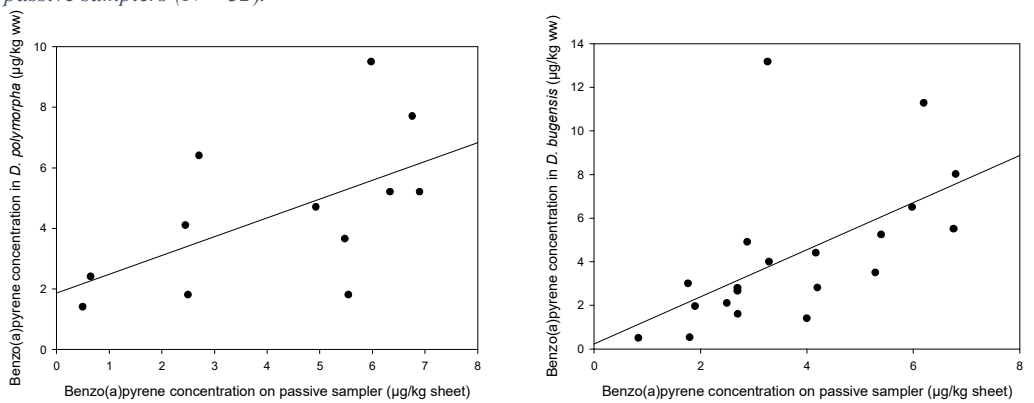


Figure 2.40: Relationship of accumulated benzo(a)pyrene concentrations in zebra mussel (left, N = *14) and quagga mussel (right,* N = *21), with concentrations in passive samplers.*

Table 2.2: Equations from linear regression for benzo(a)pyrene, comparison between biota and passive sampler.

Equation	R^2
$[Benzo(a)pyrene_{D.polymorpha}] = 0.62*[Benzo(a)pyrene_{PS}] + 1.86$	0.33
$[Benzo(a)pyrene_{D.bugensis}] = 1.08*[Benzo(a)pyrene_{PS}] + 0.23$	0.33
$[Benzo(a)pyrene_{mussel}] = 0.81*[Benzo(a)pyrene_{PS}] + 0.89$	0.28

Concentrations in biota are given in µg kg^{-1} ww and for passive samplers (PS) in µg kg^{-1} sheet.

2.3.3.5 Fluoranthene

Figures 2.41 and 2.42 show the relationship of accumulated fluoranthene concentrations in passive samplers with concentrations in freshwater mussels. No significant correlation was found between sampler and zebra mussel (R = -0.002; p = 0.99) or quagga mussel (R = 0.11; p = 0.74). This relationship was also not present for mussels in general (R = 0.05; p = 0.79).

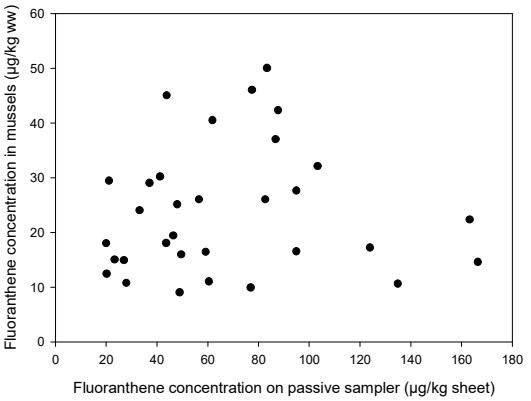


*Figure 2.41: Relationship of accumulated fluoranthene concentrations in mussels with concentrations in passive samplers (*N = *32).*

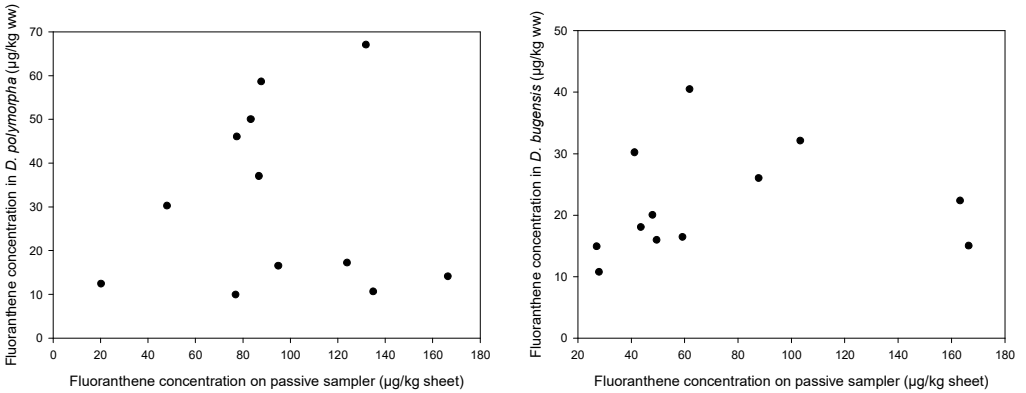


Figure 2.42: Relation of accumulated fluoranthene concentrations in zebra mussel (left, N = *14) and quagga mussel (right,* N = *21), with concentrations in passive samplers.*

2.3.3.6 Polychlorinated biphenyls (PCBs)

Scatterplots of ∑PCB concentrations in passive samplers and biota (perch and eel) are shown in *Figure 2.43*. Concentrations in passive samplers and in eel showed a significant correlation. (R = 0.46; $p < 0.01$). For perch, however, this was not the case (R = 0.22; $p = 0.29$). The equation obtained from linear regression is shown in *Table 2.3*.

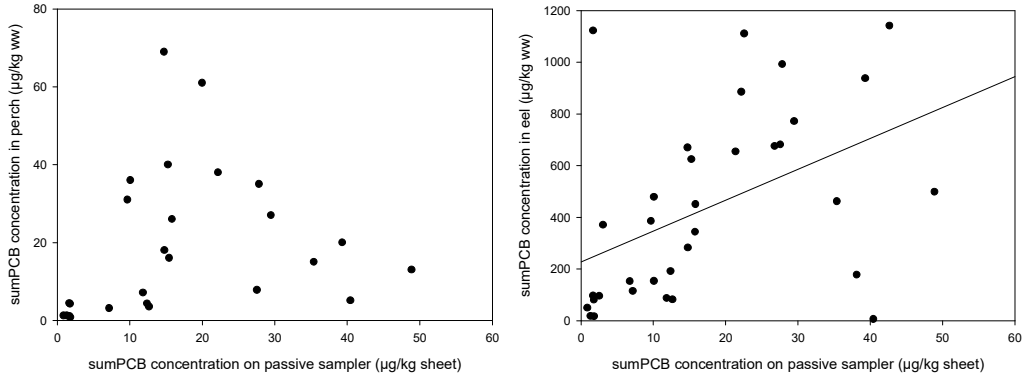


Figure 2.43: Relation of accumulated ∑PCB concentrations in perch (left, N = 29) and eel (right, N = 37), with concentrations in passive samplers.

Table 2.3: Equation from linear regression for PCB, comparison between biota and passive sampler.

Equation	R^2
$[\sum PCB_{eel}] = 11.95*[\sum PCB_{PS}] + 227.43$	0.21

Concentrations in biota are given in µg kg^{-1} ww and for passive samplers (PS) in µg kg^{-1} sheet.

2.3.4 Trophic levels

The $\delta^{15}N$ values (ratio between ^{15}N and ^{14}N stable isotopes) were determined in all exposed biota, both fish and mussels (*Appendix A6*). These values provide information about the trophic position and were higher in fish tissue (perch: 17‰ ± 2.5; eel: 16‰ ± 2.6) than in mussel tissue (zebra mussel: 9.1‰ ± 0.45; quagga mussel: 7.1‰ ± 2.1; Asian clam: 5.8‰ ± 1.0). They were similar between the two fish species. In general, $\delta^{15}N$ values of Asian clam were lower than those of quagga mussel. Results for zebra mussels were overall higher than those of quagga mussels.

The mussels were considered as primary consumers when calculating trophic levels for the fish pools. In this way, a site-dependent trophic level was determined for both perch and eel. This resulted in trophic levels between 2.8 and 6.2 (perch: 4.3 ± 0.72; eel: 4.1 ± 0.74). Variation between sites was larger than between species.

2.4 Discussion

2.4.1 General trends in compliance with the EQS_{biota}

Exceedances of the EQS_{biota} were detected in both fish species at almost all locations for Hg and ∑PBDE. A slightly lower exceedance percentage was found for dioxins and cis-heptachlor epoxide in the muscle tissue of eel and PFOS in both species. Furthermore, sporadic exceedances were found for HCB and HBCD in eel and for cis-heptachlor epoxide in perch. Finally, both benzo(a)pyrene and fluoranthene standards were exceeded in mussels exposed in about 30% of the sampling locations. The highest factor of exceedance was found for ∑PBDE and cis-heptachlorepoxide, in both perch and eel muscle tissue. This is an indication that the concentrations of these pollutants might have problematic consequences for the aquatic environment and might result in secondary poisoning through consumption for top predators (i.e. birds of prey, otters) and even a human health risk.

In general, the Zeeschelde showed both in perch and eel the highest number of pollutants with an exceedance of their respective EQS_{biota}. Furthermore, more exceedances were measured in eel than in perch. For perch, the lowest number of exceedances was measured in the Leopoldkanaal, canal Duinkerke-Nieuwpoort and canal Bocholt-Herentals. For eel, this was the case in the Dijle.

2.4.1.1 Comparison to previous Flemish monitoring studies on eel

HCB concentrations in eels in the present study were between 0.12 and 12 µg kg^{-1} ww (median: 2.7 µg kg^{-1} ww). Maes et al. (2008) reported higher concentrations between 0.0026 and 192 µg kg^{-1} ww (median: 5.9 µg kg^{-1} ww) in 2839 eels caught between 1994 and 2005 at 365 locations in Flanders. In a study on 185 Flemish waterways, which investigated the relationship between accumulated concentrations in eel, caught

between 1994 and 2009, and ecological water quality, measured HCB concentrations in muscle ranged between 0.20 and 341 µg kg^{-1} ww (Van Ael et al., 2014). In the current study, these concentrations were between 2.0 and 155 µg kg^{-1} lw (median: 24 µg kg^{-1} lw).

In the current study, mercury concentrations in eels were between 29 and 332 µg kg^{-1} ww (median: 129 µg kg^{-1} ww). Although the maximum concentration in eel caught between 1994 and 2005 in Flanders (eel monitoring network) was much higher at 1185 µg kg^{-1} ww, the median concentration of 117 µg kg^{-1} ww was comparable (Maes et al. 2008). In 2000, respective (mean) mercury concentrations of 150, 174 and 94 µg kg^{-1} ww were measured in eels from the IJzer, Meuse and Lower Scheldt basins (Maes et al., 2005). Van Ael et al. (2014) measured mercury concentrations between 10 and 708 µg kg^{-1} ww in eels collected between 1994 and 2009.

The ∑PBDE (ICES 6) concentrations in eels from the current study were between 2.8 and 1493 µg kg^{-1} lw (median: 64 µg kg^{-1} lw). Roosens et al. (2010) measured slightly higher concentrations between 10 and 5811 µg kg^{-1} lw (median: 81 µg kg^{-1} lw) in eels collected from 50 Flemish locations between 2000 and 2006. In the same time period, eels collected from 60 Flemish locations showed comparable ∑PBDE concentrations between 12 and 1400 µg kg^{-1} lw (median: 60 µg kg^{-1} lw) (Malarvannan et al. 2014).

HBCD concentrations in eels from the present study were between 1.1 and 2574 µg kg^{-1} lw (median: 60 µg kg^{-1} lw). Concentrations in eels caught at 50 sites between 2000 and 2006 were slightly higher with concentrations between 16 and 4397 µg kg^{-1} lw (median: 73 µg kg^{-1} lw) (Roosens et al. 2010). Malarvannan et al. (2014) published HBCD concentrations between 7.0 and 9494 µg kg^{-1} lw (median: 100 µg kg^{-1} lw) in muscle tissue of eels caught between 2000 and 2006 at 60 sites in Flemish waterbodies.

The ∑PCB (ICES 7) concentrations in eels in the current study were between 5.3 and 1321 µg kg^{-1} ww (median: 385 µg kg^{-1} ww). This was lower than what was measured in eels caught from the eel monitoring network between 1994 and 2005, i.e. 3.5-12,455 µg kg^{-1} ww (median 605 µg kg^{-1} ww) (Maes et al. 2008). In another extensive study, the sum of 6 indicator PCBs (ICES 6 PCBs; excluding PCB 118) was determined in eels

caught at 60 sampling sites from different Flemish waterbodies (Malarvannan et al., 2014). These concentrations laid between 5 and 2600 µg kg^{-1} ww.

In the current studies, all measurements for dicofol were below the LOQ, and thus also below the EQS_{biota} of 33 µg kg^{-1} ww. Furthermore, to our knowledge, no further European studies have been conducted on dicofol concentrations in freshwater fish. This all calls into question the relevance of monitoring this substance, which requires a costly analysis.

For a more detailed literature study and a comparison to other European monitoring studies on fish (and bivalves for PAHs), we refer to the individual reports of the sampling campaigns from 2015 to 2019 (Teunen et al., 2017; 2018; 2019 and 2020a) and the next chapters of this thesis. Comparison to available literature reveals that for most compounds a decrease in concentrations is visible over the last 10 to 20 years, a result of the stricter legislation on use and production of these compounds (*Chapter 1*).

2.4.1.2 Practical aspects of fishing

An attempt was made each time to collect 20 perches and 3 eels per site. As can be seen in *Appendix A2*, this was not always possible. For perch, this quota was not achievable in 75% of the cases, for eels in 32%. In addition, the size of the fish collected is also important. If the fish are too small, insufficient tissue can be collected to perform the necessary analyses. As a result, more fish will need to be pooled and the targeted two perch pools per site may not be achieved. Furthermore, due to their young age and shorter exposure to the environment, small perch will have lower accumulated concentrations as opposed to larger fish. Therefore, a correction based on length, a proxy for age, is recommended in the future.

Ultimately, the targeted two perch pools could be made for 59% of the sites (75% of the sites had at least one perch pool). Since three pools per site were harvested each time, a shortage of perch meant that multiple eel pools were made. This caused eel pools to consist of only one or two individuals and thus reflect individual measurements rather than pooled data. Unfortunately, this leads to gaps in the dataset.

2.4.2 Standardisation using lipid content and dry residue

Concentrations in eel were higher than those in perch for all pollutants except PFOS. This is logic for lipophilic pollutants that accumulate in higher concentrations in the fattiest fish, i.e. eel. For PFOS, slightly higher concentrations were measured in perch. This difference was even significant for dry weight concentrations. PFOS has a high affinity for proteins and does not exhibit the typical behaviour of a lipophilic pollutant (Jones et al., 2003; Zhong et al., 2019). The fact that concentrations are higher in perch than in eel could be an indication that, apart from protein content, other factors (e.g. diet, lifestyle, habitat use) influence the accumulation of perfluorinated compounds in these fish species. Similarly, Hg shows a high affinity for sulphur-based amino-acids and thiol groups in proteins (Amlund et al., 2007).

A correction based on lipid content in both fish species resulted in similar concentrations for ∑PBDE and ∑PCB. This underlines the lipophilic nature of these pollutants. For HCB and HBCD, eel concentrations were still significantly higher than perch concentrations, although the difference was smaller than for ww or dw concentrations. Other (a)biotic factors may possibly play a role here as well (e.g. diet, lifestyle, available food sources; Foekema et al. 2016). Whereas for eels specifically the juvenile 'yellow eel' stage was caught, for perch a wide range of length/age was collected. In addition, we have to take into account that the low lipid concentrations in perch (≤ 1%) might result in a larger error when standardising for lipid content. However, for Hg and PFOS, it was found that this correction resulted in significantly higher concentrations in perch than in eel. According to the proteonophilic nature of both Hg and PFOS, a standardisation based on dry weight residue is more appropriate.

2.4.3 Effects of trophic level

Due to a lack of data from primary producers, the $\delta^{15}N$ values of primary consumers, i.e. mussels, were used in the calculation of the trophic level. The $\delta^{15}N$ values in exposed mussels were between 8.3‰ and 9.9‰ for zebra mussels and between 4.9‰ and 12.8‰ for quagga mussels. In a Dutch study, $\delta^{15}N$ values of 12.1-13.9‰ and 14.3-15.8‰ were measured in quagga and zebra mussels, respectively (Foekema et al., 2016).

To allow comparison between the different field campaigns, the trophic level was determined using an average $\delta^{15}N$ value for zebra mussel from the 2016-2017 campaign (Teunen et al., 2018). This was justified by the very low variation between sites (9.1‰ ± 0.45).

The trophic levels in perch and eel in the current study ranged between 2.8 and 6.2, with a negligible difference between both fish species from the same location. Because of the similar trophic levels between species, we did not expect a difference in accumulation due to biomagnification effects. However, differences among sites were greater than between species. Therefore, underlying mechanisms need to be further investigated before substantiated statements on the effect of trophic level can be made. The measured trophic levels of perch and eel indicated a predator level. Furthermore, a similar value of 4.4 was found in eels from the Camargue (Roche et al., 2009). In a Dutch study, a trophic level between 3 and 3.5 was measured in perch (Foekema et al., 2016). Fliedner et al. (2018) found values between 3.7 and 3.8 for perch in Germany. The use of (top) predators is important to determine the concentrations to which the highest trophic levels (secondary poisoning) and humans are exposed through consumption.

2.4.4 Comparison bioaccumulation vs. passive samplers

The current project included a preliminary interpretation of the relationship between concentrations measured in biota and passive samplers. Concentrations of HCB measured in passive samplers only showed a relationship with the accumulated concentrations in perch. For eel there was no significant relationship with concentrations in the passive samplers. For ∑PBDE there was no relation between concentrations in passive samplers and in fish for both species. The ∑PCB concentrations in passive samplers were only significantly related to accumulated concentrations in eel.

Benzo(a)pyrene concentrations in passive samplers were correlated with concentrations in freshwater mussels. Fluoranthene, however, showed no correlation with accumulation in freshwater mussels.

In general, we found in literature that passive samplers show a stronger relationship with accumulated concentrations in lower trophic levels (e.g. mussels) (Smedes, 2010; Verweij et al., 2004). For fish, which usually occupy higher trophic levels, this relationship may be less pronounced due to the more complex uptake and processing of pollutants (e.g. effect of biomagnification, uptake and elimination rate of hydrophobic substances) (Smedes et al. 2010). In a Dutch study, significant relationships were found between accumulated PAHs in zebra mussels and on silicon rubber samplers (Smedes 2010). The same was true for PCB concentrations in eel. Verweij et al. (2004) found no clear correlation between accumulated concentrations of PCBs and organochlorine pollutants (e.g. HCB, heptachlor epoxide) in caged carp and measured concentrations in passive samplers.

The present link between biota and passive samplers shows the opportunity of the complementary character of these two monitoring methods. These different matrices can provide additional information, which can contribute to the interpretation and evaluation of the status in the aquatic environment. In this way, potentially harmful pollutants and their effects can be identified more efficiently. Where measurements in biota reflect the situation in situ, effects of biomagnification and bioaccumulation (or elimination) after long exposure, passive samplers provide a more standardised, less invasive working method and can give more information on bioavailability and bioaccumulation of pollutants directly from the environment.

The University of Antwerp developed the concept of an active passive sampler (Amato et al. 2018, 2019). This application aims to address some existing disadvantages of using passive samplers. For example, the device contains several sorbents, allowing a larger combination of pollutant groups to be measured. In addition, the 'active' component, being a water pump, ensures a constant/controllable water flow (flow rate). In this way, it is also possible to calculate loads, which is not possible with the standard passive sampler. In future monitoring campaigns the device can be deployed as an additional link between biota and previously used passive samplers.

2.4.5 Effects of known general pollution sources and exposure routes

An overview of the origin and use of the various pollutants was elaborately discussed in the introduction of this thesis. Information on known pollution pathways was taken from the texts of the background document SGBP Inventory Priority Substances, reference year 2018 (VMM, 2018).

For **hexachlorobenzene**, the highest average concentrations in perch and eel were measured in the Grote Nete and Leie I, respectively. These results were already found for eel by Belpaire et al. (2008). Hexachlorobenzene was monitored between 2016 and 2018 in effluents from a broad set of companies (98) within the textile, waste processing, metal, chemical, dairy, breweries, paper sectors and at waste water treatment plants (WWTPs) (16) spread across Flanders (VMM, 2018). At none of these points a concentration above the LOQ of 0.1 µg L^{-1} was measured.

The highest mean **hexachlorobutadiene** concentrations in perch were measured in the Zenne I. In eel this was in the Zuid-Willemsvaart. Since this pollutant was not detected in industrial wastewater or surface water, no sources could be identified (VMM, 2018).

The highest average **mercury** concentration was measured in perch from Zenne I and in eel from Demer VII. Maes et al. (2008) already found the highest concentrations in eel from the Zenne, but in eel from the Demer their concentrations were the lowest. Atmospheric deposition and erosion/runoff of contaminated soil together account for about 95% of emissions to surface water in the Scheldt and Meuse river basin (VMM, 2018).

Brominated diphenyl ether (**∑PBDE**) concentrations in perch were on average the highest in the Upper Scheldt IV. In eel this was the case in the Upper Scheldt I. Since PBDEs are mainly used as flame retardants on textiles, both Upper Scheldt I and IV are under strong influence of the present textile industry (e.g. Tournai) (Roosens et al. 2010).

Hexabromocyclododecane concentrations were on average the highest in both perch and eel from Upper Scheldt I. This may be explained by the use of this pollutant as a

flame retardant and also strong influences from the textile industry (Roosens et al. 2010). However, for certain textile companies that used this pollutant in the past, HBCD is still measured in their wastewater, probably due to lag effects (VMM, 2018).

Benzo(a)pyrene concentrations were highest in the mussels suspended in the Albert Canal. The highest fluoranthene concentration was measured in the Zenne II. It was already known that the sediment of the Zenne contains high background concentrations of PAHs. Furthermore, atmospheric deposition and transport together contribute for more than 90% to the total emissions of PAHs to the surface water of the Scheldt and Meuse river basins (VMM, 2018).

PFOS reached the highest concentrations on average in perch from the Zenne II and in eel from the Melsterbeek. In the Scheldt and Meuse river basins, household and industrial wastewater were found to contribute equally to emissions to surface water (VMM, 2018). PFOS was found in wastewater from various sectors, including food, chemicals, paper, textiles, metals and waste processing.

Dicofol concentrations were nowhere above the LOQ. After its ban in 2009, it was not detected in surface water anywhere in Flanders between 2016 and 2018 by the Flanders Environment Agency (http://geoloket.vmm.be/Geoviews/).

The highest average concentrations for **cis-heptachlor epoxide** in perch were measured in Dender I. In eel, this was in Upper Scheldt II+III. Relevant sources of **heptachlor** and heptachlor epoxide are currently not well understood. Because of the high persistence of these substances, however, it is thought that they may be secondary to (historically) contaminated sediment (VMM, 2018).

Dioxins in perch were on average the highest in the Sea Canal Brussels-Scheldt. In eel the concentrations were on average the highest in the Sea Scheldt IV. This may be due to historical pollution of sediment (erosion/neglect) or atmospheric transport over longer distances (VMM, 2018).

Average ∑**PCB** concentrations in perch were highest in Demer VII and in eel in Zenne II. Both Demer VII and Zenne II are locations with high known historical concentrations

in the sediment. Since PCB concentrations in both industrial wastewater and surface water were not detectable, no potential sources could be identified (VMM, 2018).

In general, the Zenne, Demer VII and several points in the Scheldt appear to be frequent recurring points of high pollution. Frequently recurring sources of pollution are atmospheric deposition (Hg and PAHs) and rearrangement of historical concentrations in the sediment (Hg, cis-heptachlor epoxide, dioxins and ∑PCB). In addition, industry provides an important (local) input of PFOS (various sectors and households), ∑PBDE (textiles) and HBCD (textiles). Overall, points sources (historical or active) might result in large differences between locations that are relatively close to each other. This was for example observed for Hg.

2.5 Conclusion and implications for bioaccumulation monitoring in the future

Mercury and ∑PBDE concentrations exceeded the EQS_{biota} at all monitoring sites and are thus substances that reach problematic high concentrations according to the biota standards. The EQS_{biota} for ∑PBDE is so low that this concentration is well below the LOQ. In addition, for PFOS, dioxins and cis-heptachlor epoxide also potential problem cases were revealed. Exceedances with the largest factor were measured for ∑PBDE and cis-heptachlor epoxide.

Results of the analyses performed within the bioaccumulation monitoring network underline the importance of the species selection used for the analyses. Firstly, it is clear that most of the priority substances accumulate higher concentrations in the fish species with the highest fat content, i.e. eel. For PFOS, however, the opposite was true. Additional account should be taken of fat content and dry residue. Correction on the basis of fat content was found to result in comparable concentrations between the two species for ∑PBDE and ∑PCB or a smaller gap for HCB and HBCD. For PFOS and Hg, concentrations were higher in perch after this correction. Overall, additional research is required to understand the factors and pathways involved in the accumulation of these pollutants.

Although the use of passive samplers already appears to be a good predictor for accumulation of benzo(a)pyrene, HCB (in perch) and ∑PCB (in eel), this relationship was absent for HCBD, ∑PBDE and fluoranthene. The use of passive samplers is promising as a complement to bioaccumulation monitoring. However, further research and elaboration of these complementary techniques is required.

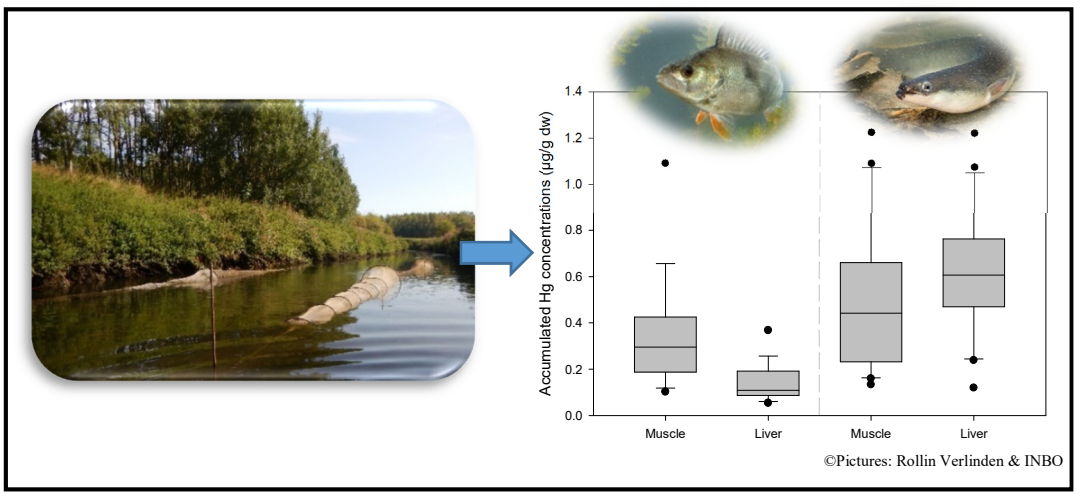
The Zenne, Demer and several points from the Scheldt were often recurring points of high accumulation of the investigated pollutants. For PAHs and Hg, atmospheric deposition is largely responsible for the pollution (over large distances). In addition, rearrangement of historically contaminated sediment plays a major role for the concentrations of Hg, cis-heptachlor epoxide and dioxins in the aquatic environment. Finally, concentrations of PFOS, ∑PBDE, and HBCD are mainly attributed to point pollution from industry (and households for PFOS).

Acknowledgements

First of all, we would like to thank the fishing teams of Linkebeek and Brussels (INBO) for the fish collection, with special thanks to Gerlinde Van Thuyne, Johan Coeck, Isabel Lambeens, Linde Galle, Thomas Terrie, Adinda De Bruyn, and Yves Maes (INBO) for additional support. In addition, we would like to thank Dr. Valentine Mubiana (UA) for mercury analyses. The lab of VMM is thanked for analyses of HCB, HCBD, PBDEs, PAHs, PCBs, and heptachlor(epoxide). Furthermore, our thanks go to Dr. Malarvannan Govindan (UA) for assistance in sample preparation of HBCD analyses. Finally, the members of the steering committee (Maarten De Jonge, Ward De Cooman, Martin Verdievel, Liesbet Poppe and Sofie Cattoir - all VMM) are thanked for the follow-up of the project. This study was made possible by the financial support of the Flanders Environment Agency

Chapter 3

Mercury accumulation in muscle and liver tissue and human health risk assessment of two resident freshwater fish species in Flanders (Belgium): a multilocation approach



Published as:

<u>Lies Teunen</u>, Claude Belpaire, Gudrun De Boeck, Ronny Blust, Lieven Bervoets, 2022. Mercury accumulation in muscle and liver tissue and human health risk assessment of two resident freshwater fish species in Flanders (Belgium): a multilocation approach. ***Environmental Science and Pollution Research 29,*** **7853-7865. https://doi.org/10.1007/s11356-021-16215-0.**

Abstract

Detrimental effects of chemical pollution-primarily caused by human activities-on aquatic ecosystems have increasingly gained attention. Because of its hydrophobic qualities, mercury is prone to easily bioaccumulate and biomagnify through the food chain, decreasing biodiversity and eventually also affecting humans. In the present study, accumulated mercury concentrations were measured in muscle and liver tissue of perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*) collected at 26 sampling locations in Flemish (Belgian) waterbodies, allowing a comparison of these species within a variety of environmental situations. Furthermore, effects of size and weight have been assessed, expected to influence accumulation and storage of pollutants. Mercury concentrations in perch ranged up to 1.7 μg g^{-1} dw (median: 0.29 μg g^{-1} dw) in muscle and from 0.02 to 0.77 μg g^{-1} dw (median: 0.11 μg g^{-1} dw) in liver tissue. For eel, these concentrations were between 0.07 and 1.3 μg g^{-1} dw (median: 0.39 μg g^{-1} dw) and between 0.08 and 1.4 μg g^{-1} dw (median: 0.55 μg g^{-1} dw) respectively. We found a correlation of accumulated mercury with length in perch, independent of location. Furthermore, a significant difference in accumulated mercury concentrations between the targeted species was measured, with the highest mean concentrations per dry weight in eel liver and muscle tissue. In perch, higher concentrations were found in muscle compared to liver tissue, while in eel, liver tissue showed the highest concentrations. These findings were further considered with concentrations corrected for lipid content, excluding the fat compartment, which is known to a hold negligible portion of the total and methyl mercury concentrations. This confirmed our previous conclusions, except for mercury concentrations in eel. Here there was no longer a significant difference between muscle and liver concentrations. Finally, health risk analyses revealed that only frequent consumption of local eel (> 71 g day^{-1}) could pose risks to humans.

Keywords: Biomonitoring; European eel; European perch; Hg; Internal distribution; Pollution.

3.1 Introduction

Mercury (Hg) is a naturally occurring element, but is widely applied on a global scale in industry (i.e. production of car components), gold mining, households (i.e. batteries), and agriculture (i.e. pesticides). These activities cause it to be introduced in aquatic ecosystems through among others erosion and both industrial and domestic discharges (Horowitz et al., 2014; Kidd and Batchelar, 2012; Selin, 2009). The largest portion, however, originates from atmospheric deposition as a result of fuel combustion, causing long-range transport (Horowitz et al., 2014; Pacyna et al., 2010; Pirrone et al., 2010). Elevated Hg concentrations have previously been found in aquatic environments of remote areas (Durnford et al., 2010; Fitzgerald and Mason, 1998). Due to its highly persistent character, Hg remains present in the environment.

Mercury can be present in the aquatic environment in dissolved or particle bound state. For piscivorous or omnivorous fish, dietary intake is the main exposure route (Bradley et al., 2017; Hall et al., 1997; Régine et al., 2006). Methylmercury is readily bioavailable and therefore causes a strong biomagnification through the food chain. Because of its high affinity for sulphur-based amino acids and thiol groups in proteins it will easily pass through the gut and can be transported via blood to different organs (Amlund et al., 2007; Bradley et al., 2017; Ribeiro et al., 1999). The muscle tissue is known to be the major sink for MeHg. The gut, however, shows a poor absorption for Hg (II). Therefore, the highest inorganic Hg concentrations are often found in the intestine (Peng et al., 2016). Since the liver acts as a demethylation and re-distribution organ, this might also be a target tissue for both organic and inorganic mercury (Havelková et al., 2008; Régine et al., 2006). A secondary pathway is uptake of dissolved mercury (mainly in its inorganic Hg (II) form) through the gills. In general, the majority (80–100%) of mercury in fish muscle tissue consists of MeHg (Bloom, 1992; Chvojka et al., 1990; Golzadeh et al., 2020; Kannan et al., 1998; Nguetseng et al., 2015). High concentrations of MeHg are further due to the low elimination rates and higher accumulation efficiency compared to inorganic Hg (II) (Bradley et al., 2017; Peng et al., 2016; Wang and Wang, 2018; Wang and Wong, 2003).

Mercury can act as a potent neurotoxicant, especially in its organic, methylated form (i.e. methylmercury - MeHg), and will interfere both with perceptive systems (i.e. vision, hearing) and movements (i.e. immobility, uncontrollable movements), even at low concentrations in humans (Clarkson, 1992; Karagas et al., 2012). Exposed fish can experience deleterious effects on their growth, development, and reproduction (Beckvar et al., 2005; Scheuhammer et al., 2007). Furthermore, high accumulated mercury concentrations have been found in piscivorous predators due to biomagnification, ultimately leading to mortality. These observations were made, among others, in European otter (*Lutra lutra*), mink (*Mustela vison*), and Eurasian bittern (*Botaurus stellaris*) (Macdonald and Mason, 1994; Newton et al., 1994; Scheuhammer et al., 2007; Wiener et al., 2003; Yates et al., 2005).

Perca fluviatilis (European perch) and *Anguilla anguilla* (European eel) in its "yellow" eel stage are frequently used for monitoring purposes (Batchelar et al., 2013; Belpaire and Goemans, 2007b; de Boer and Brinkman, 1994; Havelková et al., 2008; Ion et al., 1997; Van Ael et al., 2014), despite the IUCN status of eel as "critically endangered" (Pike et al., 2020). These are very common and widespread species in Europe, allowing for a straightforward comparison of accumulated concentrations between different countries (Belpaire and Goemans, 2004; Belpaire and Goemans, 2007a; Bignert et al., 2011). Furthermore, they are resident, creating a reliable image of a relatively restricted area and they are relatively tolerant to pollution (Belpaire et al., 2008; Järv, 2000). Because of their high trophic levels, they may accumulate high concentrations of pollutants, inducing possible toxic effects to their predators (Belpaire and Goemans, 2007b; Wiener et al., 2003).

In general, older individuals, having experienced a longer exposure time, tend to have accumulated higher mercury concentrations (Batchelar et al., 2013; Cizdziel et al., 2002; Durrieu et al., 2005; Gewurtz et al., 2011; Park and Curtis, 1997; Szefer et al., 2003; Weis and Ashley, 2007). Larger fish also tend to eat larger prey, containing higher mercury concentrations. Furthermore, size-related biokinetics might play a role. A reduced growth efficiency in larger individuals, for example, diminishes the effect of somatic growth dilution, resulting in higher concentrations (Dang and Wang, 2012). Finally, it has been

shown that MeHg is eliminated slower in older fish (Lescord et al. 2018). Although the increase of Hg accumulation with size has been researched elaborately, there is a lack of studies regarding a multiple population approach.

Within the present study, we selected a broad variation of locations with different environmental backgrounds, in order to study general trends of Hg accumulation in multiple populations throughout Flanders (Belgium), instead of investigating a specific local study situation. Internal distribution of mercury over liver and muscle tissue and comparison between two important freshwater monitoring species, *Perca fluviatilis* and *Anguilla anguilla* was the main focus of this study. The impact of species, fish length, weight, and sample site (background) on internal Hg levels was assessed, using generalized linear mixed models. Furthermore, analyses were repeated with a correction of the mercury concentration, excluding seasonality in lipid content. Finally, a human health risk assessment was performed based on consumption of muscle tissue of both perch and eel.

In general, we hypothesize that (1) higher mercury concentrations are to be expected in fish with higher weight and size, independent of location; (2) eel accumulates higher mercury concentrations, as it is a bottom dwelling species exposed to mercury contamination in sediment particles; (3) higher accumulated total Hg concentrations are to be found in muscle compared to liver tissue.

3.2 Material and methods

3.2.1 Study area and sample selection

A total of 26 sampling locations were selected in Flanders (Belgium). Sites were selected in order to fulfil the reporting requirements for the Water Framework Directive as coordinated by the Flanders Environmental Agency. Although some locations were situated in the same water body, we interpreted them as independent because of local point sources or different surroundings (i.e. industry and agriculture). Typology of sampling sites included canals, rivers and streams, brooks and polder water courses. Perch and eel where caught by the Institute for Nature and Forest Research between 2013 and 2016 and

euthanized with MS-222 (Acros Organics, Geel, Belgium). Eels were collected in their juvenile, yellow eel stage and a length class of 45–55 cm was targeted, while for perch the largest sizes were targeted. Fish were sampled using electrofishing and/or fykes, depending on the depth and characteristics of the water body. However, we did not manage to collect both species at all sites. Sampling locations are indicated in *Figure 3.1* and *Table 3.1*, as well as the total number of fish collected per site.

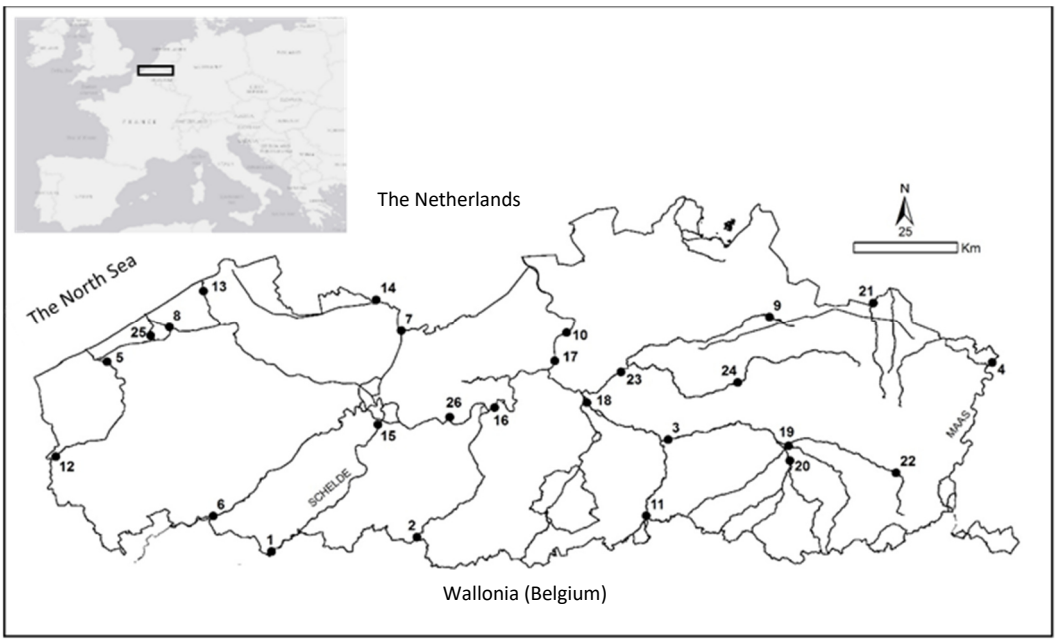


Figure 3.1: Sampling locations in Flanders (Belgium).

3.2.2 Fish sampling

A total of 300 perch and 100 eel were caught and individually analysed (*Table 3.1*). Before dissection, 3 different length (± 0.1 cm) measures and the weight (± 0.1 g) of individual fish were determined. Length measures included total length, fork length (tip of the snout to posterior end of the middle caudal rays) and standard length (tip of the snout to the midlateral posterior edge of the hypural plate). For eel, however, only total length was recorded. Fish were dissected, muscle (N = 397) and liver tissue (N = 308) isolated, weighed (Mettler AT261 DeltaRange, Mettler-Toledo) and frozen at -20°C until further processing. Muscle samples were taken in the dorsal part, behind the dorsal fin for perch

and in the dorsal part, opposite to the anus for eel. Aliquots for all muscle and liver samples were taken to perform total mercury and lipid analyses.

Table 3.1: European perch (N = 300) and European yellow eel (N = 100) were collected in 26 sampling locations between 2013 and 2016. This study was carried out in Flanders (Belgium).

No.	Sampling Site	Water body	Lambert X coordinate	Lambert Y coordinate	Sampling year	*N*	
						Eel	*Perch*
1	Pecq	Boven-Schelde I	79181	157135	2015	3	20
2	Geraardsbergen	Dender I	114132	160631	2015	3	20
3	Werchter	Demer VII	174581	184472	2015	3	9
4	Kinrooi	Maas I+II+III	252525	203301	2015	4	21
5	Nieuwpoort	IJzer III	39617	203488	2015	3	19
6	Wevelgem	Leie I	65139	165773	2015	3	14
7	Zelzate	Kanaal Gent-Terneuzen	110399	211142	2015	0	20
8	Oostende	Kanaal Gent-Oostende III	54608	212041	2015	3	20
9	Retie	Kleine Nete I	198974	214563	2015	3	17
10	Antwerpen	Zeeschelde IV	150151	210616	2015	11	0
11	Sint-Joris-Weert	Dijle I	169300	165850	2015	3	0
12	Poperinge	IJzer I	27250	180320	2016	1	20
13	Blankenberge	Blankenbergse vaart	62799	220991	2016	3	6
14	Oostburg	Leopoldkanaal I	104330	218850	2016	3	20
15	Gent	Boven-Schelde IV	104745	188127	2016	3	18
16	Dendermonde	Zeeschelde II	132788	192322	2016	4	3
17	Hemiksem	Zeeschelde III + Rupel	147328	203675	2016	3	0
18	Mechelen	Getijdedijle-Getijdezenne	155010	193500	2016	3	4
19	Herk-de-Stad	Herk + Kleine Herk	203500	182930	2016	2	0
20	Herk-de-Stad	Melsterbeek I+II	203850	179330	2016	2	0
21	Neerpelt	Dommel	223950	218080	2016	2	15
22	Bilzen	Demer I	229423	176366	2016	1	4
23	Lier	Polder van Lier	163177	201016	2015	5	25
24	Westerlo	Laakdal	191289	198420	2015	4	25
25	Camerlinckxgeleed	Camerlinckxgeleed	50720	209853	2015	5	0
26	Bergenmeersen	Boven-Schelde	122024	189948	2013	20	0

N: sample size.

3.2.3 Mercury analysis

In the present study, total Hg (tHg) was measured and used as a proxy for MeHg, being the predominant form in fish muscle (80-100%) (Golzadeh et al., 2020; Nguetseng et al., 2015). Samples were freeze-dried (Heto PowerDry LL 3000, Thermo Scientific) and dry/wet weight ratios were determined before digestion in a 1:3 (v/v) mixture of HNO_3 (69%) and HCl (37%) (Aqua Regia). Digestion was conducted in a pressurized microwave

digestion system, Discover SP-D (CEM Corporation, Matthews, NC 28106, USA). Analysis was performed using a high-resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS; Element XR, Thermo Scientific, Bremen, Germany). Procedural blanks were incorporated. Reference material used was freeze-dried mussel tissue (NIST-2976; National Institute of Standards and Technology, USA; certified concentration: 61.0 ± 3.6 μg kg^{-1} dw). Recoveries ranged from 70 to 136%. Concentrations in batches with recoveries below 90% or above 110% were corrected for this error by dividing the measured concentration by the proportion of the recovery. The method quantification limit for mercury was 0.005 μg L^{-1}. Mercury concentrations below LOQ (0.01 μg g^{-1} dw) were set at ½ of the LOQ (Bervoets et al., 2004; Custer et al., 2000). Overall, concentrations below LOQ were only detected in muscle tissue of perch (2% of samples).

Most studies report accumulated mercury concentrations on a wet weight basis. There is, however, a large variation in the dry weight/wet weight ratio for European eel (0.22 to 0.74 in the present study), leading to more variable datasets and interpretations, based on the water content in and on the fish tissue. Therefore, we suggest a more robust method using dry weight based mercury concentrations.

3.2.4 Lipid determination and lipid based correction

Total lipid percentage was determined in muscle (N = 230) and liver (N = 91) tissue of perch and in muscle (N = 63) and liver (N = 62) tissue of eel. Lipid extraction from lyophilized samples was based on the Bligh and Dyer method (Bligh and Dyer, 1959). A chloroform/methanol/water 5:5:2 (v/v/v) mixture was used. After centrifugation, lipids were isolated in the chloroform fraction. Addition of sulphuric acid (95%) induced a colour change when samples were heated to 200°C, for 15 min. The optical density was measured using a spectrophotometer (ELX 808 IU Ultra Microplate Reader, Bio-Tek Instruments Inc.) at 405 nm. Samples were analysed in duplicate. Calculations were performed using a calibration curve with a stock solution of glycerol tripalmitate (98%) dissolved in chloroform (99%).

Seasonal variation in lipid content, due to for example food availability and reproduction, may have a strong effect on measured Hg concentrations in different fish tissues and species. A higher lipid content will have an effect on the total weight of the tissue or individual and therefor affect the Hg concentration per weight unit. However, mercury is known to almost exclusively accumulate in proteins (Amlund et al., 2007). Therefore, we chose to correct for lipid percentage, excluding this portion from the total tissue weight, based on the approach of Kahilainen et al. (2016). This correction was performed using the following formula:

$$tHg_{corr} = Hg_{tot} / (1 - lipid_{prop}) \quad (\mathbf{3.1})$$

where tHg_{corr} is the lipid-corrected total mercury concentration (μg g^{-1} dw), Hg_{tot} is the measured total mercury concentration (μg g^{-1} dw), and $lipid_{prop}$ is the lipid content proportional to the tissue weight.

3.2.5 Human health risk assessment

Muscle tissue of fish, being the commonly eaten part, is often considered in human health risk analysis. Several international organizations have estimated safe methylmercury concentrations on a wet weight basis and defined them as the Minimum Risk Level (MRL; ATSDR (Agency for Toxic Substances and Disease Registry), 2018), the US EPA Reference Dose (RfD; UNEP, 2008) and the Provisional Tolerable Weekly Intake (PTWI; FAO/WHO, 2010), respectively 0.3 μg kg^{-1} body weight day^{-1}, 0.1 μg kg^{-1} body weight day^{-1} and 1.6 μg kg^{-1} body weight $week^{-1}$. The maximum tolerable amount of both fish to be eaten per day, taking into account all of the above reference values, without potential human health risk was calculated for an average adult of 70 kg. The previous values, although set for methylmercury, can be used for total mercury as well. As mentioned before, in fish over 90% of total mercury is assumed to be in its methylated form (Bloom 1992; Chvojka et al. 1990; Kannan et al. 1998). All of these calculations were performed on both species for each location separately (*Appendix B: Table B.8*) and on the entire dataset (*Table 3.2*).

Another widely used tool to determine human health risks is calculation of the Hazard Quotient (HQ; USEPA, 1989). The HQ is usually defined as the ratio of the estimated daily intake (EDI), in relation to the tolerable daily intake (TDI) of a pollutant. If the HQ exceeds one, this suggests potential health effects. In order to determine the EDI, median accumulated mercury concentration per location were multiplied by the known median consumption of the local adult population. In Flanders, mainly recreational fishermen are exposed to contaminated fish through consumption of their catch. Therefore, results based on an interview of fishermen were included, with a mean consumption of 2.7 g of perch day^{-1} and 18 g of eel day^{-1} (ANB-VF/2015/4 2016). As for TDI, converted concentrations for an average adult of 70 kg were used for MRL, RfD and PTWI. Bilau et al. (2007) performed a risk analysis for the worst-case scenario, based on the amount of eel that was taken home by recreational fishermen. It was calculated that people always taking home the eel caught, would consume an average of 71.14 g day^{-1} if they ate everything themselves. This worst-case scenario was also included in the present analysis (*Appendix B: Table B.9*).

For all calculations concerning human health, concentrations were recalculated on a wet weight base (μg g^{-1} ww) using the dry/wet weight ratio measured in both species (*Appendix B: Tables B.4 and B.5*).

3.2.6 Statistical analysis

Statistical analyses were performed using the software package R (R version 3.3.2; R Core Team 2016). A Spearman correlation test was conducted between weight and length measures, between dry and wet weight based concentrations and between concentrations in liver and muscle tissue. To investigate different accumulated mercury concentrations in respectively species, tissues (muscle/liver) and age indicators (length/weight) linear mixed models were composed for each of these variables with location as random intercept. For these analyses, an F-test with Kenward-Roger Degrees of Freedom Approximation was used to determine the significance of the variables, comparing the full model (including one explanatory variable) to the reduced model (without explanatory variable). Furthermore, these analyses were repeated with Hg concentrations corrected for

the lipid content. Finally, a prediction equation to extrapolate Hg concentrations between both fish species, was created using a linear regression model. Significant outliers were removed using the Grubb test in Graphpad. Overall significance levels were considered at a *p*-value < 0.05.

3.3 Results

3.3.1 Total Hg accumulation and internal distribution

Individual mercury concentrations in perch were found to range from <0.01 to 1.7 µg g^{-1} dw (median: 0.29 µg g^{-1} dw; <0.001-0.35 µg g^{-1} ww) and from 0.02 to 0.77 µg g^{-1} dw (median: 0.11 µg g^{-1} dw; 0.003-0.19 µg g^{-1} ww), respectively in muscle and liver tissue (*Appendix B: Tables B.6 and B.8*). For eel, the Hg concentrations in muscle tissue were between 0.07 and 1.3 µg g^{-1} dw (median: 0.39 µg g^{-1} dw; 0.03-0.43 µg g^{-1} ww), while there was a concentration range from 0.08 to 1.4 µg g^{-1} dw (median: 0.55 µg g^{-1} dw; 0.02-0.29 µg g^{-1} ww) measured in liver tissue (*Appendix B: Tables B.6 and B.8*). A significant difference in accumulated concentrations was found between both species in muscle tissue ($F = 20.26$; $p < 0.001$), as well as in liver tissue ($F = 336.54$; $p < 0.001$), with the highest concentrations in eel for both tissues (*Figure 3.2*). For perch, a significant difference between liver and muscle accumulated concentrations was found ($F = 127.25$; $p < 0.001$). Accumulated mercury concentrations in muscle were higher than those in liver. Also for eel, a significant difference in mercury concentrations between tissues was found ($F = 6.30$; $p < 0.05$), however this time with the highest concentrations in the liver tissue (*Figure 3.2*). In all of the above analyses, the effect of location was taken into account, using linear mixed models. A correlation of Hg concentrations in liver and muscle tissue was detected for both species ($r \geq 0.56$; $p < 0.001$) (*Appendix B: Tables B.2 and B.3*).

The median accumulated Hg concentrations per location in eel and perch in both liver and muscle tissue were compared in order to identify extrapolation possibilities between the species. A significant correlation was found for wet weight concentrations in muscle tissue ($r = 0.66$; $p < 0.01$). Linear regression resulted in the following equation ($R^2 = 0.44$):

$$[Hg_{perch\ (ww)}] = 0.36 * [Hg_{eel\ (ww)}] + 0.01 \qquad (\mathbf{3.2})$$

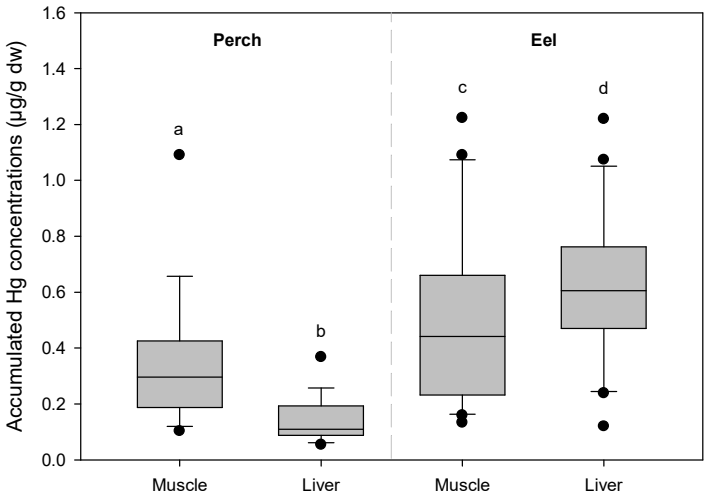


Figure 3.2: Boxplots accumulated mercury concentration in perch and eel, muscle and liver tissue. Median concentrations per location were used. Different letters stand for a significant difference ($p < 0.05$*).*

3.3.2 Effects of fish size

All length measures were highly correlated ($r \geq 0.99$; $p < 0.001$), as well as dry weight and wet weight based concentrations ($r \geq 0.92$; $p < 0.001$) (*Appendix B: Table B.1*). For all analyses hereafter, total length (TL) was used as length measure. Fish weight (W) showed an exponential increase with increasing length for both eel ($W = 9.4205e^{0.0062TL}$; $R^2 = 0.94$) and perch ($W = 0.8374e^{0.0252TL}$; $R^2 = 0.96$).

Mercury accumulation increased with increasing length for perch in both muscle ($F = 184.61$; $p < 0.001$) and liver tissue ($F = 72.44$; $p < 0.001$) (*Figure 3.3; Appendix B: Table B.10*). For eel, no significant effect of length could be found for Hg accumulation in muscle tissue ($F = 3.67$; $p = 0.06$) or liver tissue ($F = 0.30$; $p = 0.59$) (*Figure 3.3; Appendix B: Table B.10*). For these analyses as well, influences of locations were included (mixed model). Furthermore, fat content in muscle tissue of eel increased significantly with both length ($F = 13.24$; $p < 0.001$) and weight ($F = 19.10$; $p < 0.001$). Increasing length ($F = 32.71$; $p < 0.001$) and weight ($F = 16.99$; $p < 0.001$) of perch, however, resulted in a slight but significantly decreasing fat content. Liver fat content in perch was affected by neither length ($F = 0.66$: $p = 0.42$) nor weight ($F = 1.88$; $p = 0.17$). The total lipid content in liver

of eel significantly increased with increasing length (F = 4.55; $p < 0.05$) and weight (F = 13.90; $p < 0.001$).

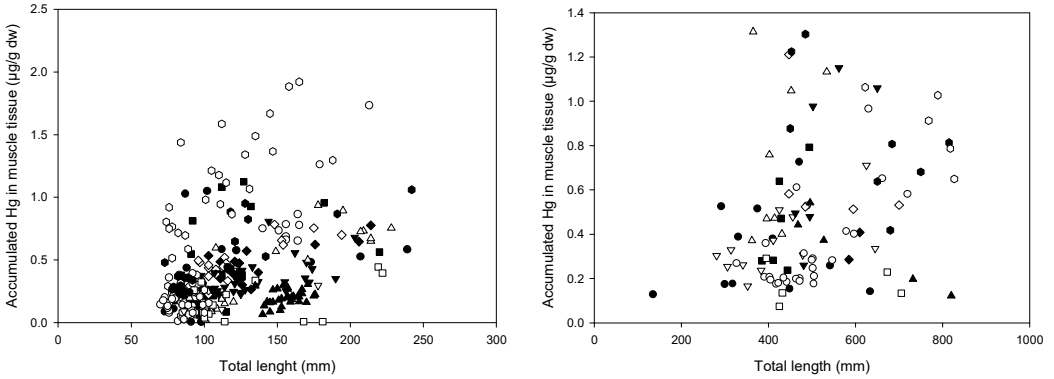


Figure 3.3: Regression between total length of the individual and the accumulated mercury concentration in muscle for perch (LEFT; F = 184.61, p *< 0.001) and for eel (RIGHT; F = 3.67,* p *= 0.06). Every symbol refers to a different location.*

3.3.4 Correction for lipid content

Total lipid content in perch ranged from 0.50 to 2.5 % (median: 0.92%) in muscle tissue and from 1.6 to 4.2 % (median: 2.4%) in liver tissue (*Appendix B: Tables B.4 and B.5*). In eel, lipid percentages ranged from 1.6 to 28 % (median: 7.6%) in muscle tissue and from 1.8 to 10 % (median: 2.7%) in liver tissue (*Appendix B: Tables B.4 and B.5*). The results demonstrated that muscle tissue of eel contained a significant higher lipid concentration than muscle tissue of perch (F = 356.8; $p < 0.001$; *Appendix B: Tables B.4 and B.5*). The same was true for liver tissue (F = 7.3; $p < 0.01$).

Additionally, mercury concentrations were corrected for lipid content (µg g^{-1} dw), excluding the lipid proportion from the total tissue weight. This caused concentrations to lay between <0.01 and 1.3 µg g^{-1} dw (median: 0.26 µg g^{-1} dw) in muscle tissue of perch. For liver tissue in this species, concentrations were calculated to be between 0.02 and 0.79 µg g^{-1} dw (median: 0.17 µg g^{-1} dw). For eel concentrations were between 0.09 and 1.4 µg g^{-1} dw (median: 0.48 µg g^{-1} dw) and between 0.08 and 1.4 µg g^{-1} dw (median: 0.56 µg g^{-1} dw) for muscle tissue and liver tissue respectively.

Concentrations between both species still differed significantly for both muscle (F = 53.30; $p < 0.001$) and liver tissue (F = 132.64; $p < 0.001$). The highest concentrations were

still measured in eel, both for muscle and liver tissue (*Appendix B: Table B.7*). The difference in accumulated concentrations between muscle and liver was only significant for perch. In perch the highest concentrations were measured in muscle tissue ($F = 44.40$; $p < 0.001$), whereas in eel there was no significant difference between liver and muscle concentrations ($F = 2.28$; $p = 0.13$) (*Appendix B: Table B.7*).

Finally, after correction for lipid content a significant increase of accumulated mercury with size could still be detected in muscle ($F = 114.71$; $p < 0.001$) and liver tissue ($F = 13.14$; $p < 0.001$) of perch. For both muscle ($F = 0.86$; $p = 0.41$) and liver tissue ($F = 0.89$, $p = 0.35$) in eel this effect was once again absent.

3.3.5 Human health risk assessment

Mercury concentrations (µg g^{-1} ww) in the muscle tissue were used to perform a human health risk analysis. None of the sampled fish showed an exceedance of the WHO guideline for human consumption of mercury, namely 0.5 µg g^{-1} ww for perch and 1 µg g^{-1} ww for eel (EC, 2006; EC, 2008a). *Table 3.2* contains the maximum amount of fish (g) to be eaten per day, for an adult weighing 70 kg, without posing health risks (MADC). This value was determined on the pooled dataset of all sample locations, using both median and 95% percentile values of perch and eel. The MRL, RfD and PTWI concentrations were interpreted. Clearly, the recommended amount of eel is considerably lower than that of perch. Furthermore, the hazard quotient (HQ) was determined, based on the annual consumption of caught fish by fishermen. Maximum tolerated daily intake of fish (TDI) was far above the estimated daily intake dose (EDI), resulting in an $HQ < 1$ for perch. For eel on the other hand, MADC's were very low. A low EDI, however, still resulted in $HQ < 1$. The highest concentration measured in eel (0.43 µg g^{-1} ww in 'Kanaal Gent-Oostende III') gave an HQ of 1.12 for RfD, possibly posing a health risk through consumption. For the results of the above for the mean mercury concentrations on each location, we refer to *Appendix B: Table B.8*.

Table 3.2: Determination of human health risk through consumption of contaminated fish in Flanders. Maximum amount (g) of contaminated fish muscle a 70 kg person can consume per day without posing health risks (MADC) were calculated for the median and 95th percentile of the observed mercury concentrations in fish muscle tissue in Flemish water bodies, based on MRL (ATSDR, 2018), RfD (UNEP, 2008) and PTWI (FAO/WHO, 2010). The Hazard Quotient (HQ) was determined by dividing the estimated daily intake (EDI) for perch (2.7 g day^{-1}) and eel (18 g day^{-1}) with the MADC.

	Hg concentration in muscle tissue (µg g^{-1} ww)		**MADC (g/day/70 kg adult) and HQ**					
			MRL		RfD		PTWI	
	50th	**95th**	**50th**	**95th**	**50th**	**95th**	**50th**	**95th**
Perca fluviatilis	0.06	0.21	357 (0.01)	100 (0.03)	119 (0.02)	33 (0.08)	274 (0.01)	76 (0.04)
Anguilla anguilla	0.11	0.31	185 (0.10)	68 (0.27)	62 (0.30)	23 (0.81)	142 (0.13)	52 (0.35)

In the worst case scenario for eel in Flanders the average amount consumed was 71.14 g day^{-1} (Bilau et al., 2007). This is almost 4 times higher than the average estimated daily intake dose (EDI) for eel. Therefore, with this consumption rate, this would result in a HQ > 1 for RfD on the median Hg concentration in eel of this study. For the 95th percentile concentrations this leads to HQ values of 1.05, 3.1 and 1.4 respectively for MRL, RfD and PTWI, by dividing 71.14 g day^{-1} by the MADC. Consequently, this would mean that for MRL, RfD and PTWI values respectively 8%, 68% and 24% of the locations on average resulted in a HQ > 1, posing a possible threat to human health (*Appendix B: Table B.9*).

Additionally, the European Biota Quality Standard (EQS_{biota}), a threshold concentration for protection of the integrity of aquatic ecosystems under the Water Framework Directive, namely 0.02 µg g^{-1} ww for Hg (EU, 2013), was exceeded in every sampled location, indicating potential health risks to the food web, mainly on top predators.

3.4 Discussion

3.4.1 Total Hg accumulation and internal distribution

In the present study, measured concentrations in muscle tissue ranged from <0.01 to 1.7 µg g^{-1} dw (<0.001-0.35 µg g^{-1} ww) in perch and from 0.07 to 1.3 µg g^{-1} dw (0.03-0.43 µg g^{-1} ww) in eel. These results fall within ranges reported in other studies on Flemish waterbodies. Bervoets et al. (*subm.*) measured Hg concentrations between 0.58 and 1.1 µg g^{-1} dw in perch and between 0.28 and 0.94 µg g^{-1} dw in eel from the Winterbeek (Flanders, Belgium). Furthermore, a preliminary monitoring study published results ranging from 0.04 to 0.93 µg g^{-1} ww in perch and from 0.05 to 0.32 µg g^{-1} ww in eel from 16 different water bodies in Flanders (De Jonge et al., 2014). Other studies, based on data from the Eel Pollution Network of Flanders, reported concentrations between 0.005 and 1.2 µg g^{-1} ww in eel muscle tissue (Belpaire and Goemans, 2007b; Maes et al., 2005; Maes et al., 2008). Comparable results were found in other European studies, with concentrations in muscle tissue ranging from 0.03 to 1.4 µg g^{-1} ww for perch (Dusek et al., 2005; Foekema et al., 2016; Jirsa et al., 2014; Łuczyńska et al., 2016; Noël et al., 2013; Petkovšek et al., 2012; Svobodová et al., 1999; Szefer et al., 2003) and from 0.001 to 0.79 µg g^{-1} ww for eel (Downs et al., 1999; Edwards et al., 1999; Eira et al., 2009; Genç and Yilmaz, 2017; Has-Schön et al., 2006; Has-Schön et al., 2008; Noël et al., 2013). A very elaborate Canadian study reported median Hg concentrations of 0.32 and 0.14 µg g^{-1} ww in American eel (*Anguilla rostrata*) and yellow perch (*Perca flavescens*) respectively (Depew et al., 2013).

Furthermore, in the present study, liver concentrations were found to be between 0.02 and 0.77 µg g^{-1} dw (0.003 - 0.19 µg g^{-1} ww) for perch and between 0.08 and 1.38 µg g^{-1} dw (0.02-0.29 µg g^{-1} ww) for eel. European studies on freshwater systems reported liver concentrations between 0.03 and 1.03 µg g^{-1} ww in perch (Jirsa et al., 2014; Petkovšek et al., 2012; Svobodová et al., 1999) and between 0.007 and 2.23 µg g^{-1} ww in eel (Downs et al., 1999; Eira et al., 2009; Genç and Yilmaz, 2017; Has-Schön et al., 2006; Has-Schön et al., 2008).

Overall, in the present study, higher median Hg concentrations were accumulated in eel compared to perch. This is in line with observations from other Flemish studies (Teunen et al., 2020b: *Chapter 2*; Bervoets et al. *subm.*). A considerable amount of the ingested mercury is diet or particle bound, available in its organic form and will be transported to the muscle tissue (Bradley et al., 2017; Hall et al., 1997; Régine et al., 2006). Nonetheless, it is noteworthy that in the present study most perch were rather small, 59% was smaller than 12 cm. This might have contributed to the lower mercury concentrations in perch. Since perches reach adulthood at a mean length of around 11 cm (fishbase.se), most of the sampled fish are considered juveniles. Besides a shorter exposure time, their diet consist mostly of zooplankton, in contrast to macro-invertebrates and small fish for adult perch (Lappalainen et al., 2001). Smaller fish have been shown to contain a lower relative MeHg percentage due to somatic growth dilution caused by a higher growth rate of these smaller fish, a more readily absorption of the aqueous (dissolved) Hg (II) and a faster excretion of MeHg (Lescord et al., 2018). Therefore, we should take into account that the proportional contribution of Hg (II) to the total Hg concentrations might be higher in small than in larger individuals. Since the total Hg concentration in muscle tissue is largely still comprised of MeHg, we consider this a good proxy for MeHg. In liver tissue, however, inorganic mercury might have a larger contribution to total mercury concentrations, due to demethylation processes. On the other hand, higher total mercury concentrations in eel compared to perch might be due to the specific bottom-dwelling habitat of this species and therefore bioaccumulation of sediment-bound mercury (Edwards et al., 1999). Bacteria that live in the sediment are known to take part in the methylation process, creating a more direct exposure of this bioavailable compound (Macalady et al., 2000).

In perch, higher concentrations were found in the muscle tissue compared to liver tissue, even after correcting for lipid content. This is in line with existing literature on perch (Jirsa et al., 2014; Svobodová et al., 1999; Voigt, 2007). These results confirm the high affinity of (methyl)mercury for muscle tissue. In eel, however, higher accumulated concentrations were detected in the liver compared to muscle tissue. This is in line with the results found by Genç and Yilmaz (2017). Eira et al. (2009) and Has-Schön et al. (2006, 2008), on the other hand, found higher accumulated Hg concentrations in muscle

than in liver tissue of eel. After correction for lipid content in the present study, however, there was no longer a significant difference between both tissues for eel. The accumulation and (acute) detoxification role of the liver might result into high concentrations stored in liver tissue (Havelková et al., 2008; Kružíková et al., 2013; Scheuhammer et al., 2007). Higher muscle concentrations, on the other hand, reflect that deposited mercury is slowly evacuated over a long-term period, due to overall lower accumulated mercury concentrations in lightly- or non-contaminated sites. This effect might explain individual cases with higher concentrations accumulated in liver tissue than in muscle tissue (*Appendix B: Tables B.4 and B.5*). However, the influence of other factors needs to be taken into account (e.g. environmental factors, diet, and bioavailability). In the present study, using linear mixed models, site-related effects were incorporated.

The larger variation in eel concentrations could be explained by the variable fat content of these fish as well. Monitoring studies in Flanders reported fat percentages between 1.3 and 32% in eel, compared to between 0.18 and 1.02% in perch (Teunen et al., 2017, 2018). Lower lipid concentrations might indicate lower food availability and quality. Because of the high biomagnification potential of (Me)Hg, larger and more numerous prey might lead to higher accumulated concentrations (Dang and Wang, 2012). Furthermore, difference in internal distribution between both species might point out a different inter-organ transport and toxicological response. Processes of internal distribution and biokinetics (elimination and assimilation) can be species-dependent (Peng et al., 2016; Ribeiro et al., 1999), resulting in different concentrations and distribution patterns. The results in the present study underline the importance of taking into account the large variation caused by environmental and species-specific life history traits.

Finally, we found a possible extrapolation of wet weight muscle concentrations in perch to wet weight muscle concentrations of eel (*Eq. 3.2*). This implies that, for monitoring purposes, it might no longer be needed to catch both species. Instead, analysis of one species can be used to predict concentrations in the other. This relationship is even strengthened through the resident nature of these species. However, also other criteria determine to a large extent the suitability of a species for monitoring purposes

(occurrence, distribution, habitat choices, density, catchability, size) (Belpaire and Goemans, 2007a).

3.4.2 Effects of length

For perch, an increase in accumulated Hg concentrations, in both muscle and liver tissue, was found with increasing length, independent of the background. This effect was still present after correction on lipid content. An increased accumulation as effect of age (i.e. weight or length) is often detected in perch (Driscoll et al., 1995; Järv et al., 2013; Łuczyńska, 2005; Łuczyńska et al., 2016; Svobodová et al., 1999; Szefer et al., 2003; Voigt, 2007). This confirms the hypothesis of bioaccumulation with older individuals, who were exposed for a longer period of time, showing higher concentrations of mercury. Furthermore, larger individuals usually occupy a higher trophic level, being exposed to higher concentrations through diet, because of the biomagnification effect of mercury (Olsson et al., 2000).

Seasonal variation in accumulated concentrations has been reported, caused by differences in food availability, summer growth (diminishing Hg concentrations), reproduction (lipid-rich egg production) and changes in trophic position (Braaten et al., 2014). Nevertheless, there was no significant effect of length or weight on accumulated Hg in muscle or liver tissue of eel, even after correction for lipid content. This is according to the results for muscle tissue found by Noël et al. (2013) and Genç and Yilmaz (2017). However, other studies did report a positive correlation of accumulated mercury with length and weight parameters in muscle tissue of eel (Downs et al., 1999; Edwards et al., 1999). It is therefore important to note that we selected on a specific life stage (i.e. yellow eel) and targeted a limited size range. Finally, the relation between Hg accumulation and length showed to be location (background) dependent.

3.4.3 Human health risk assessment

Although the Hg concentration in muscle tissue of both perch and eel did not exceed the WHO guideline for human consumption, the maximum recommended consumption (g) per day is rather low, especially for eel (often less than 100 g of eel per day). Nonetheless,

due to a very low consumption rate, even for fishermen, the HQ was lower than one for both species at all locations. Polak-Juszczak and Nermer (2016) likewise reported that the largest eels might pose a health risk for Hg.

Including the worst case scenario for eel, however, it became clear that these few fishermen consuming a higher amount of eel have a high chance of experiencing detrimental effects of accumulated mercury concentration. Due to the variation of MeHg contribution to tHg, however, using total Hg as a proxy for MeHg, might lead to an overestimation of the risks. It was reported that large fish consumers in Flanders showed higher Hg levels in hair and blood (Croes et al., 2014). Moreover, based on high PCB (Polychlorinated biphenyl) concentrations measured in fish from Flemish water bodies, the recommendation already exists not to consume fish caught in Belgium (Maes et al., 2008).

In every sample location an exceedance of the EQS_{biota} was observed for both fish species. A widespread exceedance of this standard was already found in monitoring reports of perch and eel in the Flemish water bodies (Teunen et al., 2017, 2018; De Jonge et al., 2014). On a global scale, this is a reoccurring finding as well, even on isolated locations (Durrieu et al., 2005; Guhl et al., 2014; Mataba et al., 2016; Noël et al., 2013; Van Ael et al., 2014; Verhaert et al., 2019; Wyn et al., 2010). It should however be noted that this is a highly conservative threshold, which is well be below calculated critical body residues for risk of MeHg toxicity (Depew et al., 2012; Fuchsman et al., 2016). There have been indications that the current EQS_{biota} might be too strict and not ecologically relevant (Teunen et al., 2018, 2017). Van Ael et al. (2014) demonstrated that, even with high accumulated Hg concentrations in eel, a good ecological quality could still be observed in aquatic ecosystems.

3.5 Conclusion and implications for management purposes

The results observed in the present study confirmed the suitability of both European 'yellow' eel and European perch as monitoring species, as they accumulated high concentrations of mercury. Furthermore, these species are rather easy to collect and provide sufficient tissue to perform the analyses needed. However, it is important to note that a large portion of the perch collected where very small, potentially skewing the results.

A significant difference in accumulated mercury concentrations between targeted species was found, with the highest concentrations in eel. In perch, higher concentrations were found in muscle compared to liver tissue. For eel, the opposite was found. The correlation of accumulated mercury with length in perch confirmed the fact that mercury concentrations increase with fish size.

Based on the findings, some implications for management purposes can be made. First, for perch, muscle tissue seems to be the most relevant tissue to evaluate both the environmental exposure risk and the risk for human health through consumption of fish contaminated with mercury. For eel, on the other hand, higher concentrations were found in liver tissue. The difference between both tissues, however, is small and even disappeared when correcting for lipid content. In order to be able to compare both species and include human health risk, it is more relevant to continue monitoring in muscle tissue. Accumulated concentrations, although relatively high, did not pose any direct threat to human health through average fish consumption. However, it is not recommended to consume over 100 g a day of fish caught in Flemish water bodies based on accumulated Hg concentrations.

The fact that mercury accumulation increases with size, should be taken into account during selection of appropriate monitoring species and sizes of the individuals (i.e. biota-monitoring). Therefore, it is important to select fish of a certain length or weight range. Alternatively, a correction for size can be implemented.

We would like to stress the importance of using linear mixed models when including fish from different sampling sites in order to more correctly take into account site-specific environmental effects of fish inhabiting a specific location.

Acknowledgements

This study was partly funded by the Flanders Environment Agency. We wish to thank dr. Valentine Kayawe Mubiana and Steven Joosen (University of Antwerp) for their help with the metal analysis. Furthermore the fishing crews of the INBO are thanked. Finally, we thank Jasper Foets and Edith Swerts for part of the data collection.

Chapter 4

PFAS accumulation in indigenous and translocated aquatic organisms from Belgium, with translation to human and ecological health risk

Published as:

Lies Teunen, Lieven Bervoets, Claude Belpaire, Maarten De Jonge, Thimo Groffen, 2021. PFAS accumulation in indigenous and translocated aquatic organisms from Belgium, with translation to human and ecological health risk. *Environmental Sciences Europe 33*: 39. https://doi.org/10.1186/s12302-021-00477-z.

Abstract

Background

Despite specific restrictions on their production and use, per- and polyfluoroalkyl substances (PFAS) are still omnipresent in the environment, including aquatic ecosystems. Most biomonitoring studies have investigated the PFAS concentrations in indigenous organisms, whereas active biomonitoring has only been used sporadically. In the present study, accumulated PFAS concentrations were measured in indigenous fish, European perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*) and in translocated freshwater mussels (*Dreissena bugensis* and *Corbicula fluminea*) at 44 sampling locations within the main water basins of Flanders, the northern part of Belgium. Finally, both human health risk and ecological risk were assessed based on accumulated concentrations in fish muscle.

Results

Among locations, ΣPFAS concentrations ranged from 8.56 – 157 ng g^{-1} ww (median: 22.4 ng g^{-1} ww) in mussels, 5.22 – 67.8 ng g^{-1} ww (median: 20.8 ng g^{-1} ww) in perch, and 5.73 – 68.8 ng g^{-1} ww (median: 22.1 ng g^{-1} ww) in eel. Concentrations of PFOA and PFTeDA were higher in mussels compared to fish, whereas for PFDA and PFUnDA the opposite was true. A comparison of concentrations on a wet weight basis between both fish species showed significantly higher PFDoDA, PFTrDA, PFTeDA and PFOA concentrations in eel compared to perch and significantly higher concentrations of PFDA and PFOS in perch. In mussels, PFAS profiles were dominated by PFOA and showed a higher relative contribution of short-chained PFAS, while PFAS profiles in fish were dominated by PFOS. Furthermore, all mussel species clearly occupied a lower trophic level than both fish species, based on a stable isotope analysis.

Conclusions

Biomagnification of PFDA, PFUnDA and PFOS and biodilution of PFOA and PFTeDA were observed. Translocated mussels have been proven suitable to determine which PFAS are present in indigenous fish, since similar PFAS profiles were measured in all biota. Finally, mean PFAS concentrations in fish did pose a human health risk for eel, although tolerable daily intake values for perch were close to the reported daily consumption rates in Belgium and exceeded them in highly contaminated locations. Based on the ecological risk of PFOS, the standard was exceeded at about half of the sampling locations (58% for perch and 44% for eel).

Keywords: Active biomonitoring, Passive biomonitoring, perfluoroalkyl substances, European perch, European eel, bivalves

4.1 Background

Since the beginning of the 20th century, the increased emission of anthropogenic chemicals has led to a dramatic environmental impact (Corlett, 2015). Perfluoroalkyl substances (PFAS) have been produced at large scale for more than 60 years. Their lipophobic and hydrophobic properties make them suitable for a wide range of applications; as surfactants in surface coatings for textiles, soil repellents, food contact paper, cleaning products, and fire-fighting foams (Buck et al., 2011). The manufacturing and use of PFAS has resulted in a global contamination of these chemicals in the environment, wildlife and humans (Butt et al., 2010; Giesy and Kannan, 2002; Groffen et al., 2019a; Houde et al., 2006b).

Due to their persistence, potential health effects and global distribution, multiple manufacturers decided to phase-out the production of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (USEPA, 2000; EPA, 2006). In addition, other regulatory measures have been taken, such as the inclusion of both of these PFAS in the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009 and 2019, respectively (Stockholm Convention, 2008, 2019). Nonetheless, as PFAS are highly resistant to degradation, high environmental concentrations of some PFAS persist (Land et al., 2018).

In the Belgian terrestrial environment among the highest concentrations of multiple PFAS have been reported (Groffen et al., 2017, 2019a). However, in the Belgian aquatic environment the spatial distribution of PFAS has been studied less frequently and targeted only PFOS (Hoff et al., 2005, 2009). As fish consumption is an important route of PFAS pollution in humans in Flanders (Colles et al., 2020), it is important to investigate the spatial distribution of PFAS in the Belgian aquatic environment in order to determine potential human and ecological health risks. Furthermore, these studies used passive biomonitoring (PBM) on indigenous organisms, and were performed on a limited number of sampling sites. Studies measuring PFAS using active biomonitoring (ABM) with translocated individuals, on the other hand, have only sporadically been done (Babut et al., 2020). This technique allows for the exposure of the same species with a controlled

background condition in every sampling location, creating a more standardized sample collection. In addition, individuals of similar size can be exposed during the same pre-defined time (Bervoets et al., 2005b).

Therefore, as a baseline study the aim of this study was to investigate the current spatial distribution of PFAS in the aquatic environment of Flanders, Belgium, using both ABM (translocated mussels) and PBM (indigenous fish). Furthermore, to test for biomagnification of individual PFAS compounds, a comparison of accumulated concentrations between primary consumers and top predators was made. Thirdly, we examined the suitability of mussels in ABM, by comparing accumulation profiles in fish species with those in the mussels. Finally, we investigated the potential environmental risk and health risks to human through the consumption of PFAS-contaminated fish.

4.2 Materials and method

4.2.1 Sampling locations and sample collection

A total of 44 sampling locations were selected within the main water basins of Flanders, the northern part of Belgium (*Figure 4.1*). These locations were characterized as canals, rivers and streams. The nature and number of biota samples are indicated in *Table 4.1*. All sampling locations were selected within the existing monitoring network implemented for the Water Framework Directive and showed a variation with respect to anthropogenic pressure (e.g. urban, rural, industrial areas).

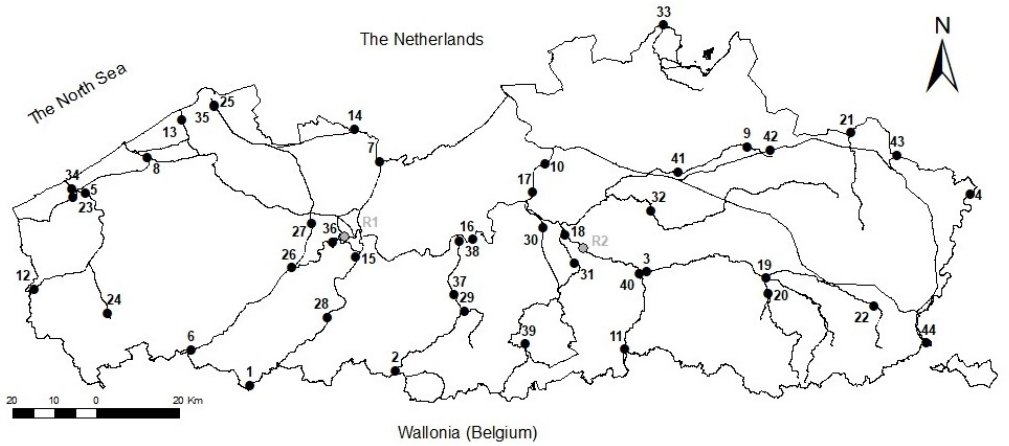


Figure 4.1: Overview of the sampling sites. A more detailed overview of sampling locations can be found in Table 4.1.

4.2.2 Fish

Fish collection was performed by the Research Institute for Nature and Forest (INBO) between 2015 and 2018. European perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*) in its yellow eel stage were caught using electrofishing, with a maximum of 4 eels and 20 perches per location. However, we were not able to catch both species at every sampling point. Both are sedentary, predatory fish with a diet mainly consisting of invertebrates and small fish (Laffaille et al., 2005; Rask, 1986; Yalçin-Özdilek and Solak, 2007). Juvenile yellow eels were collected targeting a length class of 45 – 55 cm. In deeper water bodies, additionally fykes (90 cm diameter and a total length of 22 m) were installed and harvested after 48 h. For a more detailed overview of the fishing procedure and equipment we refer to Belpaire et al. (2000). The fish were sorted on the field and bycatch was released. The perch and eel were frozen for transport and stored at -20°C until further processing.

*Table 4.1: List of sampling locations in support of Figure 4.1. Number of samples per location (*N*) are indicated for perch (*Perca fluviatilis*), eel (*Anguilla anguilla*) and mussels (*Dreissena bugensis *or alternatively* Corbicula fluminea *and* Mytilus edulis*).*

No.	Water body	City	*Perca fluviatilis (N)*	*Anguilla anguilla (N)*	*Dreissena bugensis (N)**
1	BOVEN-SCHELDE I	Spiere-Helkijn	20	3	5
2	DENDER I	Geraardsbergen	22	3	5
3	DEMER VII	Werchter	8	3	5
4	MAAS I+II+III	Kinrooi	21	4	5
5	IJZER III	Nieuwpoort	19	3	5[a]
6	LEIE I	Wevelgem	14	3	5
7	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	Zelzate	21	0	5[a]
8	KANAAL GENT-OOSTENDE III	Oostende	20	3	0
9	KLEINE NETE I	Retie	17	3	5
10	ZEESCHELDE IV	Antwerpen	0	11	0
11	DIJLE I	Oud-Heverlee	0	3	5
12	IJZER I	Poperinge	20	1	5
13	BLANKENBERGSE VAART + NOORDEDE	Blankenberge	6	3	0
14	LEOPOLDKANAAL I	Oostburg	20	3	0
15	BOVEN-SCHELDE IV	Gent	18	3	5
16	ZEESCHELDE II	Kastel	3	4	5
17	ZEESCHELDE III + RUPEL	Hemiksem	0	3	0
18	GETIJDEDIJLE-GETIJDEZENNE	Mechelen	4	3	0
19	HERK + KLEINE HERK	Herk-de-Stad	0	2	0
20	MELSTERBEEK I+II	Herk-de-Stad	0	2	0
21	DOMMEL	Neerpelt	15	2	0
22	DEMER I	Bilzen	4	1	0
23	KANAAL DUINKERKE-NIEUWPOORT	Koksijde	20	3	5[a]
24	KANAAL IEPER-IJZER	Ieper	0	3	0
25	LEOPOLDKANAAL II	Brugge	6	3	5[a]
26	LEIE III	Deinze	10	4	5
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	Nevele	9	4	5
28	BOVEN-SCHELDE II+III	Oudenaarde	0	3	5
29	BELLEBEEK	Liedekerke	0	3	5
30	ZEEKANAAL BRUSSEL-SCHELDE	Willebroek	20	3	5
31	ZENNE II	Zemst	7	4	5 + 5[a]
32	GROTE NETE III	Heist-op-den-Berg	16	4	5
33	MARK (Maas)	Hoogstraten	18	4	5
34	HAVENGEUL IJZER	Nieuwpoort	0	2	5[b]
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	Brugge	20	3	5
36	TOERISTISCHE LEIE	Gent	20	3	5

Table 4.1 (continued).

37	DENDER IV	Aalst	20	3	5
38	DENDER V	Dendermonde	20	6	5
39	ZENNE I	Beersel	17	0	5
40	DIJLE IV	Wijgmaal	0	3	5
41	KLEINE NETE II	Herentals	0	3	4
42	KANAAL BOCHOLT-HERENTALS	Mol	20	0	5
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (partly) + KANAAL BRIEGDEN-NEERHAREN	Bocholt	20	3	4
44	ALBERTKANAAL	Kanne, Riemst	20	2	0

* As for mussels, the number of samples used for analysis instead of the total number of exposed mussels is used. [a] Asiatic clam (*Corbicula fluminea*) or [b] blue mussel (*Mytilus edulis*) were used instead of quagga mussels. R1 (Nekker) and R2 (Blaarmeerse) are reference locations where mussels were collected. Different water body numbers (e.g. IJzer I, IJzer II,…) were in line with WFD classification. Mussels collected in 2017 were exposed in locations 23-33, collected in 2017 in locations 34-44 and collected in 2019 in the remaining locations.

4.2.3 Mussels

Non-native quagga mussels (*Dreissena bugensis*) were collected in the recreational lake the Nekker in Mechelen between 2017 and 2019. This area was selected based on the absence of any known pollution sources and hence, low concentrations of PFAS were expected. Furthermore, low concentrations of organic micropollutants (polychlorinated biphenyl's (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs)) measured by Bervoets et al. (2005b) reflect the general absence of industrial and household influence.

At least two weeks prior to exposure, the mussels were acclimated to the current climate in a semi-natural pond (mesocosm structure, University of Antwerp, Belgium), filled with dechlorinated tap water. A subset of 5-10 randomly selected mussels was analysed before exposure as to determine background concentrations. In order to reduce undesired spread of this exotic species to the sampling locations, the exposure took place during autumn and winter, since quagga mussel reproduction declines at low water temperatures (Wong et al., 2012). At locations with high salinity (N = 5; mean EC20: > 2.4 mS cm^{-1}), Asiatic clams (*Corbicula fluminea*) were exposed, as quagga mussels would not survive the higher salinity. These clams were collected from the recreational lake the Blaarmeerse in Ghent (2017 and 2018) and in the Nekker in Mechelen (2019). Unfortunately, insufficient

individuals could be collected from the Blaarmeerse in order to determine background concentrations. However, we expected little difference with previously measured concentrations in zebra mussels (*Dreissena polymorpha*) which showed very low ∑PFAS concentrations, namely 8.2 ng g^{-1} ww (unpublished data; *Appendix C: Table C.1*). Due to the very high and fluctuating salinity (EC20: 22.9 ± 14.4 mS cm^{-1}) in the harbour channel of the IJzer, local populations of blue mussel (*Mytilus edulis*) attached to a wharf were collected and analysed. For an overview of the used species per location and exposure year, we refer to *Table 4.1*.

A total of 70 to 75 quagga mussels per location were exposed during six weeks in two polyethylene cages, each consisting of two attached pond baskets (11 x 11 x 22 cm; mesh size of 2x4 mm), allowing free water circulation (Bashnin et al., 2019; Bervoets et al., 2005b; Smolders et al., 2002). When Asiatic clams were used, 25 to 30 individuals were exposed, because of their larger size. Cages were attached to bridges or solid structures on the bank using metal chains and locks, at a depth of at least 1m below the water surface. After recollection, mussels were depurated for at least 15h in particle free water from the respective sampling location at 15-20°C. Mussels were frozen at -20°C until further processing. Per location, three to five mussels were randomly selected for PFAS analysis on each individual. Lipid content and polyaromatic hydrocarbon (PAHs) concentrations were determined in the remaining (pooled) mussels as part of a large monitoring study (Teunen et al., 2020b: *Chapter 2*).

4.2.4 Sample preparation

A total of 515 perch and 132 eel were collected. Fish in poor conditions or visibly damaged, were discarded. Muscle samples of ±1g per individual were taken from the mid dorsal part of the body, opposite to the anus. Fish were pooled per species per location and homogenised using a stainless steel kitchen mixer (Bosch, MSM65PER). This resulted in 33 perch pools and 41 eel pools. The composition of the pools is presented in *Table 4.1*.

Although often higher PFAS concentrations are measured in liver tissue (Houde et al., 2006b; Valsechi et al., 2020), in the present study we chose to measure them in muscle tissue. This facilitates the calculation of human health risk and environmental monitoring (of hydrophobic compounds) in terms of secondary poisoning by top predators.

Mussel soft tissue was removed from the shell and weighed (up to 0.0001 g; Mettler AT261 DeltaRange, Mettler-Toledo). Furthermore, the tissue condition index (CI), as a measure of health, of individual mussels and clams was calculated as CI = tissue wet weight (TWT in gram)/shell dry weight (Park et al., 2012) and is displayed in Table S1. For fish, no CI was calculated since no individual samples were included in the present study and condition range would be very dependent on the size of collected fish. The mussel tissue was further homogenized using a TissueLyer LT (Qiagen, GmbH, Germany) with stainless steel beads (5 mm; Qiagen GmbH, Germany).

4.2.5 Chemical extraction and analyses

All used PFAS abbreviations are according to Buck et al. (2011). Fifteen PFAS were selected as target analytes, including 4 perfluoroalkyl sulfonic acids (PFSAs) and 11 perfluoroalkyl carboxylic acids (PFCAs). The target analytes and isotopically mass-labelled internal standards (ISTDs; MPFAC-MXA, Wellington Laboratories, Guelph, Canada), used in the quantification of these analytes, are illustrated in *Appendix C: Table C.2*. During the chemical extractions, HPLC grade acetonitrile (ACN; LiChrosolv, Merck Chemicals, Belgium), Milli-Q (MQ; 18.2 mΩ; TOC: 2.0 ppb; Merck Millipore, Belgium) and ammonium hydroxide (Filter Service N.V., Belgium) were used.

The extraction procedure followed the method described by Powley et al. (2005) with modifications. The homogenized fish muscle (0.80 ± 0.27 g) and mussel soft tissue (0.23 ± 0.07 g) samples were weighed into 50 mL polypropylene (PP) tubes and spiked with 10 ng of the ISTD mixture. After addition of 10 mL of ACN, the samples were vortex-mixed thoroughly and sonicated for 3 x 10 min (Branson 2510), with vortexing in-between the time periods. Hereafter, the samples were left overnight on a shaking plate (135 rpm, room temperature, GFL 3020, VWR International, Belgium). After centrifugation (4°C, 2400 rpm, 10 min, Eppendorf centrifuge 5804R, rotor A-4-44), the supernatant was transferred

to a 15 mL PP tube and dried to 0.5 mL using a rotational-vacuum-concentrator (Eppendorf concentrator 5301, Hamburg, Germany). The concentrated extract was transferred to a PP Eppendorf tube containing 50 mg of graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Belgium) and 50 µL of glacial acetic acid, to eliminate pigments. In addition, 2 x 250 µL of ACN, used to rinse the 15 mL PP tubes, was added to these Eppendorf tubes. After vortex-mixing, the extracts were centrifuged (4°C, 10000 rpm, 10 min, Eppendorf centrifuge 5415R; Rotor F45-24-11). The supernatant was dried completely using the rotational-vacuum-concentrator, and reconstituted with 200 µL of 2% ammonium hydroxide in ACN. The samples were vortex-mixed for at least 1 min and filtrated through an Ion Chromatography Acrodisc 13 mm Syringe Filter with 0.2 µm Supor polyethersulfone Membrane (VWR International, Belgium) attached to a PP auto-injector vial.

4.2.6 UPLC-TQD analysis

Ultra-performance liquid chromatography coupled tandem ES(-) mass spectrometry (UPLC-MS/MS, ACQUITY, TQD, Waters, Milford, MA, USA) was used to analyse the PFAS. The target analytes were separated using an ACQUITY BEH C18 column (2.1 x 50 mm; 1.7 µm, Waters, USA). An ACQUITY BEH C18 pre-column (2.1 x 30 mm; 1.7 µm, Waters USA) was inserted between the solvent mixer and injector to retain PFAS contamination originating from the system. The injection volume was set at 10 µL with a flow rate of 450 µL min^{-1}. 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. To identify and quantify the target analytes, multiple reaction monitoring (MRM) of two diagnostic transitions per target analytes (*Appendix C: Table C.2*) was used. This allowed us to confirm the absence of false-positives in the samples.

4.2.7 Quality control and assurance

As quality control for the PFAS analyses, one procedural blank (10 mL of ACN) was analysed per batch of 10 – 20 samples. Additionally, per 11 samples, one reference sample

of sterilized fish muscle tissue (pike-perch (*Sander lucioperca*), QUASIMEME Laboratory Performance Studies; Van Leeuwen et al., 2011) was included. All measurements were within ranges of the inter-laboratory study results of the reference material (*Appendix C: Table C.7*). To prevent cross-over contamination between samples during the UPLC-TQD analyses, ACN was injected on a regular basis to rinse the column. The concentrations in the blanks were all below the limit of quantification (LOQ). Individual LOQs were determined in the actual samples, hence taking into account possible matrix effects, based on the signal-to-noise (S/N) ratio of 10 and are displayed in *Table 4.2*. LOQs for PFBS and PFHxS were much higher compared to the other compounds, probably due to suboptimal extraction conditions. PFAS profiles might therefore not be accurate, as bioaccumulation of PFHxS was expected in fish (Munoz et al., 2017). Due to the high LOQ values, these data are possibly lacking. The target analytes were quantified using their corresponding ISTD (*Appendix C: Table C.2*), with exception of PFPeA, PFHpA, PFTrDA, PFTeDA, PFBS and PFDS for which no ISTD was present. These analytes were quantified using the ISTD closest in terms of functional group and carbon chain length, as has been validated by Groffen et al. (2019b). Method recoveries for the fish samples varied between 41% (PFBA) and 96% (PFOA). In mussel tissue, the recoveries varied between 53% (PFHxS) and 115% (PFNA).

4.2.8 Stable isotope analysis

Stable isotope analysis were performed on the pooled fish muscle and mussel samples per species and per location. After freeze-drying at -55°C, between 0.5 and 1 mg of homogenized tissue samples were encapsulated in pre-weighted 5 x 8 mm tin (Sn) capsules to determine nitrogen (N) and carbon (C) concentrations, as well as $\delta^{15}N$ and $\delta^{13}C$ (Verhaert et al., 2013). Stable isotopes were measured using an EA1110 elemental analyser coupled to a Thermo DeltaV Advantage IRMS with a Conflo IV interface at the Department of Earth and Environmental Sciences, KULeuven (Belgium). For the calibration, a combination of IAEA-600 (caffeine), a leucine and a freeze-dried tune muscle tissue standard were used. These latter two standards were previously calibrated

with certified reference standards and the estimated precisions for $\delta^{15}N$ and $\delta^{13}C$ were better than 0.05‰ and 0.13‰, respectively.

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

$$\delta^{15}N \text{ or } \delta^{13}C = [(R\_sample/R\_reference)-1] \times 1000 \quad (\mathbf{4.1})$$

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

The $\delta^{15}N$ was divided by 3.4, the mean trophic fractionation of $\delta^{15}N$ (Borgå et al., 2011), to estimate the trophic level (TL) of the organisms (*Appendix C: Table C.3*). A side note should be made that, using this method, a relative rather than an absolute TL was calculated, not taking into account site-specific baseline levels and food chain. However, since $\delta^{15}N$ of the lower trophic levels (i.e. mussels) showed limited variation between locations, indicating comparable food chain structures, we believed the used method is justified. Relations between TL and levels of bioaccumulated substances could only be assessed for PFOA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA and PFOS, as these were the only compounds that were detected in more than 50% of the samples (further motivation in the statistical analyses section). Trophic magnification factors (TMFs) (i.e. the change in contaminant concentrations per trophic level) for the target analytes were determined based on the TLs and the logarithmically transformed concentrations of the analytes (more details in the statistical analyses section).

4.2.9 Human health and ecological risk assessment

The maximum edible amount of fish, which a person of 70 kg could consume per day without potential health risks, was calculated for PFOA and PFOS based on the minimal risk levels (MRLs) proposed by the ATSDR (2019) and based on the MRL levels of the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM)(EFSA, 2020). The EFSA MRL value was determined on the sum of PFOA, PFOS, PFHxS and PFNA. In the present study, however, PFHxS measurements from 2016 were missing (11 sample locations) and both PFHxS and PFNA had >97% of measurements below LOQ. Due to the high LOQ values, especially for PFHxS, using ½

LOQ would probably give an overestimation of the risk. Therefore, the EFSA 2020 guideline was compared against the sum of PFOS and PFOA. The MRL values are proposed for oral, intermediate intake. The maximum edible amount of fish which can be consumed per day without potential health risks was calculated based on *Formula 4.2*.

$$Q = W * M/C \qquad (\mathbf{4.2})$$

With M = MRL for oral intake of the substance (ng kg^{-1} body weight day^{-1}), W = weight of a person (kg), Q = maximum amount of contaminated organisms that a person can consume per day without risking health effects (g day^{-1}) and C = observed concentrations of a substance in an organism (ng g^{-1} ww).

Furthermore, accumulated PFOS concentrations in fish were tested for compliance against the European Environmental Quality Standards for biota (EQS_{biota}), namely 9.1 µg kg^{-1} ww (EU, 2013).

4.2.10 Statistical analyses

The statistical analyses were performed using R Studio (version 3.2.2) and the level of significance was set at $p \leq 0.05$ (adjusted *p*-values). The normality assumptions of the residuals were examined using the Shapiro-Wilk test. Concentrations below the LOQ were given a value of LOQ/2 (Bervoets et al., 2004). Whenever the quantified concentrations of an analyte were below the LOQ in more than 50% of the samples at a certain location, or in a certain species, these data were excluded from the statistical analyses in order to minimize left-skewing of the data due to overleverage by left-censored data. Significant differences in CI of the mussels among locations were investigated using a One-Way ANOVA followed by Tukey's Honestly Significant Difference test for post-hoc analysis. These tests were also used to investigate differences in stable isotope concentrations, as well as in TLs, among species. Spearman rank correlation tests were used to investigate correlations between the CI of the mussels and the accumulated PFAS concentrations as well as between the PFAS concentrations in the organisms and the $\delta^{13}C$ and $\delta^{15}N$ concentrations. For this analysis, mean values per location were determined. Paired two-sample T-tests, or Paired Wilcoxon signed rank

tests in case of non-normality, were used to test for differences in PFAS concentrations between both fish species and between the fish species and mussels. We used Stable Isotope Bayesian Ellipses in R (SIBER) to compare the isotopic niche area of each species as well as isotopic niche overlap among the species. This technique has been proven useful to compare isotopic niche widths among and within communities (Jackson et al., 2011). Trophic magnification factors (TMFs) were determined based on a linear regression model between the TLs and the logarithmically transformed concentrations of the target analytes. The TMFs were calculated as 10^b, where b is the slope of the linear model (Borgå et al., 2011).

4.3 Results

4.3.1 PFAS concentrations and profiles

Mean background ΣPFAS (i.e. the sum concentration of all the target analytes, with values <LOQ replaced by LOQ/2) concentrations for mussels collected from the Nekker (reference location) were 21.88 ng g^{-1} ww for quagga mussels and 20.79 ng g^{-1} ww for Asian clams. The spatial distributions of ΣPFAS concentrations in the mussel and fish species are displayed in *Figures 4.2-4.4*. The ΣPFAS concentrations ranged from 8.56 – 157 ng g^{-1} ww in mussels (median ΣPFAS concentration of 22.4 ng g^{-1} ww), 5.22 – 67.8 ng g^{-1} ww in perch (median ΣPFAS concentrations of 20.8 ng g^{-1} ww), and between 5.73 – 68.8 ng g^{-1} ww in eel (median ΣPFAS concentration of 22.1 ng g^{-1} ww). Detailed information on PFAS concentrations in the mussels and fish at each individual location is reported in *Appendix C: Table C.1* for mussel and *Appendix C: Table C.4* for perch and eel.

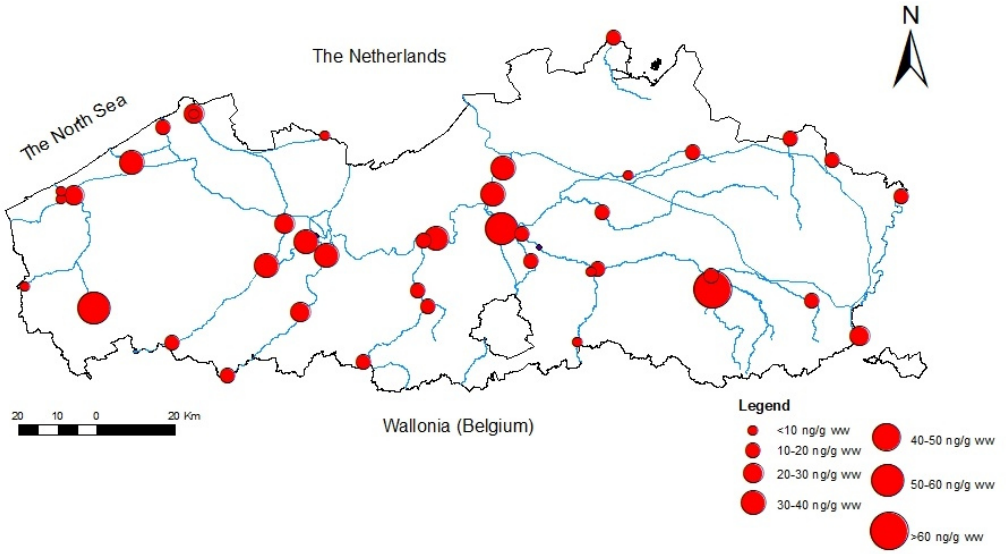


Figure 4.2: Sum of PFAS concentrations measured in eel muscle tissue in Flanders (Belgium). Increased size of the circles indicates higher accumulated concentrations (ng g^{-1} ww).

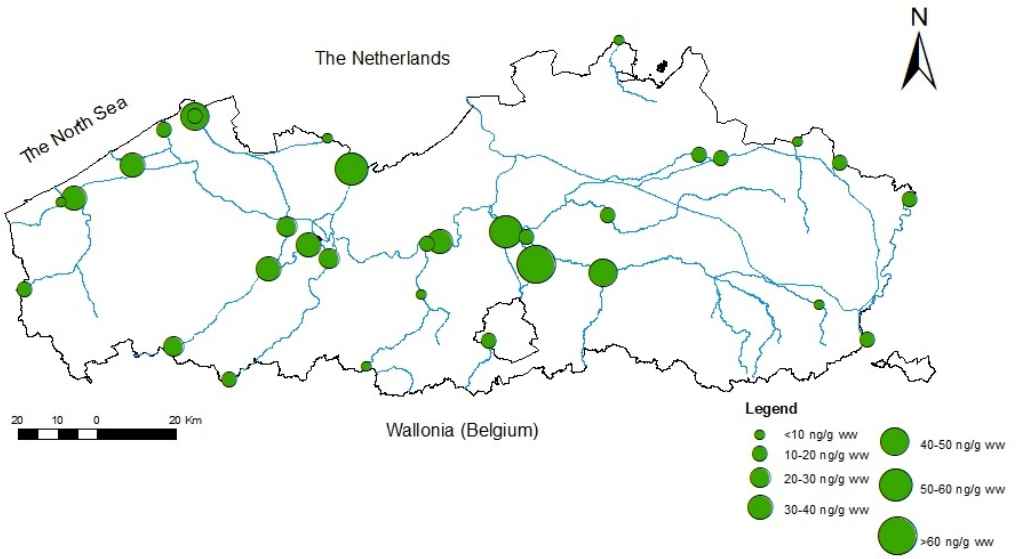


Figure 4.3: Sum of PFAS concentrations measured in perch muscle tissue in Flanders (Belgium). Increased size of the circles indicates higher accumulated concentrations (ng g^{-1} ww).

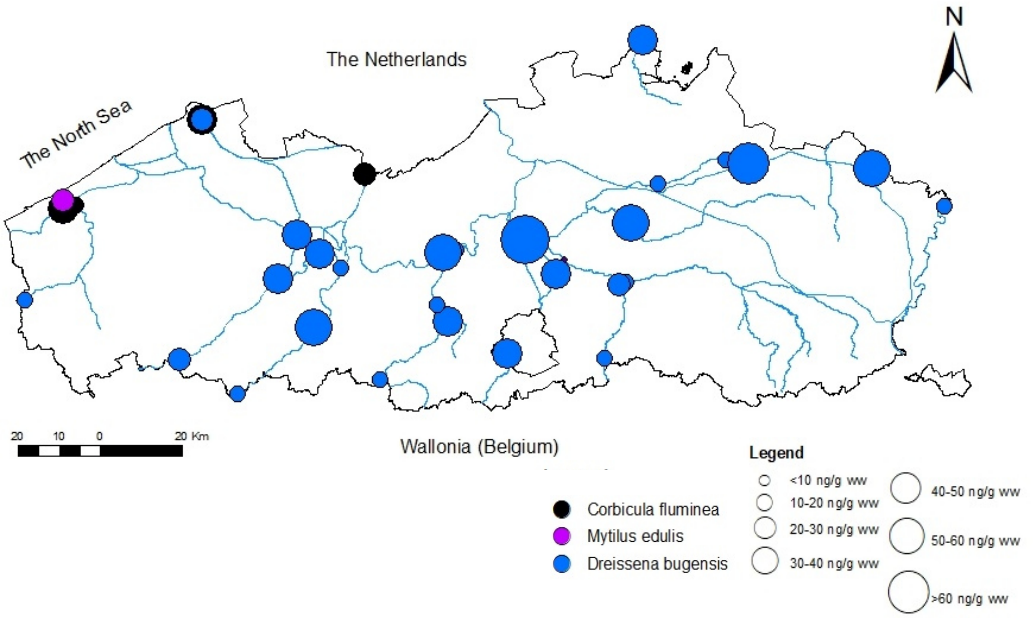


Figure 1.4: Sum of PFAS concentrations measured in mussel tissue in Flanders (Belgium). Increased size of the circles indicates higher accumulated concentrations (ng g^{-1} ww). Different colours represent different species.

The mean and median PFAS concentrations over the sites are displayed for each species in *Table 4.2* and compared in *Figure 4.5*. As PFOA, PFDA, PFUnDA, PFDoDA and PFTeDA were detected in more than 50% of both the mussels and fish muscle tissue (of both species), only these compounds were compared among mussels and fish. In addition, PFTrDA and PFOS were detected in more than 50% of the muscle samples of both fish species and hence we also compared the concentrations of these analytes between eel and perch. Significant differences in PFAS concentrations between mussels and perch were observed for PFOA ($p < 0.001$), PFDA ($t_{13} = -4.187$; $p = 0.001$), PFUnDA ($p = 0.030$) and PFTeDA ($p = 0.002$). The PFOA and PFTeDA concentrations were higher in the mussels, whereas the concentrations of PFDA and PFUnDA were significantly higher in perch. The PFDoDA concentrations did not significantly differ between perch and mussels ($p = 0.326$). Similarly to the perch, the PFOA concentrations in eel were also significantly lower than those in the mussels ($p < 0.001$), whereas PFDA ($t_{17} = -2.244$; $p = 0.038$) and PFUnDA ($p = 0.021$) concentrations were higher in the eel. No differences between eel and mussels were observed for PFDoDA ($p = 0.265$) and PFTeDA (0.167). Between the

fish species, significant differences were observed for PFOA ($p < 0.001$), PFDA ($t_{28} = 3.17$; $p = 0.004$), PFDoDA ($p < 0.001$), PFTrDA ($p < 0.001$), PFTeDA ($p < 0.001$) and PFOS ($p = 0.016$). The PFOA, PFDoDA, PFTrDA, PFTeDA concentrations were higher in eel than in perch, while PFDA and PFOS concentrations were higher in perch. The PFUnDA concentrations did not differ between the fish species ($p = 0.629$).

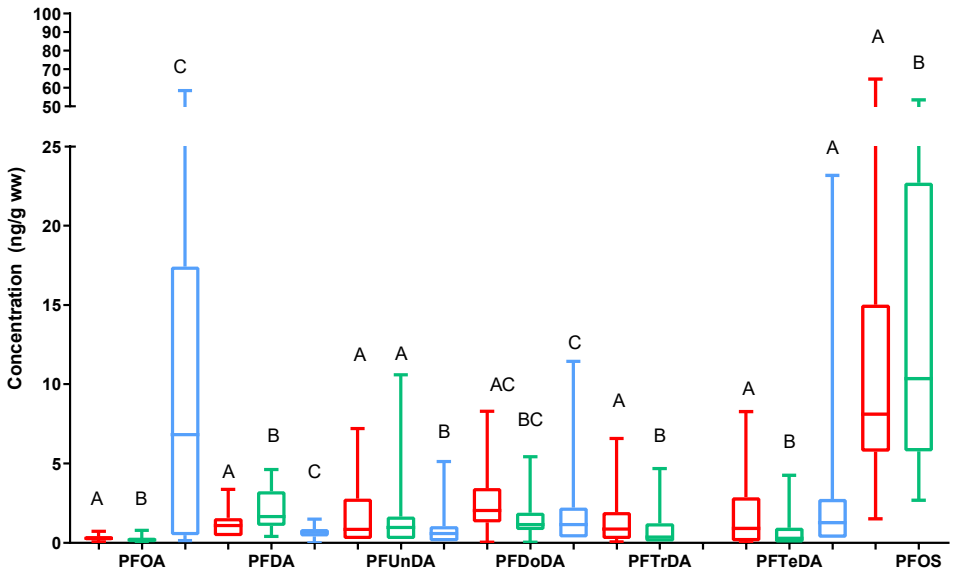


Figure 4.5: Comparison of PFAS muscle concentrations in ng g^{-1} wet weight among eel (red bars; N = 41), perch (green bars; N = 33) and mussels (blue bars; N = 178). Differences in concentrations among the organisms are depicted by different letters for a specific compound. Only compounds with a detection frequency >50%, in at least two of the three organisms, are included. Hence, the PFTrDA and PFOS concentrations in mussels are excluded from the figure.

The PFAS profiles in the mussel, perch and eel are displayed in *Figure 4.6*. Regarding the mussels, we did not distinguish among the different species and grouped them all together, as no large differences in PFAS profiles were observed among the three mussel species (*Appendix C: Figure C.1*). The PFOA contribution in the blue mussels was slightly larger than those in the quagga mussel and Asiatic clam, but this is likely the result of a smaller sample size (due to the collection in only one location) of this species compared to the others. The PFAS profile of the mussels was dominated by PFOA, whereas in both fish species PFOS was the dominant compound. Furthermore, the relative contribution of short-chained PFAS, i.e. PFBA, PFPeA, PFHxA and PFBS was higher in the mussels than

in the fish species. Between the fish species, no major differences in detected PFAS compounds were observed, although PFHxA was only detected in eel. However, the relative contribution of the detected compounds did sometimes differ between species. The PFAS profiles of the mussels contained a higher contribution of PFBA ($F_{2,252} = 5.57$; $p = 0.004$), PFOA ($F_{2,252} = 49.1$; $p < 0.001$) and PFTeDA ($F_{2,252} = 12.8$; $p < 0.001$) compared to both fish species. The opposite, with a higher contribution in the fish than in mussel, was true for PFNA ($F_{2,252} = 18.5$; $p < 0.001$), PFDA ($F_{2,252} = 18.9$; $p < 0.010$) and PFOS ($F_{2,252} = 821$; $p < 0.001$). In addition, the PFHxA contribution was significantly higher in mussels than in eel ($p < 0.001$). The PFUnDA ($F_{2,233} = 1.96$; $p = 0.143$), PFDoDA ($F_{2,252} = 3.03$; $p = 0.169$) and PFTrDA ($F_{2,252} = 2.89$; $p = 0.057$) contributions were similar between mussels and fish. Between the fish species, contributions of PFDA ($F_{2,252} = 18.9$; $p = 0.022$) and PFOS ($F_{2,252} = 821$; $p < 0.001$) were higher in perch compared to eel. The opposite was true for PFDoDA ($F_{2,252} = 3.03$; $p = 0.039$). Contributions of PFBA, PFOA, PFNA, PFUnDA, PFTrDA, PFTeDA and PFDS did not differ between eel and perch ($p > 0.179$).

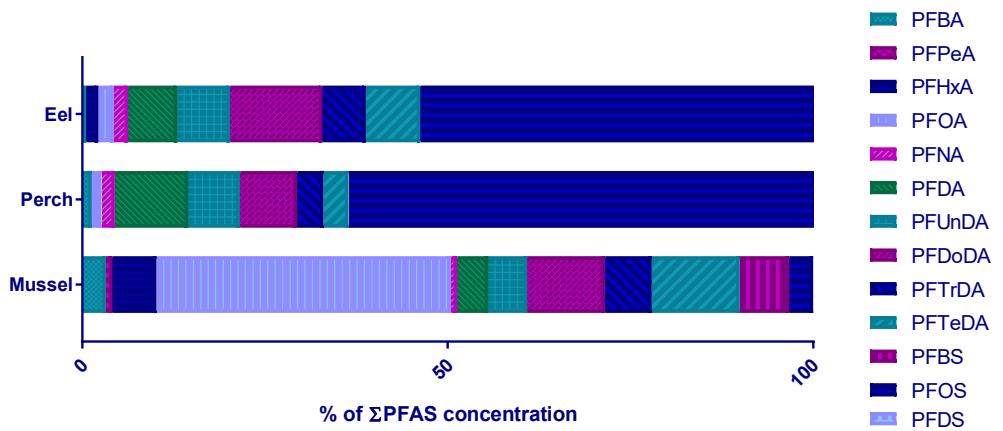


Figure 4.6: PFAS profiles in eel (N = 78), perch (N = 86) and mussels (N = 178) collected across Flanders. PFHpA and PFHxS were excluded from the figure as concentrations of these PFAS were <LOQ in all samples.

Table 4.2: Individual limits of quantification (LOQ; ng g^{-1} ww) and concentrations (mean, median and range; ng g^{-1} ww) for the target analytes in mussel tissue and fish muscle tissue across Flanders. Significant differences in mean analyte concentrations among the organisms are indicated by different capital letters. Only compounds that were detected in more than 50% of the samples of a particular species were included in these analyses.

	LOQ		**Perch (*N* = 33)**				**Eel (*N* = 41)**				**Mussel (*N* = 181)**			
	Fish	Mussel	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
PFBA	0.135	0.259	<LOQ	<LOQ	<LOQ	0.159	<LOQ	<LOQ	<LOQ	0.219	0.330	<LOQ	<LOQ	5.283
PFPeA	0.531	0.185	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.658
PFHxA	0.365	1.409	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.876	<LOQ	<LOQ	<LOQ	1.601
PFHpA	0.143	0.432	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFOA	0.106	0.269	0.159$^{(A)}$	0.175	<LOQ	0.787	0.287$^{(B)}$	0.276	<LOQ	0.727	10.2$^{(C)}$	6.278	<LOQ	58.6
PFNA	0.489	0.176	<LOQ	<LOQ	<LOQ	0.730	<LOQ	<LOQ	<LOQ	0.608	<LOQ	<LOQ	<LOQ	0.639
PFDA	0.824	0.188	1.978$^{(A)}$	1.645	<LOQ	4.624	1.030$^{(B)}$	0.956	<LOQ	2.502	0.561$^{(C)}$	0.464	<LOQ	3.047
PFUnDA[a]	0.452	0.192	1.940$^{(A)}$	0.965	<LOQ	10.6	1.968$^{(A)}$	1.051	<LOQ	7.202	0.719$^{(B)}$	0.607	<LOQ	5.136
PFDoDA	0.081	0.676	1.692$^{(AC)}$	1.145	<LOQ	5.429	2.533$^{(BC)}$	1.975	<LOQ	8.289	1.687$^{(C)}$	1.162	<LOQ	11.4
PFTrDA	0.128	0.665	0.879$^{(A)}$	0.361	<LOQ	4.693	1.471$^{(B)}$	0.771	<LOQ	6.579	1.079	<LOQ	<LOQ	7.933
PFTeDA	0.017	0.617	0.773$^{(A)}$	0.289	<LOQ	4.259	1.971$^{(B)}$	1.571	<LOQ	8.278	2.300$^{(B)}$	1.322	<LOQ	23.2
PFBS[a]	5.598	1.253	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.809	<LOQ	<LOQ	147.5
PFHxS[a]	7.202	7.177	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFOS	0.880	0.258	15.8$^{(A)}$	10.4	2.672	53.5	12.7$^{(B)}$	8.027	1.521	64.6	0.395	<LOQ	<LOQ	5.608
PFDS	0.003	2.478	0.008	<LOQ	<LOQ	0.138	0.010	<LOQ	<LOQ	0.204	<LOQ	<LOQ	<LOQ	<LOQ

[a]in 2016 PFUnDA, PFBS and PFHxS were not analysed in the fish samples; therefore, the LOQ and concentrations of these analytes were determined on the samples from all years, excluding 2016.

One-way ANOVA results showed that only the CI of the quagga mussels exposed at the Dijle (*Figure 4.1, Loc. 40*) was significantly higher than those at the Zenne (*Figure 4.1, Loc. 31*) ($F_{28,144}$ = 2.44; p = 0.030), while no other differences among locations were observed for the quagga mussels. The Asiatic clams did not differ in CI among the six locations ($F_{6,26}$ = 2.19; p = 0.077). There was no significant correlation between the CI of the mussels (regardless of species) and concentrations of PFOA (p = 0.991), PFDA (p = 0.950), PFUnDA (p = 0.687), PFDoDA (p = 0.928) and PFTeDA (p = 0.747), nor with ΣPFAS concentrations (p = 0.517). When looking at only the quagga mussels, the PFOA (p = 0.843), PFDA (p = 0.680), PFUnDA (p = 0.590), PFDoDA (p = 0.732), PFTeDA (p = 0.307) and ΣPFAS (p = 0.843) concentrations were not significantly correlated to the CI of the mussels. For all other PFAS, the detection frequencies were below 50% and hence, no correlation with the CI was investigated.

4.3.2 Isotopic niche overlap, trophic levels and associations with PFAS concentrations

The $\delta^{13}C$ and $\delta^{15}N$ values are reported in *Appendix C: Table C.1* for the mussels, and Table *Appendix C: Table C.4* for the fish species. The SIBER analysis revealed that the isotopic niches of perch and eel, as well as those of the quagga mussels and Asian clams, overlapped (*Figure 4.7; Appendix C: Table C.5*). Both mussel species had no, or very limited, overlap in isotopic niche with the fish species. This difference in isotopic niche between the mussel species and the fish species was mainly the result of significant differences in $\delta^{15}N$ values, which were higher in both fish species compared to the mussel species ($F_{3,130}$ = 120, p < 0.001). The $\delta^{13}C$ did only differ between the quagga mussels and the eel, with higher $\delta^{13}C$ values in the mussels ($F_{3,130}$ = 3.18; p = 0.026). The median corrected standard ellipse area (SEAc), representing the isotopic niche width, was larger in eel (23.84‰²), compared to perch (11.00‰²), while the SEAc of the Asian clams (13.46‰²) was higher than those of the quagga mussels (8.39‰²) (*Appendix C: Figure C.2)*.

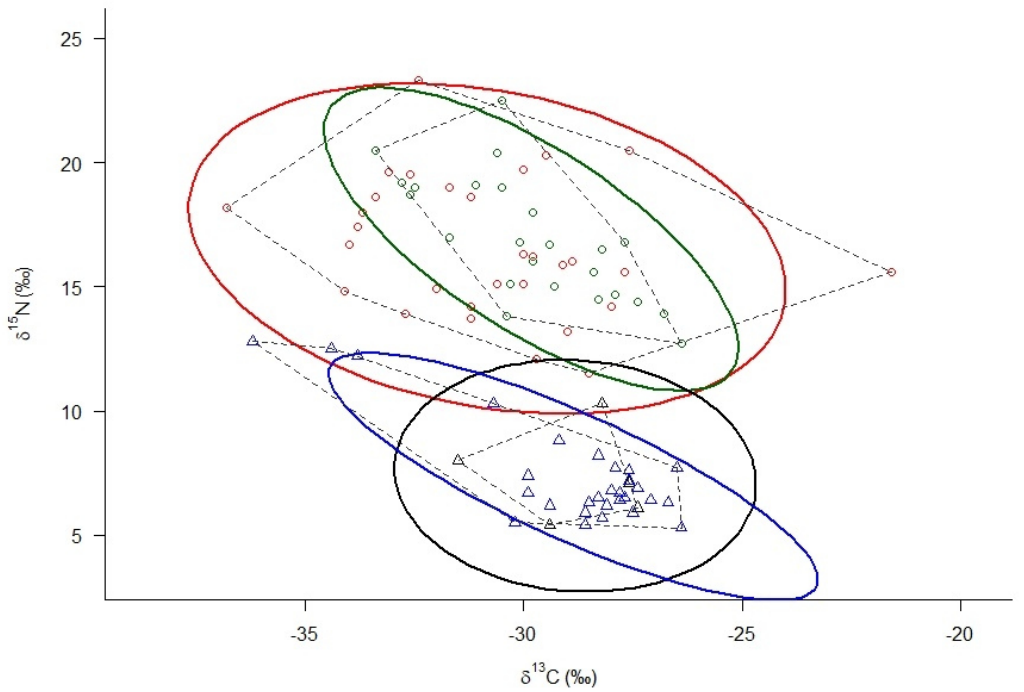


*Figure 4.7: Isotopic niche overlap among perch (*Perca fluviatilis*, green ellipse,* N = *24), eel (*Anguilla anguilla*, red ellipse,* N = *31), quagga mussel (*Dreissena bugensis*, blue ellipse,* N = *30) and Asian clam (*Corbicula fluminea*, black ellipse,* N = *5).*

The trophic levels of the organisms are displayed in *Appendix C: Table C.3*. The TLs for the mussel species were significantly lower than for the fish species ($F_{3,84}$ = 100; p < 0.001). Differences in TL between the quagga mussels and the Asian clams, as well as between the perch and eel, were not significant. The TL of all organisms was negatively related to the PFOA (slope (b) = -0.381; R^2 = 0.476; p < 0.001) and PFTeDA (b = -0.137; R^2 = 0.062; p = 0.011) concentrations, and positively related to the concentrations of PFDA (b = 0.101; R^2 = 0.212; p < 0.001), PFUnDA (b = 0.097; R^2 = 0.116; p = 0.002) and PFOS (b = 0.499; R^2 = 0.638; p < 0.001). No relationships between TL and PFDoDA (p = 0.791) and PFTrDA (p = 0.409) were observed. TMFs were only calculated for the compounds that were significantly related with TLs and are displayed in *Appendix C: Table C.6*.

Significant negative relations have been observed between the PFOA concentrations in mussels and perch (t_{16} = -2.31; p = 0.035; R^2 = 0.20), whereas concentrations of PFTrDA

(t_{16} = 4.77; p < 0.001; R^2 = 0.56) and PFTeDA (t_{16} = 4.54; p < 0.001; R^2 = 0.54) were positively related between both organisms. No relationships between the PFAS concentrations in perch and mussels have been observed for PFDA, PFUnDA, PFDoDA and PFOS (p > 0.05). Between mussels and eels, significant positive relationships were observed for PFDoDA (t_{19} = 2.16; p = 0.044; R^2 = 0.15), PFTrDA (t_{19} = 6.87; p < 0.001; R^2 = 0.70), PFTeDA (t_{19} = 6.89; p < 0.001; R^2 = 0.70), and PFOS (t_{19} = 4.83; p < 0.001; R^2 = 0.53). The concentrations of PFOA, PFDA and PFUnDA (p > 0.05) were not related between eel and mussel. Finally, between both fish species, a positive relationships was observed for PFUnDA (t_{11} = 9.91; p < 0.001; R^2 = 0.89), PFDoDA (t_{14} = 7.37; p < 0.001; R^2 = 0.78), PFTrDA (t_{14} = 16.8; p < 0.001; R^2 = 0.95), PFTeDA (t_{14} = 5.53; p < 0.001; R^2 = 0.66) and PFOS (t_{14} = 3.18; p = 0.006; R^2 = 0.38). The concentrations of PFOA and PFDA (p > 0.05) were not related between eel and perch.

Negative correlations were observed between the $\delta^{13}C$ values and concentrations of PFOS (ρ = -0.405; p < 0.001), PFDA (ρ = -0.410; p < 0.001), PFUnDA (ρ = -0.317; p = 0.007), PFDoDA (ρ = -0.277; p = 0.009) of all organisms, as well as between values of $\delta^{15}N$ and concentrations of PFOA (ρ = -0.659; p < 0.001). The $\delta^{13}C$ concentrations were positively correlated with concentrations of PFOA (ρ = 0.275; p = 0.009), whilst the $\delta^{15}N$ concentrations were positively correlated with those of PFOS (ρ = 0.753; p < 0.001), PFDA (ρ = 0.439; p < 0.001) and PFUnDA (ρ = 0.363; p = 0.002). No correlations were observed between $\delta^{13}C$ and PFTrDA (p = 0.306), $\delta^{13}C$ and PFTeDA (p = 0.302), and between $\delta^{15}N$ and concentrations of PFDoDA (p = 0.548), PFTrDA (p = 0.644) and PFTeDA (p = 0.101). For all other PFAS, no correlations were examined as detection frequencies were <50%.

4.3.3 Human health risks

Based on the mean concentrations and the concentration ranges in the eel and perch, the maximum edible amounts of both fish species per day (g) for a person of 70 kg have been calculated and reported in *Table 4.3*. This value was determined on the pooled dataset of all sample locations. Calculated using the mean concentrations of the sum of PFOS and

PFOA, a person of 70 kg should consume maximally 6.4 g of eel and 5.0 g of perch per day without a potential health risk. A worst-case scenario, using the maximum concentrations detected in the fish species, revealed that humans should not consume more than 0.68 g of eel and 0.82 g of perch per day. For the above results the MRLs by the EFSA Panel on Contaminants in the Food Chain (EFSA, 2020) were used, since they are the most strict. For calculations based on ATSDR (2019) MRLs for PFOS and PFOA individually we refer to *Table 4.3*.

Table 4.3. Minimal Risk Levels (MRLs) and maximum edible amounts (g day^{-1}) of fish muscle tissue, which a 70 kg person can consume per day without health risks (Q-values), determined for mean concentrations and concentration ranges (min-max, between brackets) in perch and eel across Flanders.

	ATSDR (2019)		*EFSA (2020)*[a]
	PFOA	PFOS	Sum PFOA, PFOS, PFNA and PFHxS
MRL (ng kg^{-1}day^{-1})	3	2	0.63
Concentration (ng g^{-1} ww) in eel	0.304 (<LOQ – 0.727)	13 (1.52-65)	13 (1.64-65)
Maximum edible amount of eel per day (g) for a 70 kg person (Q-value)	872 (289 – 3962)	22 (2.17-92)	6.4 (0.68-27)
Concentration (ng g^{-1} ww) in perch	0.195 (<LOQ – 0.787)	16 (2.67 – 53)	16 (2.88-54)
Maximum edible amount of perch per day (g) for a 70 kg person (Q-value)	1700 (267 – 3962)	16 (2.62 – 52)	5.0 (0.82-15)

[a] Q-values for the EFSA (2020) MRL were calculated on the sum of PFOA and PFOS due to missing data and more than 97% measurements <LOQ for the other analytes.

4.4 Discussion

4.4.1 Spatial distribution

Our results confirmed a wide distribution and bioavailability of PFAS in the aquatic environment. The canal Brussel-Schelde (*Figure 4.1, Loc. 30*) showed high accumulated concentrations in all biota. This canal is subject to direct influence from intensive industrial activities. The highest PFAS concentrations in perch were measured in the Zenne river (*Figure 4.1, Loc. 31*), known for its very high background pollution and influences from Brussels (Teunen et al., 2020b: *Chapter 2*). Furthermore, high concentrations were measured in mussels deployed in the canal Bocholt-Herentals (*Figure 4.1, Loc. 42*). This canal is a connection between the Meuse and Scheldt basin, both known for large effects of industrial as well as household waste water as a source of PFAS (Teunen et al., 2020b: *Chapter 2*). The Melsterbeek, however, showing high accumulated concentrations in eel, flows through a more agricultural region. Here, contamination with PFAS might be caused by agriculture, households or undefined point sources. However, our conclusions on this part are mere qualitative and based on personal interpretation and experiences of the general monitoring network of the Flanders Environment Agency. Further investigation using data on population density, area of industrial surfaces and emission indices could be used to investigate the relationship between accumulated concentrations in biota and possible sources with a more quantitative approach.

The use of PFOS is restricted since 2009 (Stockholm Convention, 2008). This should eventually lead to a decrease of this substance in the environment. Previous studies on the water bodies used in the present study indeed showed higher concentrations of PFOS. A preliminary monitoring study from 2013 reported PFOS concentrations in muscle tissue of eel of 15, 33, 7.2 and 34 µg kg^{-1} ww in the upper-Scheldt, canal Ieper-IJzer, Kleine Nete and Demer respectively (De Jonge et al., 2014). The present study showed concentrations of 5.6, 14.5, 12 and 11.3 respectively at the same sampling locations in eel. These results showed a clear decrease and possibly revealed the effects of phasing-

out the use of these compounds to the environment, with the exception of the Kleine Nete, which remained stable.

Compared to previous studies on yellow perch (Perca flavescens), the PFOS (118.6 ± 29 ng g^{-1} ww) and PFUnDA concentrations (3.8 ± 1.2 ng g^{-1} ww) in New Jersey, USA, were considerably higher than those reported in the present study, whilst, on the contrary, concentrations of PFDA (1.1 ± 0.4 ng g^{-1} ww) and PFDoDA (0.7 ± 0.2 ng g^{-1} ww) were lower in New Jersey (Goodrow et al., 2020). The concentrations of PFOS, PFUnDA, PFTrDA, PFDA, PFDoDA and PFOA were higher in the present study compared to those reported in perch collected in Finnish rivers (3.4 ng g^{-1} PFOS, 1.0 ng g^{-1} PFUnDA, 0.45 ng g^{-1} PFTrDA, 0.5 ng g^{-1} PFDA, 0.23 ng g^{-1} PFDoDA and 0.03 ng g^{-1} PFOA; Junttila et al., 2019) and to those reported in shad (*Alosa agone*), European whitefish (*Coregonus lavaretus*), burbot (*Lota lota*), rainbow trout (*Oncorhynchus mykiss*), perch, roach (*Rutilus rutilus*), brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) in glacial lakes from the Alps in France, Switzerland and Italy (6.0 ng g^{-1} PFOS, 0.3 ng g^{-1} PFUnDA, 0.5 ng g^{-1} PFDA and 0.3 ng g^{-1} PFDoDA (Valsecchi et al., 2020). A monitoring study in the Netherlands measured PFOS concentrations in bream (*Abramis brama*), roach, perch and pike-perch between 4.9 and 120 ng g^{-1} ww (Foekema et al. 2016), which were higher than those reported in the present study. In the North Rine-Westfalen basin in Germany, eel PFOS concentrations ranged between 8.3 and 49 ng g^{-1} ww (Guhl et al. 2014). The PFOS concentrations in the Loire estuary ranged from 17.9 to 39.0 ng g^{-1} ww (Couderc et al., 2015). Kwadijk et al. (2010) examined the distribution of multiple PFAS in eel from The Netherlands and reported PFOS concentrations ranging from 7 to 58 ng g^{-1} ww. In Lake Möhne, Germany, PFOS concentrations of 37 – 83 ng g^{-1} ww have been reported in eel, whereas PFOA concentrations ranged up to 2.3 ng g^{-1} ww (Hölzer et al., 2011). These concentrations are comparable to those reported in eel in the present study. Compared to other studies investigating PFAS concentrations in mussels, the ΣPFAS concentrations were higher in the present study than reported in previous studies in fresh water mussels from Spain (Fernández-Sanjuan et al., 2010), and marine mussels from the Netherlands (Zafeiraki et al., 2019), Spain (Gómez et al., 2011; Zabaleta et al., 2015), and Denmark (Bossi et al., 2008), but lower than those reported in marine mussels from Portugal (Cunha

et al., 2005). Comparison to literature, revealed a large variation of PFAS concentrations measured in biota, both on a European and global scale. High concentrations might be due to the presence of different sources of PFAS contamination (e.g. point sources, diffusive emission sources). On the other hand, differences between species could be explained by their diet (Babut et al., 2017).

4.4.2 PFAS bioaccumulation, -magnification and -dilution

Longer chain PFAS preferentially partition to sediments, as the water solubility of PFAS is inversely proportional to the length of the carbon chain, while short chain compounds remain dissolved in the water (Labadie and Chevreuil, 2011; Prevedouros et al., 2006). The carbon chain length, as well as the identity of the anionic functional group of PFAS, are related to their bioaccumulative potential, with PFSAs being more bioaccumulative than PFCAs with the same fluorinated carbon chain length (Conder et al., 2008). Furthermore, PFCAs and PFSAs that contain at least eight fluorinated carbons (i.e. PFNA and PFOS and longer compounds) have a greater bioaccumulative potential (Conder et al., 2008). Although shorter chain PFAS can also bioaccumulate, they have a much smaller bioaccumulation potential and their bioaccumulation is mainly related to elevated concentrations in the water column (Conder et al., 2008; Goodrow et al., 2020). This might also explain the larger contribution of short-chain PFAS in mussels compared to fish, since both isotopic niche analysis and TL confirmed that the mussels occupied a lower position in the trophic food chain.

The differences in PFAS profiles between the mussels and fish species are likely also the result of different ways of exposure, caused by the sampling strategy and experimental design of this study. As the mussel cages were placed in the water column, without contact to the sediment, the mussels have been exposed solely to the water and suspended material, whilst the fish have been exposed to both the water and sediment. Although, both species are considered to be spending time in close relation to the sediment compartment (Westrelin et al., 2018; Belpaire and Goemans, 2007a), eel shows a more bottom-dwelling lifestyle. Consequentially, the dominance of hydrophilic PFCAs and PFSAs, with less than eight fluorinated carbons (i.e. PFOA, PFHxS and shorter PFAS),

was expected in the mussels. Similarly, the fish species have also been exposed to sediments and hence to longer chain PFAS with a higher bioaccumulative potential. This also explains why PFOA concentrations were significantly higher in the mussels compared to both fish species, whilst the opposite pattern was often observed for longer chain PFAS. On the other hand, it has been shown that biotransformation (the degradation of PFAS precursors to, for example, PFOS) efficiency increased with increasing trophic level, from invertebrates to fish (Babut et al., 2017).

Dietary differences between perch and eel could explain the differences in PFAS concentrations and profiles between both fish species. Although on average, the TLs of both species did not differ, eels have a slightly broader range of TLs compared to perch. Despite that both are predatory species, feeding primarily on invertebrates and small fish species (Rask, 1986; Yalçin-Özdilek and Solak, 2007), there are differences in the feeding ecology between both species in the studied populations. The broader isotopic niche (indicated by SEAc) indicates that, despite the overlap in isotopic niche area between both species, eels have a more diverse and flexible diet, which might consist of different invertebrate or fish species, compared to perch (Belpaire et al., 1992). For example, De Meyer et al. (2018) showed that head morphology of eel (broad-headed vs. narrow-headed) could influence diet, trophic level and therefore pollutant accumulations. Furthermore, the diet of both species is known to depend on their size, as size-dependent diet segregation of both species has been reported before (Ezzat and El-Seraffy, 1977; Rask, 1986; Yalçin-Özdilek and Solak, 2007). This segregation is also known to vary widely among populations (Rask, 1986), which could also explain dietary differences, and hence differences in exposure, between perch and eel. Additionally, spatial differences in diet may occur depending on local ecological variation in species composition and food availability. Finally, biotransformation of PFAS can be species-specific (Babut et al., 2017; Galatius et al., 2013), probably due to specific proteins involved in the process (Ng and Hungerbühler, 2013), resulting in different contamination profiles.

A positive correlation between accumulated concentrations in mussels and fish was found for PFTrDA, PFTeDA, and PFOS and PFDoDA in eel. This positive relationship reflects the possibility of mussels to predict the pollutant pressure at different locations, since high

concentrations at a specific location are found in fish as well as mussels. With a negative correlation, as was the case for PFOA in perch, the relationship between species is contradictory, and mussels will not be able to predict high pollution levels in fish (and therefore a risk of secondary poisoning). Furthermore, all significant relationships between both fish species showed to be positive, which is a logical consequence since they occupied similar trophic levels of the same food web in each location.

The TMFs in the present study were compared to those of other studies on freshwater ecosystems, and were higher than those reported by Loi et al. (2011) for PFOS (TMF = 1.3) and lower than those for PFUnDA (TMF = 1.7), although the general trend of biomagnification was comparable to the present study. Furthermore, the TMFPFOS calculated in the present study is in line with other European studies on lake and river food chains as reported by Rüdel et al. (2020a). On the other hand, Lescord et al. (2015) reported a negative relation between accumulation and trophic level for PFOS, PFNA and PFUnDA in a foodweb from the high Arctic. In a study on alpine lakes in Northern Italy, a TMFPFOS of 3 was found (Mazzoni et al., 2020). However, when they analysed a fish-only food web this value became not significant and lower than one. The presence of TMFs greater than 1 for PFOS, PFDA and PFUnDA was expected, as the biomagnification of these PFAS has been reported before (Houde et al., 2006a; Munoz et al., 2017). The observed biodilution for PFOA and PFTeDA could, in case of PFOA, be explained by differences in exposure between the mussels and fish species, as was described above. Regarding PFTeDA, its biodilution may be associated with its large molecular size, limiting the penetration of cell membranes (Conder et al., 2008; Hong et al., 2015). However, since species of different locations were compared, we might need to take into account the possible effect of location (ecological quality, physicochemical parameters) rather than just bare biomagnification and –dilution effects on bioaccumulated concentrations. This is in agreement with Munoz et al. (2017), who stated that the PFAS chemical structure is not be exclusively predictive of TMFs, since they are also influenced by trophic web characteristics.

4.4.3 Suitability of mussels in active biomonitoring of PFAS

The PFAS profiles in the mussels did not differ much among the three species (*Appendix C: Figure C.1*). Furthermore, isotopic niche determination showed an overlapping niche for both quagga mussels (*D. bugensis*) and Asian clams (*C. fluminea*). Therefore, it is appropriate to use both species in monitoring studies on locations with varying salinity and extrapolate the results. Although the PFAS profile of the blue mussels (*M. edulis*) differs slightly from those of the quagga mussels (*D. bugensis*) and the Asian clams (*C. fluminea*), this is likely the result of a smaller dataset for the first species. Therefore, more research, using a larger sample size, is necessary to fully confirm the suitability of using blue mussels simultaneously with the other two species.

Regarding their suitability in active biomonitoring, our results show that mussels can provide an overview of the contaminants present in the environment to which fish are exposed. The PFAS compounds that have been detected in the mussels were similar to those detected in the fish species, although concentrations differed, as was explained above.

Both ABM using mussels and PBM using indigenous fish, have their assets and liabilities. As the mussels provided a short time pollution profile (exposure during six weeks), fish allowed the integration of a lifetime exposure. Short time exposure might be influenced by seasonal variations in bioavailability, which is cancelled out using indigenous species. Furthermore, the numerous measurements below LOQ in mussels might give an underestimation of the situation. The bioavailability, however, is made clear through biomagnification in the accumulated concentrations of fish. Measuring in fish also may give additional information towards risk assessment for species of higher trophic levels feeding on fish, such as predatory birds or mammals (including humans). On the other hand, from an ethic perspective, the use of invertebrates might be encouraged. PFAS analysis can be done on a small amount of tissue and therefore on individual mussels, so no large numbers are needed. The results in the present study confirm the possibility of extrapolation between mussels and fish and between both fish species.

4.4.4 Human health risks

The maximum recommended amount of eel that could be consumed without posing health risks, according to the ATSDR guidelines (2019), was lower than that of perch concerning PFOA contamination, but for PFOS the opposite was true. Nonetheless, for PFOS the differences between both species (ca. 1.4 times) were smaller than for PFOA (ca. 2 times). On the other hand, when using the EFSA value (2020), the maximum recommended amounts of both fish were much lower. This sensitive value was determined with a decreased immunoresponse after consumption as a critical human health response and is to be tested against the sum of PFOS, PFOA, PFNA, and PFHxS. However, as stated in the materials section, the sum of PFOS and PFOA was used in the present study. Although, this might have led to an underestimation of the actual risk, we believe it to be a good estimation since PFOS had the largest contribution to the total PFAS sum.

Due to consumption of their catch, in Flanders mainly recreational anglers and their families may be exposed to contaminated fish. A mean consumption of 2.7 g of perch per day and 18 g of eel per day was reported in an interview on anglers (ANB-VF/2015/4). The maximum recommended amounts of fish that could be consumed without posing health risks (Q-values; *Table 4.3*), calculated using the mean concentrations in the fish species, were higher than the mean consumption amounts in Flanders for both species using the ATSDR (2019) MRL. The Q-values for eel were ca. 50 and 1.2 times higher than the reported consumption amount, for PFOA and PFOS respectively. For perch, this was ca. 630 and 6 times higher. On the other hand, using the stricter EFSA (2020) MRL, Q-values were below the reported consumption amount for eel and only 2 times higher for perch.

However, when using a worst-case scenario, based on the maximum concentrations in both fish species, health risks due to PFAS contamination are expected from the consumption of both fish species. In this worst case scenario, the maximum edible amount of perch per day was 2.62 g day^{-1} and 0.82 g day^{-1} (*Table 4.3*), calculated using the ATSDR (2019) PFOS MRL value and the EFSA (2020) MRL values, respectively. For

eel, these Q-values in the worst-case scenario are 2.17 g day^{-1} and 0.68 g day^{-1}, respectively, which are 8 to 26 times lower than the average eel consumption in Flanders.

Therefore, it is likely that the local recreational fishermen have a high chance of experiencing detrimental effects of accumulated PFAS concentrations. Evidentially, calculations were performed on mean consumption rates, indicating individuals exist that consume more. For these people even more locations might pose a health risk, since the Q-values for perch were very close to the mean consumption rate. PFOS accumulation in humans has been associated with multiple hepatotoxic, neurotoxic, reproductive, immunotoxic and thyroid disruptive effects, which could lead to severe diseases and even death (as reviewed by Zeng et al., 2019). Even at very low concentrations, PFAS can alter the lipodome, disrupting lipid and weight regulation (Gorrochategui et al., 2014). The Flemish government, however, already discourages consumption of eel and other predatory fish from Flemish waterbodies due to high concentrations of other pollutants (e.g. PCBs) (Maes et al., 2008).

4.4.5 Ecological health risk

Under the Water Framework Directive (WFD) the EU (EU, 2013) defined Biota Quality Standards (EQS_{biota}) for freshwater, threshold concentrations for protection of the integrity of aquatic ecosystems and specifically for prevention of secondary poisoning and human health risk. For perfluoroalkyl substances, 9.1 µg PFOS kg^{-1} ww was set as the (human health based) threshold ($EQS_{biota,\ hh}$). For eel and perch respectively, 44% and 58% of sampling locations exceeded the $EQS_{biota,\ hh}$, indicating potential health risks to the food web and to top predators (including humans) through fish consumption. In a German monitoring study, PFOS was above the $EQS_{biota,hh}$ in 33% of the locations in perch muscle (Rüdel et al., 2020b). However, the current $EQS_{biota,\ hh}$ is based on the previous EFSA tolerable daily intake (TDI) value for PFOS of 150 ng kg^{-1} body weight, which can be converted to 10.5 µg per day considering a 70 kg person (EFSA, 2008; Mazzoni et al., 2019). This value is more than 200 times higher than the sensitive EFSA group TWI value (EFSA, 2020) used in the human health risk determination in the present study. Furthermore, the EQS_{biota} was calculated considering a mean European daily fish

consumption of 115g (EU, 2011b), while Belgium is known to have a lower fish consumption compared to other European countries (Altintzoglou et al., 2011). All this leads to the conclusion that the current EQS_{biota} for PFOS might underestimate the risk for human health consequences through fish consumption, especially for Belgium, and needs to be revised.

On the other hand, the higher EQS of PFOS of 33 µg kg^{-1} ww (EU, 2011b) was determined specifically for protection of top predators against secondary poisoning ($EQS_{biota,\ secpois}$). Comparison to this standard resulted in an exceedance for 7% of the sampling locations for eel and 15% for perch. It was, however, stated that when determining the risk for secondary poisoning it is more appropriate to use whole fish measurements instead of fillet (EC, 2014). An average conversion factor between both matrices of about 3 was determined for perch (Rüdel et al., 2020b; Fliedner et al., 2018). This would increase the exceedance in perch to 45% of all locations.

Furthermore, the setup of our study is in line with general recommendations for biota monitoring under de WFD (EC, 2014). All biota used in the present study are considered good biomonitor species. However, in order to estimate the risk for secondary poisoning, taking into account biomagnification effects, the use of top predators (TL of 4 in freshwaters) is recommended. Both fish species included in the present study could be classified as such (TL_{perch}: 4.97 ± 0.15; TL_{eel}: 4.86 ± 0.14). Furthermore, their widespread (European) occurrence and limited home range allow for good monitoring practices (Belpaire and Goemans, 2007a; Fliedner et al., 2018). Although, within the present study a limited size range was targeted, differences in ranges and mean fish sizes between locations were detected (Teunen et al., 2020b: *Chapter 2*). This might affect the mean PFAS concentrations per location and comparison between locations, since accumulated concentrations increase with size and age (EC, 2014). As stated before, due to the high affinity of PFAS for proteins, liver tissue might have been a better matrix for sole monitoring purpose. However, since human health risk assessment was an important focus of the present study, muscle tissue was considered a more appropriate matrix. Finally, a standardization of hydrophobic compounds was proposed in the Guidance Document (EC,

2014). For mercury and perfluorooctane sulfonate (PFOS), which do partition to proteins in contrast to the other lipophilic priority compounds, a standardization to a default dry weight fraction of 26% was recommended. This approach was not included in the present study. However, the standardization had a very limited effect on or even increased the variability of measured concentrations (Fliedner et al., 2018, Valsecchi et al., 2020). Furthermore, Valsecchi et al. (2020) reported that dry weight standardization, as a proxy for protein content, for PFOS is inappropriate because PFAS bind to specific proteins.

4.5 Conclusion

Based on both ABM and PBM, our results show that PFAS are widely bioavailable across Flanders' aquatic environment. The highest concentrations were measured nearby known densely populated areas, probably with both industry and households being the main sources for PFAS pollution. The PFAS concentrations in the fish species were, in general, comparable to those reported in other industrialized and populated regions in Europe and the USA.

Although biomagnification as well as biodilution have been observed, it should be stated that this was examined on a combination of translocated and indigenous organisms, which have been exposed to different sources throughout the ABM period. Therefore, the outcomes could differ when using indigenous invertebrates, whose concentrations would reflect not only the exposure through the water, but also through the sediment. Nonetheless, translocated mussels have been proven suitable to determine which PFAS are present in indigenous fish, as PFAS profiles were similar among the different species.

Human health risks due to the consumption of PFAS-contaminated fish are expected, especially for eel. Based on the average concentrations, the recommended amounts of fish that could be consumed without posing health risks were lower than the mean consumption amounts in Flanders for eel, but not for perch. However, regarding perch, this difference was very small. Hence, when looking at a worst-case scenario, calculated using the maximum detected concentrations in fish, these recommended consumption amounts were much lower than the mean consumption amounts in Flanders.

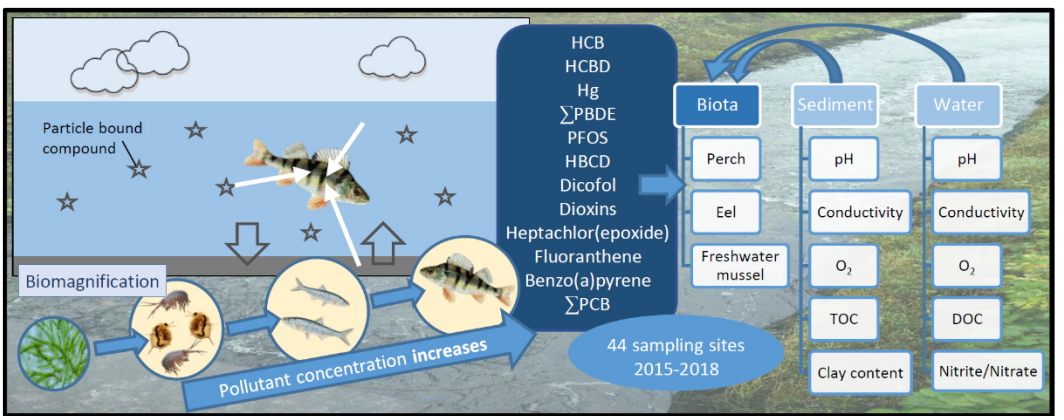
Acknowledgements

This study was partly funded by the Flanders Environment Agency and the Research Foundation Flanders (FWO, grants G018119N and 12ZZQ21N). Thimo Groffen is a postdoctoral researcher of the Research Foundation of Flanders (FWO).

We thank Tim Willems (University of Antwerp) for the UPLC analysis and Prof. Dr. Steven Bouillon (KULeuven) for the stable isotope analysis. The technical crew of INBO Linkebeek is acknowledged for their help in fish sampling.

Chapter 5

Effect of abiotic factors and environmental concentrations on the bioaccumulation of persistent organic and inorganic compounds in freshwater fish and mussels



Published as:

Lies Teunen, Maarten De Jonge, Govindan Malarvannan, Adrian Covaci, Claude Belpaire, Jean-François Focant, Ronny Blust, Lieven Bervoets, 2021. Effect of abiotic factors and environmental concentrations on the bioaccumulation of persistent organic and inorganic compounds to freshwater fish and mussels. *Science of the Total Environment 799*, 149448. https://doi.org/10.1016/j.scitotenv.2021.149448.

Abstract

Many aquatic ecosystems are under persistent stress due to influxes of anthropogenic chemical pollutants. High concentrations can harm entire ecosystems and be toxic to humans. However, in case of highly hydrophobic compounds, their low water solubility precludes direct measurement in water, and thus alternative monitoring strategies are needed. In the present study, we investigated the extent to which bioaccumulated concentrations of persistent compounds can be predicted by concentrations in environmental compartments (water and sediment). Due to their high biomagnification potential, Hg and PFOS were included in this analysis as well. At 44 field locations in Flanders (Belgium), we monitored the concentrations of 11 priority compounds and their derivatives, included in the Water Framework Directive, in both sediment and water (where feasible) and biota (European perch, European eel and freshwater mussels). Besides, some sediment (i.e. total organic carbon (TOC) and clay content) and water characteristics were measured (i.e. pH, oxygen level, conductivity, nitrate, nitrite and dissolved organic carbon (DOC)). Measurements of HCB, HCBD, cis-heptachlorepoxide, HBCD and PFOS in sediment and ∑PCB in water showed a lower detection frequency than in fish samples. While PCB profiles were comparable between all matrices, for PBDE clear differences were detected between sediment and fish profiles, with BDE99 contributing the most for sediment (34%) and BDE47 for fish (≥ 44%), followed by BDE99 for perch (28%) and BDE100 for eel (25%). Water concentrations for PFOS and benzo(a)pyrene were predictive of respective bioaccumulated concentrations. HCB, ∑PCB and ∑PBDE, concentrations in fish were dependent on sediment concentrations and negatively related to organic compound levels ($p < 0.05$). Furthermore, pH and nitrite were negatively associated with accumulated concentrations in eel for HCB and PFOS, respectively ($p < 0.05$). Significant relationships between bioaccumulation and sediment and/or water concentrations strengthened the basis for surrogate monitoring methods. Finally, the extrapolation potential of Hg, ∑PBDE, PFOS, HBCD and ∑PCB between both fish species offered new opportunities in extrapolating different European monitoring frameworks.

Keywords: POPs, Metals, European perch, Yellow eel, OCPs, Water Framework Directive

5.1 Introduction

Persistent organic pollutants (POPs) and metals may harm entire aquatic ecosystems due to losses of habitat and biodiversity and can cause chronic or acute toxicity to aquatic organisms (EC, 2008b). Originating from various anthropogenic activities (i.e. industry, agriculture, side products of combustion), pollutants may end up in the environment via discharge, leaching, erosion and atmospheric deposition (Schweitzer and Noblet, 2018). Although the use and production of many of the pollutants have declined substantially over the last decades, due to the Stockholm Convention, historical contamination is still omnipresent in the aquatic environment (Belpaire and Goemans, 2007b; Maes et al., 2008).

Hydrophobic organic compounds (HOCs) will not easily dissolve in the water and therefore are not to be measured so straightforwardly in the water phase (Belpaire and Goemans, 2007b; Jürgens et al., 2013). Additionally, weak or non-existent relationships between environmental concentrations and accumulated levels in aquatic organisms can lead to an underestimation of the risk. Therefore, monitoring water or sediment does not guarantee sufficient protection of the aquatic environment (Weltens et al., 2002). Whereas concentrations of these pollutants in abiotic compartments, especially in the water column, are often below the detection limit, they are still ubiquitous and easily detectable in biota (Belpaire et al., 2008; Weltens et al., 2002). Mercury and PFOS (perfluorooctane sulfonate), on the other hand, are known to have a high affinity for proteins and are less hydrophobic (Amlund et al., 2007; Zhong et al., 2019). However, through their chemical characteristics, HOCs, mercury and PFOS accumulate and biomagnify through the food chain, eventually reaching high, harmful concentrations in top predators and potentially being toxic to humans via consumption (EU, 2013; Lavoie et al., 2013; Van Ael et al., 2013; Wu et al., 2009).

Both water variables (e.g. oxygen content, pH, conductivity, dissolved organic carbon (DOC)) and sediment characteristics (e.g. clay content, total organic carbon (TOC)) can influence the bioavailability of pollutants. High DOC or TOC levels might result in higher organic complexation reducing the bioavailability of lipophilic compounds (Dittman and

Driscoll, 2009; Li et al., 2015b; Moeckel et al., 2014). Salinity and pH are known factors to influence chemical speciation of metals and organic compounds and induce structural and morphological modifications in organisms, affecting bioavailability and accumulation efficiency of these compounds (Dittman and Driscoll, 2009; Spry and Wiener, 1991). Furthermore, high environmental acidity reduces biodiversity and the general quality of the ecosystem (Driscoll et al., 2001; Watras et al., 1998).

In the present study, European perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*) in its yellow eel stage were selected as suitable monitoring species. They fulfil the essential monitoring purpose requirements (Belpaire and Goemans, 2007a; Teunen et al., 2020b: *Chapter 2*). Both species accumulate high HOCs concentrations because of their high position in the aquatic food chain. Furthermore, they are known resident species with a broad habitat range because of low sensitivity to environmental pollution and poor water quality. Their restricted home range allows for local contamination monitoring (Ovidio et al., 2013). Usually, eel concentrations are among the highest recorded in freshwater biota because of their high lipid content and bottom-dwelling lifestyle (Belpaire and Goemans, 2007a; Palstra et al., 2006). Because of the absence of a reproductive cycle during its juvenile yellow eel stage, seasonal fluctuation in accumulation patterns is limited (Belpaire and Goemans, 2007a).

Concerning polycyclic aromatic hydrocarbons (PAHs), their high elimination rate in fish, however, might underestimate the accumulated concentrations present in the food chain (EC, 2013). Hence, PAHs are recommended to be measured in bivalves or crustaceans instead (EC, 2013). Active biomonitoring, using translocated individuals, often has been used for monitoring bioaccumulative pollutants (Babut et al., 2020; Catteau et al., 2021). This standardized sampling technique is based on the exposure of a particular species - with controlled low background concentrations and sizes or conditions - to different sampling locations, reflecting the local pollution load. The Dreissena bivalve genus has often been used for this purpose (Bashnin et al., 2019; Teunen et al., 2021a: *Chapter 4*; Potet et al., 2018).

The effect of general abiotic factors, such as DOC/TOC, on bioavailability and thus bioaccumulation of lipophilic compounds has been studied in the past (Dittman and Driscoll, 2009; Moeckel et al., 2014). Contrastingly, to the best of our knowledge, no detailed studies looking into the effects of numerous abiotic factors and environmental concentrations on bioaccumulation of a large set of priority hydrophobic organic compounds on a vast collection of sample locations have ever been performed. In Flanders (Belgium), an extensive physicochemical and biota monitoring network allowed us to investigate the influence of environmental concentrations and characteristics on the accumulation of HOCs in the aquatic food chain – mainly focussing on the highest concentrations reached in top predators through biomagnification – in a very broad range of aquatic ecosystems. Our study's general innovative aspect is evaluating the relationship between environmental and accumulated concentrations of a large group of hydrophobic priority compounds over a vast number of sample locations with varying backgrounds and contamination levels, taking into account water quality parameters and sediment characteristics. All priority hydrophobic organic compounds (HOCs) that are of interest for monitoring in fish (and mussels), according to the Water Framework Directive (WFD), were included in the present study. Furthermore, PCBs (polychlorinated biphenyls) were included due to their highly lipophilic properties and high accumulation in predatory fish (Belpaire and Goemans, 2007b; Masset et al., 2019).

To determine the bioavailability and potential toxicity of hydrophobic organic compounds to aquatic organisms, it is imperative to identify the most relevant sample matrix to avoid over- or underestimation of environmental quality and potential human health risk assessment. The primary purpose of the present study was (1) to identify the major environmental exposure paths that affect the bioavailability of hydrophobic pollutants by relating concentrations in biota to sediment and water concentrations; (2) to evaluate the role of abiotic characteristics of water and sediment to the bioaccumulation of these pollutants and (3) to compare PCB and PBDE (polybrominated diphenyl ether) profiles between biota, water and sediment.

5.2 Material and methods

5.2.1 Sample location and target species

A total of 44 sampling locations were selected in Flanders (Belgium), reflecting extensive water body types (canals, rivers and streams) from fresh and brackish water environments (*Figure 5.1; Appendix D: Table D.1*).

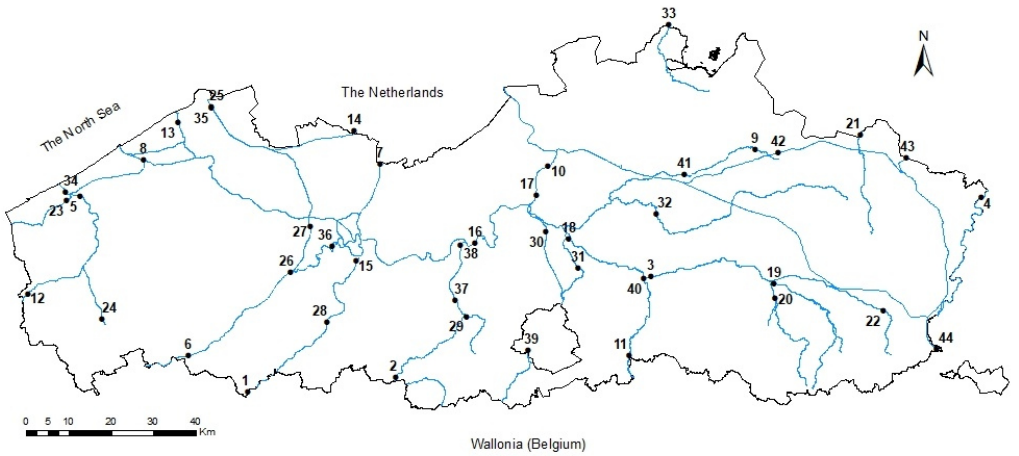


Figure 5.1. Map of Flanders (northern part of Belgium) with 44 sampling locations (2015-2018). Detailed information on sample points are indicated in Table D.1.

5.2.1.1 Fish

European eel (*A. anguilla*) in its sedentary 'yellow eel' stage and European perch (*Perca fluviatilis*) were collected from the different sites between 2015 and 2018. In total, 132 eels and 515 perches were caught using electrofishing (Deka 7000 or Deka 3000) and fyke fishing. The specific fishing method was dependent on the water body type. For a more detailed description, we refer to Belpaire et al. (2000). Fish were identified on the field, measured (total length) and weighed. Furthermore, they were sacrificed using MS-222 (Acros Organics, Geel, Belgium) and frozen for transport. A mean length between 45 and 55 cm of juvenile yellow eel was targeted. However, this was not possible for all locations. In order to have sufficient muscle tissue for analysis, the largest perches were collected. Unfortunately, it was not possible to catch both species at every sample location.

5.2.1.2 Mussels

Freshwater mussels (zebra mussel *Dreissena polymorpha* or quagga mussel *Dreissena bugensis*) were collected from reference sites. An alternative species, Asiatic clams (*Corbicula fluminea*), was exposed in locations with high salinity (mean EC20: > 2.4 mS cm^{-1}), since *Dreissena* sp. cannot survive these brackish waters. Zebra mussels were collected from the recreational lake the Blaarmeerse in Ghent (2015) and at the drinking water reservoir of the Antwerp Drinking Water Company (Waterlink) in Duffel (2016). Since zebra mussel stocks decreased, quagga mussels were used from 2017. Simultaneous exposure of both species showed a minimal variation in accumulated concentrations of benzo(a)pyrene, while fluoranthene showed a larger variation up to a factor 2 (Teunen et al., 2020). Quagga mussels were collected from the recreational lake the Nekker in Mechelen (2017 and 2018). Finally, Asiatic clams were collected from the recreational lake the Blaarmeerse in Ghent (2015-2018). Low background concentrations of organic micropollutants (PCBs, PBDEs, organochlorine pesticides and metals) were measured in mussels from these reference locations (Bervoets et al., 2005b).

The mussels were acclimated to the current environmental temperature in a semi-natural pond (mesocosm structure, University of Antwerp, Belgium; dechlorinated tap water), at least two weeks prior to exposure. Background concentrations were monitored on a subset of 5-10 mussels (*Appendix D: Table D.4*). The exposure took place during autumn and winter, as to reduce the risk of spreading these exotic species in the sampling locations since mussel reproduction is reduced at water temperatures below 12 °C (Wong et al., 2012).

The mussels were exposed for six weeks to each of the sampling locations. The set-up existed of two polyethylene cages, each consisting of two attached pond baskets (11 x 11 x 22 cm; mesh size of 2 × 4 mm), positioned 1 m below the water surface, allowing free water circulation (Bashnin et al., 2019; Bervoets et al., 2005b). Per location, a total of 70-75 and 25-30 individuals were exposed of *Dreissena* sp. and *Corbicula fluminea*, respectively. The cages were attached to bridges or solid structures on the river banks

using metal chains and locks. After recollection, mussels were depurated for at least 15 h in particle-free water from the respective sampling site at 15-20 °C.

5.2.2 Sample preparation

Fish were again weighed (Sartorius CP4202S, 0.01 g accuracy, Göttingen, Germany) and measured (total length, 1 mm accuracy) before dissection. Muscle tissue was dissected from the fish over the entire length of the body. Per location, a maximum of 20 perch and 3 eels were targeted and pooled per species (*Appendix D: Table D.1*). A total of 50 g per pool was needed to be able to perform all the analyses. Pools were homogenised in 50 mL polypropylene tubes using a stainless steel kitchen mixer (Bosch, MSM65PER) and stored at -20 °C before analysis.

The exposed mussels were dissected and pooled per location. Pools were homogenised in 50 mL polypropylene tubes using a Qiagen TissueRuptor (Qiagen, Hilden, Germany) and stored at -20 °C before analysis.

5.2.3 Analysis of biota samples

The analytical methods are reported in detail in *Appendix D1*, including quality assurance/control. The compounds measured in biota were hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), mercury (Hg), PBDEs, PFOS, hexabromocyclododecane (HBCD), dicofol, dioxins, heptachlor, trans- and cis- heptachlorepoxide, PCBs, benzo(a)pyrene and fluoranthene.

In the present study, PCB was considered as the sum of congeners PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180 (PCB ICES 7; further referred to as ∑PCB) and PBDE as the sum of BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE154 (PBDE ICES 6; further referred to as ∑PBDE). Furthermore, HBCD was calculated as the sum of α-, β- and γ-HBCD. If at least one of the congeners showed a detectable concentration, a value of ½ LOQ was used for the congeners with concentrations <LOQ (Bervoets et al., 2004; Custer et al., 2000). Dioxins were calculated as the sum of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCB-DL).

5.2.4 Analysis of water and sediment samples

The same compounds as analysed in fish were measured in water and sediment at the same locations between 2009 and 2019. These data were provided by the Flanders Environment Agency (http://geoloket.vmm.be/Geoviews/) and were available as part of their routine monitoring network. This is a licensed laboratory (as specified in the Compendium for Water sampling, measurements and Analysis (WAC)) holding a BELAC accreditation to ISO/IEC 17025 for environmental monitoring (including water and sediment) of organic compounds and metals. Furthermore, physical and chemical characteristics of water were also recorded by the Flanders Environment Agency (oxygen content, conductivity, pH, nitrate, nitrite and dissolved organic carbon (DOC)) and taken into account as predictive water variables. As for sediment, oxygen content, conductivity, pH, total organic carbon (TOC) and clay content were included. Water samples were collected and measured monthly, while for sediment, this was done yearly. To account for the effect of seasonal fluctuation, measurements of all (abiotic) environmental characteristics and concentrations were calculated as geometric means per location. No measurements were available for PBDE, HBCD, dicofol and dioxins in water. Heptachlor and dioxins data are lacking for sediment. To compare environmental concentrations and physico-chemical characteristics with accumulation of PAHs in mussels, calculations were performed on samples taken within the year of exposure were used for sediment parameters and on samples taken during the exposure period or one year difference within the same season for water parameters. This approach resulted in an adjustment of all environmental data to the short-term exposure of the mussels.

5.2.5 Statistics

Statistical analyses were performed using the software package R (R version 4.0.4; R Core Team, 2021). A Spearman correlation was performed between the abiotic parameters of water and sediment. Further statistical analysis was only performed for compounds and matrices with at least 50% of measurements above the detection limit. For each of the measured compounds, multiple regression models were constructed to establish the links between the concentration of compounds in biota and in sediment or water, as well as the

influence of abiotic factors. Using stepwise elimination of non-significant factors, a model with factors contributing to accumulation in biota was identified. Because of the skewed nature of concentration data in each compartment, they were transformed using the logarithmic function. For testing extrapolation possibilities between both fish species, a linear regression model was constructed. With this the potential to predict the accumulated concentration in one species by analysing the other one was investigated. A Kruskall-Wallis test was used to compare results for accumulated concentrations in mussels, because different species and populations were used for exposure between sample years. Significant outliers were removed using the Grubbs test in Graphpad and adjusted datasets were used for statistics and figures. Significance levels were interpreted at a p-value < 0.05.

5.3 Results

5.3.1 Results and detection frequency in different matrices

Measurements of both pollutants and characteristics in biota, sediment and water column are displayed in *Table 5.1* and *Appendix D: Table D.2 to D.4*. For a standardized comparison between different matrices, biota as well as sediment concentrations were displayed per dry weight (dw).

Table 5.1: Ranges (and median) of measured concentrations in biota (µg kg^{-1} dw), sediment (µg kg^{-1} dw) and water (ng L^{-1}). Abiotic parameters are included such as pH (-), O_2 (mg L^{-1}), conductivity (EC20; µS cm^{-1}), TOC (g C kg^{-1} dw), DOC (mg C L^{-1}), Clay (%), nitrate (mg N L^{-1}) and nitrite (mg N L^{-1}).

Parameter	*Perca fluviatilis*	*Anguilla*	Sediment	Water
HCB	<LOQ-2.5 (0.24)	0.48-33 (9.1)	<LOQ-1.4 (0.18)	<LOQ-1.3 (1.25)
HCBD	<LOQ-4.0 (1.3)	<LOQ-8.4 (<LOQ)	<LOQ-2.7 (0.5)	<LOQ
Hg	160-735 (286)	83-1526 (407)	21-2404 (94)	6.4-65 (17)
∑PBDE	<LOQ-8.4 (3.4)	0.90-285 (22)	<LOQ-8.3 (0.74)	-
PFOS	13-270 (48)	4.4-220 (27)	<LOQ-8.0 (0.25)	0.8 -14 (2.7)
HBCD	<LOQ-4.7 (1.3)	<LOQ-1106 (25)	<LOQ	-
Dicofol	<LOQ	<LOQ	<LOQ-19 (2.5)	-
Dioxins[a]	0.002-0.020 (0.007)	0.006-0.103 (0.03)	-	-
Heptachlor	<LOQ	<LOQ	-	<LOQ
tHpChlepx	<LOQ	<LOQ	<LOQ	<LOQ
cHpChlepx	<LOQ-7.8 (0.60)	<LOQ-46 (0.76)	<LOQ	<LOQ
∑PCB	3.5-700 (100)	25-4001 (1277)	<LOQ-318 (8.0)	<LOQ-9.4 (4.8)
Benzo(a)pyrene[b]	<LOQ-270 (23)	NA	<LOQ-2800 (100)	<LOQ-40 (2.6)
Fluoranthene[b]	<LOQ-1073 (169)	NA	<LOQ-7500 (250)	<LOQ-73 (11)
pH	-	-	6.8-9.1 (7.9)	7.1-8.3 (7.9)
O_2	-	-	2.8-13 (8.9)	4.4-11 (8.5)
EC20	-	-	254-10770 (848)	339-16233 (832)
TOC	-	-	1.2-50 (13)	-
DOC	-	-	-	3.7-14 (6.8)
Clay (%)	-	-	1.7-36 (6.9)	-
Nitrate	-	-	-	0.4-6.5 (3.1)
Nitrite	-	-	-	0.01-0.3 (0.09)

[a] concentrations in µg $WHO\text{-}TEQ_{2005}$ kg^{-1} dw. [b] measured in freshwater mussels instead of fish. LOQs are indicated in *Table D.9*. tHpChlepx: trans-heptachlorepoxide; cHpChlepx: cis-heptachlorepoxide.

The percentage of quantifiable concentrations (>LOQ) was determined for biota (pooled per location and per species), water and sediment (*Table 5.2*). For abiotic measurements, a geometric mean was determined per location and matrix for each compound and characteristic. Only locations with all measurements below the detection limit were scored as <LOQ (*Appendix D: Table D.6*). For some compounds in specific matrices, a significant number of sample locations resulted in values below LOQ. This was the case for HCB in water (90%) and sediment (50%), PFOS in sediment (49%), HBCD in sediment (100%) and ∑PCB in water (67%). Hg concentrations in water showed a large seasonal fluctuation, with 48% of all individual measurements being <LOQ. This was also the case for PAHs, to a lesser extent. HCBD, heptachlor and trans-heptachlorepoxide had a very low detection frequency in all tested matrices. Cis-heptachlorepoxide was easily detected in eel samples only. On the other hand, only 24% of sediment samples had dicofol concentrations above the LOQ, in contrast to biota samples (0%).

The correlation tests showed a relation between O_2 ($r^2 = 0.42$; $p < 0.05$), pH ($r^2 = 0.73$; $p < 0.001$) and conductivity (EC20; $r^2 = 0.99$; $p < 0.001$) measured in water and sediment (*Appendix D: Table D.8*). Furthermore a significant correlation was found between $pH_{sediment}$ and clay content ($r^2 = 0.42$; $p < 0.05$), between TOC and clay content ($r^2 = 0.74$; $p < 0.001$), between $O_{2,water}$ and pH_{water} ($r^2 = 0.49$; $p < 0.001$), between $O_{2,water}$ and nitrite ($r^2 = -0.64$; $p < 0.001$), between $EC20_{water}$ and nitrate ($r^2 = -0.31$; $p < 0.05$), between $EC20_{water}$ and nitrite ($r^2 = -0.32$; $p < 0.05$), between nitrate and nitrite ($r^2 = 0.56$; $p < 0.001$) and between nitrate and DOC ($r^2 = -0.48$; $p < 0.001$).

Table 5.2: Percentages of measured locations with concentrations >LOQ of hydrophobic compounds in biota (perch and eel), water and sediment and the number of measurements (N) and sample locations (n).

Compound	Perch (2015-2018)			Eel (2015-2018)			Sediment (2009-2019)			Water (2009-2019)		
	% > LOQ	*N*	n	% > LOQ	*N*	n	% > LOQ	*N*	n	% > LOQ	*N*	n
HCB	42	65	33	**100**	67	41	**50**	148	44	10	1467	30
HCBD	6.1	65	33	2.4	67	41	4.5	86	43	0	72	2
Hg	**100**	65	33	**100**	67	41	**100**	151	44	**100**[a]	2283	42
∑PBDE	**85**	65	33	**100**	67	41	**98**	149	44	NA	NA	NA
PFOS	**100**	65	33	**100**	67	41	**51**	56	41	**100**	302	26
HBCD	**61**	65	33	**95**	67	41	0	55	42	NA	NA	NA
Dicofol	0	40	29	0	26	24	24	44	38	NA	NA	NA
Dioxins	**100**	28	28	**100**	16	16	NA	NA	NA	NA	NA	NA
heptachlor	0	65	33	0	67	41	NA	NA	NA	0	1450	30
tHpClepx	0	65	33	0	67	41	0	45	31	0	7	3
cHpClepx	21	65	33	**93**	67	41	0	45	31	0	1461	30
∑PCB	**100**	65	33	**100**	67	41	**98**	133	44	33	1073	27
Benzo(a)pyrene[b]	**86**	2369	43	NA	NA	NA	**89**	150	44	**100**[a]	2015	28
Fluoranthene[b]	**98**	2369	43	NA	NA	NA	**84**	150	44	**100**[a]	2014	28

[a] large seasonal variation results in 52%, 73% and 74% of all measurements >LOQ for Hg, benzo(a)pyrene and fluoranthene respectively. [b] compounds measured in mussels instead of fish. Percentages in bold contain at least 50% of measurements >LOQ.

5.3.2 PCB and PBDE profiles

All measured matrices showed the highest concentrations for congeners PCB 153, PCB 138 and PCB 180. In general, profiles of bioaccumulated and environmental concentrations were very comparable (*Figure 5.2*). Small differences could be detected in the contribution of lower halogenated congeners to the ∑PCB (water: PCB 153 > PCB 138 > PCB 180 > PCB 101/52/28 > PCB 118; sediment: PCB 153 > PCB 138 > PCB 180 > PCB 101 > PCB 118 > PCB 52 > PCB 28; perch: PCB 153 > PCB 138 > PCB 180 > PCB 101 > PCB 52 > PCB 118 > PCB 28; Eel: PCB 153 > PCB 138 > PCB 180 > PCB 118 > PCB 101 > PCB 52 > PCB 28).

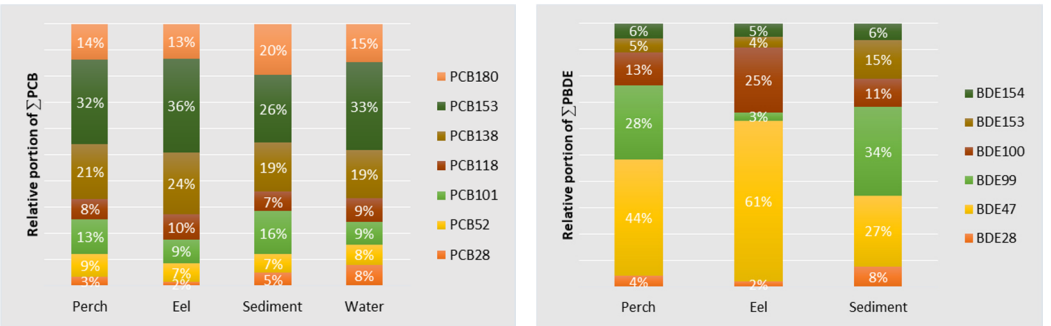


Figure 5.2: Profiles of PCBs (left) and PBDEs (right) contributions to ∑PCB and ∑PBDE in water (2009-2019), sediment (2009-2019), perch and eel (2015-2018).

For both perch and eel, BDE 47 was the main BDE congener (*Figure 5.2*). In sediment the highest concentrations were measured for BDE 99. Furthermore, a large variation existed for PBDE profiles between matrices (Sediment: BDE 99 > BDE 47 > BDE 153 > BDE 100 > BDE 28 > BDE 154; Perch: BDE 47 > BDE 99 > BDE 100 > BDE 154 > BDE 153 > BDE 28; Eel: BDE 47 > BDE 100 > BDE 154 > BDE 153 > BDE 99 > BDE 28).

5.3.3 Relationship between environmental and accumulated concentrations

Further statistical analyses and interpretations were only performed on compounds with more than 50% of bioaccumulated and environmental concentrations above LOQ. This included HCB (in eel), Hg, ∑PBDE, PFOS and ∑PCB (measured in fish) and benzo(a)pyrene and fluoranthene (measured in mussels).

Based on the correlation test results, Eq. (*5.1*) was used in a multiple regression model. Stepwise deletion was performed until all non-significant factors were removed. In the case of logic correlations - such as O_2, pH and conductivity in water and sediment and nitrate and nitrite - one of both variables was used in the multiple regression models. Water parameters were used for the characteristics measured in both abiotic matrices, since a more extensive dataset was available for water.

$$\text{Log}(\text{conc}_{\text{biota}}) = \log(\text{conc}_{\text{water}}) + \log(\text{conc}_{\text{sediment}}) + O_2 + \text{pH} + \text{EC20} + \text{TOC} + \text{clay} + \text{nitrite} + \text{DOC} \qquad (\mathbf{5.1})$$

Where $\text{conc}_{\text{biota}}$ is the bioaccumulated concentration of a specific compound in biota (µg kg^{-1} dw), $\text{conc}_{\text{water}}$ is the concentration of the same compound measured in the water column (ng L^{-1}), $\text{conc}_{\text{sediment}}$ is the concentration of that compound measured in the sediment (µg kg^{-1} dw). Furthermore, parameters added to the multiple regression models were O_2 (oxygen content; mg O_2 L^{-1} water), pH, EC20 (electrical conductivity at 20 °C; µS cm^{-1}), TOC (g C kg^{-1} dw in sediment), clay content (%), nitrite concentration (mg N L^{-1}) and DOC (mg C L^{-1}).

In these multiple regression models, accumulated ∑PBDE and ∑PCB concentrations in biota showed a positive relation with concentrations in the sediment ($p \leq 0.003$; *Table 5.3, Figure 5.3; Appendix D: Table D.10*). For PFOS, concentrations in fish could be related to water concentrations ($p \leq 0.002$). The same was true for benzo(a)pyrene concentrations in mussels and water ($p < 0.001$). Furthermore, DOC or TOC contributed significantly to the described relationship between sediment and both fish species for ∑PBDE ($p \leq 0.012$) and ∑PCB ($p < 0.001$), and for eel in HCB ($p = 0.003$). Nitrite concentration ($p = 0.028$) and magnitude of conductivity ($p = 0.012$) contributed to the relationship for PFOS in eel

and pH ($p = 0.036$) for HCB in eel. These abiotic characteristics contributed negatively to the relationship between concentrations in the environment (water or sediment) and the bioaccumulated concentrations. However, the effect of conductivity on PFOS concentrations in eel was minimal (slope of 0.0001), and therefore not included in the graphs of *Figure 5.3*. For Hg and fluoranthene, no significant ($p > 0.05$) relationships with bioaccumulated concentrations could be identified for abiotic parameters or environmental concentrations.

Table 5.3: Significant ($p < 0.05$) equations for HCB, PFOS, Hg, ∑PBDE, ∑PCB, benzo(a)pyrene and fluoranthene after stepwise deletion in multiple regression models. Significance levels of the independent parameters are indicated with letters: $^{A}p < 0.05$, $^{B}p < 0.01$, $^{C}p < 0.001$.

Compound	Significant equation	R^2 (DF)
HCB	NA	NA
	Log(eel) = 11.62 + 0.53*log(sediment)^{B} – 0.04*TOC^{B} – 1.01*pH^{A}	0.29 (37)
PFOS	Log(perch) = 3.14 + 0.80*log(water)^{C}	0.41 (20)
	Log(eel) = 3.33 + 0.59*log(water)^{B} – 0.0001*conductivity^{A} – 6.04*nitrite^{A}	0.38 (21)
Hg	NS	NS
	NS	NS
∑PBDE	Log(perch) = 2.16 + 0.29*log(sediment)^{B} – 0.15*DOC^{B}	0.48 (28)
	Log(eel) = 5.03 + 0.48*log(sediment)^{C} – 0.06*clay^{C} – 0.16*DOC^{A}	0.53 (36)
∑PCB	Log(perch) = 5.32 + 0.40*log(sediment)^{B} – 0.29*DOC^{C}	0.59 (27)
	Log(eel) = 6.30 + 0.71*log(sediment)^{C} – 0.08*TOC^{C}	0.53 (37)
Benzo(a)pyrene	Log(mussel) = 2.28 + 0.58*log(water)^{C}	0.48 (20)
	Log(*Dreissena*) = 2.61 +0.45*log(water)^{B}	0.37 (16)
Fluoranthene	NS	NS
	NS	NS

NS: not significant. NA: insufficient data above LOQ to perform statistics. Mussel includes a combination of all exposed mussels; Dreissena refers to both *Dreissena polymorpha* and *Dreissena bugensis*.

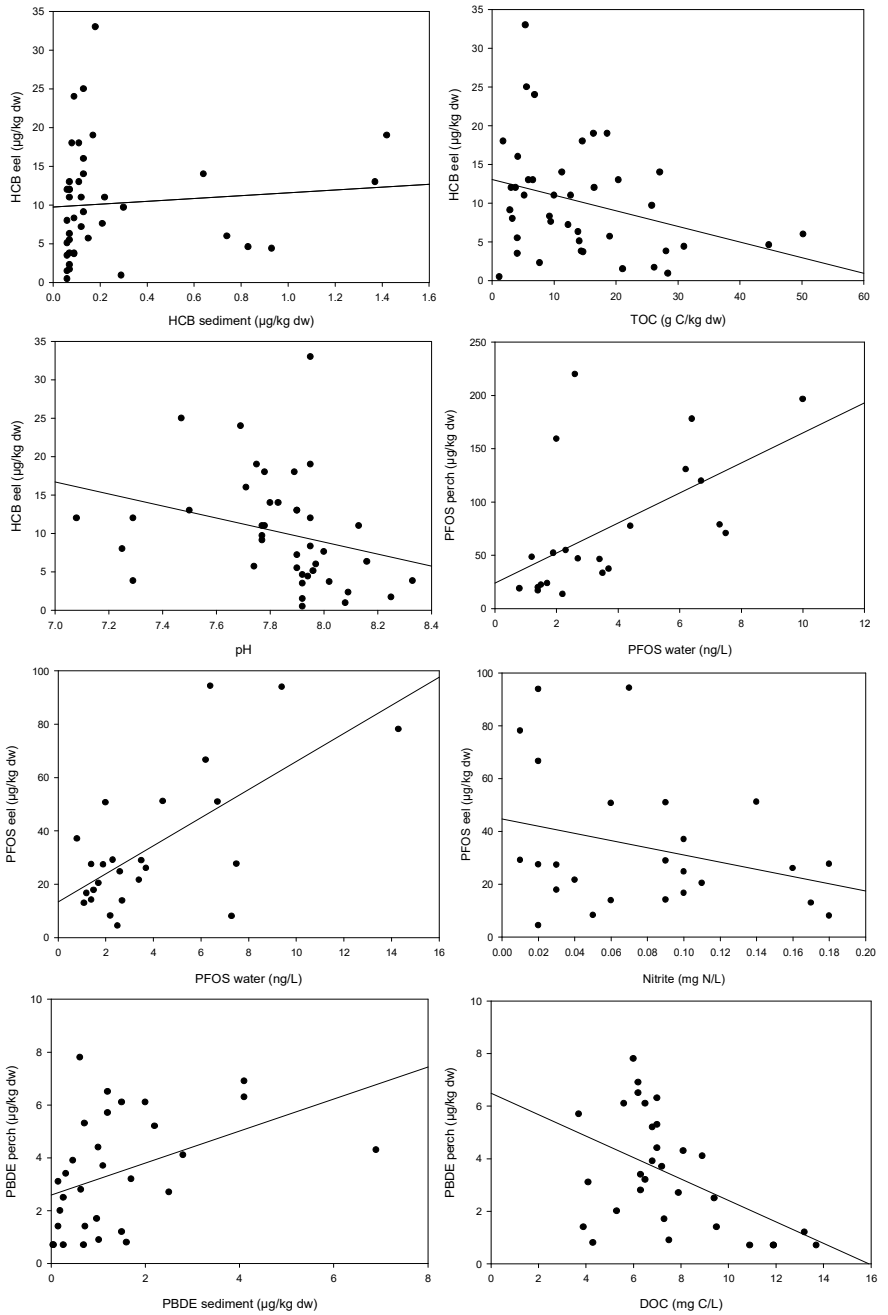


Figure 5.3: Scatterplots of abiotic factors or environmental concentrations (2009-2019) in relation to bioaccumulated concentrations in biota (2015-2018) with regression lines. Independent variables were included in the above graphs in case of significant contribution according to multiple regression models in Table 5.3. Significance levels of independent parameters are given in Table 5.3.

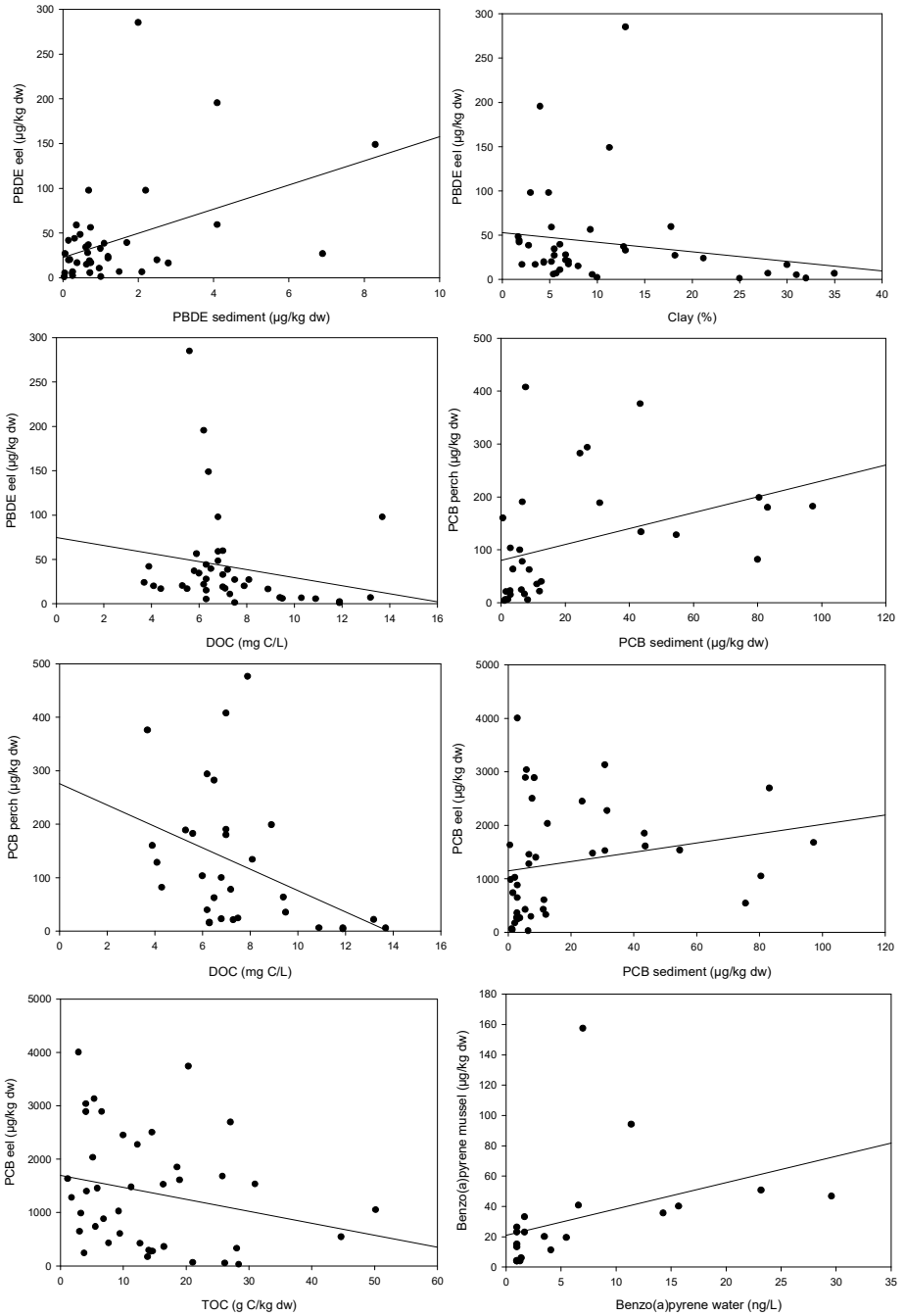


Figure 5.3 (continued).

5.3.4 Extrapolation between fish species

Linear regression models were used to test for extrapolation potential between accumulated concentrations in both fish species (*Table 5.4; Appendix D: Table D.11*). These analyses were only performed when for both species, the detection exceeded 50%. Despite the 100% detection in both perch and eel, no statistical analyses could be done for dioxins since only one species was analysed per location. We identified an equation to extrapolate concentrations between perch and eel for all other included compounds. Furthermore, it was clear that the highest concentrations were mainly measured in eel, especially in a location with a lower pollution loading (intercept >1). However, for PFOS, Hg and ∑PCB, the difference between both species decreased at highly polluted areas (slope < 1), potentially with higher accumulated concentrations in perch.

Table 5.4: Significant ($p < 0.05$) extrapolation equations for PFOS, Hg, HBCD, ∑PBDE and ∑PCB between both species. Significance levels of the independent parameters are indicated with letters: $^{A}p < 0.05$, $^{B}p < 0.01$, $^{C}p < 0.001$.

Compound	Significant equation	R^2 (DF)
PFOS	Log(eel) = 1.67 + 0.41*log(perch)^{B}	0.26 (28)
Hg	Log(eel) = 1.74 + 0.76*log(perch)^{B}	0.20 (28)
HBCD	Log(eel) = 2.68 + 1.15*log(perch)^{A}	0.16 (27)
∑PBDE	Log(eel) = 1.90 + 1.08*log(perch)^{C}	0.39 (28)
∑PCB	Log(eel) = 3.91 + 0.68*log(perch)^{C}	0.49 (28)

5.4 Discussion

5.4.1 Accumulated concentrations in biota

For comparison with literature, biota concentrations were also reported per wet weight (ww; *Table 5.5*). These calculations were performed using dry/wet weight ratios which were determined for each sample (*Appendix D: Table D.1 and D.4*). Results on bioaccumulated concentrations reported in this study are in line with reported ranges of HOCs in Flemish and European monitoring studies of perch and eel in freshwater systems. Measured concentrations of flame retardants (HBCD and PBDEs) seemed to be remarkably lower in eel and perch from Italy (Tavoloni et al., 2021) and eel from Poland (Szlinder-richert et al., 2014) compared to the present study. Accumulated concentrations of PFOS were lower in eel from Italy (Giari et al., 2015) and perch from Sweden (Åkerblom et al., 2017). Also, ∑PCB concentrations in fish from Belgium (including the present study) showed to be often higher compared to other European countries (Blanchet-letrouvé et al., 2014; Ferrante et al., 2010; Fliedner et al., 2018; Mchugh et al., 2010; Szlinder-richert et al., 2010, 2014). Finally, mercury concentrations in perch from Scandinavia were much higher than those measured in the present study (Miller et al., 2013; Negm, 2015; Sonesten, 2003). Variation in accumulation patterns and concentrations between countries, on the other hand, was to be expected to a certain level due to different pollution sources (e.g. point sources, atmospheric deposition). Furthermore, the year of sampling can be an important factor influencing results, since most of the target compounds have been banned or restricted over the past decades. Finally, due to the biomagnificating nature of these compounds, age and therefore the duration of exposure, usually shows a positive relationship with accumulated concentrations (Gewurtz et al., 2011). Furthermore, the reproductive stage is also considered an essential factor since lipophilic compounds might be excreted during the spawning of lipid-rich eggs by females (Weis and Ashley, 2007). This could result in a lower accumulated concentration in females during the reproductive season. In the present study, however, this could only be the case for perch, since eel were collected in their juvenile, non-reproductive yellow eel phase.

Table 5.5: Ranges (and median) muscle concentrations (µg kg-1 ww) of HOCs in perch and eel as measured during the present study compared to literature data from European monitoring studies.

Species	HCB	HCBD	Hg	∑PBDE	PFOS	HBCD	Dioxins[a]	∑PCB	Country	Study
Anguilla anguilla	**0.12-10 (3.1)**	**<0.5-2.1 (0.25)**	**32-332 (132)**	**0.25-106 (7.4)**	**1.5-65 (8.3)**	**<0.3-412 (9)**	**0.001-0.04 (0.008)**	**5.3-1320 (385)**	Belgium, Flanders	**Present study**
	<LOQ-62		10-535	6.9-5284[d]					Belgium, Flanders	(Belpaire & Goemans 2007a)
								11-7753 (226)	Belgium, Flanders	(Belpaire et al., 2011)
							0.057		Belgium, Flanders	(Byer et al., 2013)
	<2-19 (6.2)	<2-5	49-324 (194)	14-15	7.2-34 (24)	110-430			Belgium, Flanders	(De Jonge et al., 2014)
								5-2600 (75)[b]	Belgium, Flanders	(Malarvannan et al., 2014)
			93-173						Belgium, Flanders	(Maes et al., 2005)
	0.002-192		5.0-1185					3.5-12455	Belgium, Flanders	(Maes et al., 2008)
		<LOQ-6.9 (0.2)							Belgium, Flanders	(Roose et al., 2003)
	2.1-5.6 (3.9)			7.5-18 (8.8)[c]				433-1102 (645)	Belgium, Flanders	(Van Ael et al., 2013)
			10-708 (97)						Belgium, Flanders	(Van Ael et al., 2014)
								3.5-279	France	(Blanchet-letrouvé et al., 2014)
				0.1-18[c]	18-39			29-746[b]	France	(Couderc et al., 2015)
	3.4-50		69-314	9.2-242	8.3-49		0.006-0.045	165-1630[b]	Germany	(Guhl et al., 2014)
					37-83 (77)				Germany	(Hölzer et al., 2011)
	1.9-2.5								Great Britain	(Jürgens et al., 2013)
					<0.4-2.5 (1.0)				Italy	(Giari et al., 2015)
	<LOQ-21 (1.2)							37-518 (159)	Italy	(Ferrante et al., 2010)
				0.27-0.93 (0.50)		0.16-1.1 (0.54)			Italy	(Tavoloni et al., 2021)
				1.0-7.1[e]		1.2-15		1.9-18	Ireland	(Mchugh et al., 2010)
	0.4-23.8						0.0007-0.008	4.0-534	Poland	(Szlinder-richert et al., 2010)
				0.07-8.2		0.18-16	0.001-0.015	1.7-289	Poland	(Szlinder-richert et al., 2014)
	(<3-7.2)	(<3.3-3.9)						(5.6-10487)	Scotland	(Macgregor et al., 2010)
					7-52				The Netherlands	(Kwadijk et al., 2010)
				8.3-151		<0.1-230			The Netherlands	(Van Leeuwen and De Boer, 2008)

Perca fluviatilis	**<0.1-0.52 (0.05)**	**<0.5-0.79 (0.25)**	**32-148 (58)**	**<0.3-1.4 (0.73)**	**2.4-54 (10)**	**<0.3-1.1 (0.29)**	**0.0003-0.005 (0.001)**	**0.75-140 (18)**	**Belgium, Flanders**	**Present study**
	<2	<2	42-926 (97)						Belgium, Flanders	(De Jonge et al., 2014)
						0.42-1.6			Czech Republic	(Pulkrabová et al., 2007)
	0.26-0.33		131-509	0.7-1.4	8.1-12		0.0007-0.0015	8.2-16[b]	Germany	(Fliedner et al., 2018)
					39-150 (96)				Germany	(Hölzer et el, 2011)
			260-310						Norway	(Braaten et al., 2014)
			221-448						Finland	(Miller et al., 2013)
				<LOQ-0.024[f]		<LOQ-0.027			Italy	(Tavoloni et al., 2021)
					5.4-17				Italy	(Squadrone et al., 2015)
			20-2420						Sweden	(Sonesten, 2003)
			263-550						Sweden	(Miller et al., 2013)
			160-830		<LOQ-0.93				Sweden	(Åkerblom et al., 2017; Negm, 2015)

Concentrations in µg kg^{-1} ww. [a] concentrations in µg $WHO\text{-}TEQ_{2005}$ kg^{-1} ww. [b] PCB as sum of 6 congeners (without PCB 118). [c] PBDE as sum of 7 congeners (including BDE 183). [d] PBDE as sum of 10 congeners. [e] PBDE as sum of 11 congeners. [f] PBDE as sum of 15 congeners.

Accumulated fluoranthene concentrations in mussels from the present study (<5-107 µg kg^{-1} ww; median: 22 µg kg^{-1} ww) were higher than concentrations measured in zebra mussels from a pilot study in Flanders (9.9-10 µg kg^{-1} ww; median: 10 µg kg^{-1} ww; De Jonge et al., 2014), but lower than those previously measured in zebra mussels from the Netherlands (33-250 µg kg^{-1} ww; Hendriks et al., 1998). Although the pilot study and the present study partly covered the same sampling locations, differences in accumulated PAH concentrations might be due to the small number of locations (N = 2) in the pilot study, rather than differences in environmental concentrations and exposure. It should also be taken into account that in the present study, mussels collected from the drinking water reservoir, which were exposed in 2016 (*Appendix D: Table D.4*), showed higher background fluoranthene concentrations (21 µg kg^{-1} ww) than mussels from other reference locations (<5 µg kg^{-1} ww). This might overestimate the local pollution load. Exposed mussels from 2016, however, showed accumulated concentrations lower than the background concentrations at some locations, indicating the potential for active metabolization and elimination of fluoranthene by freshwater mussels (Thorsen et al., 2004). Furthermore, no significant difference between results of different sample years ($H_{(3)}$ = 2.34; p = 0.51), including all mussel species, was found. For benzo(a)pyrene, concentrations reported in the present study (<1-27 µg kg^{-1} ww; median: 3.0 µg kg^{-1} ww) were higher compared to the Flemish study (0.66-1.3 µg kg^{-1} ww; median: 0.98 µg kg^{-1} ww; De Jonge et al., 2014), but comparable to the Dutch results (6.0-15 µg kg^{-1} ww; Hendriks et al., 1998).

5.4.2 Detection frequency in different matrices

In the present study, water concentrations of HCB and ∑PCB were often below LOQ (*Table 5.2*). For HCB, PFOS and HBCD, this was the case in sediment. Cis-heptachlor epoxide was not detected in environmental samples. Accumulated concentrations of these compounds in biota, however, were well within the detectable range. Therefore, we conclude that the accuracy of the current method for environmental samples is not suitable for predicting the bioaccumulated concentrations, since environmental concentrations were below the LOQ. For dicofol, the opposite was true; it could only be quantified in

sediment samples. This might be a result of a higher LOQ value for biota compared to sediment rather than the absence of dicofol in biota. Previous studies showed higher accumulated dicofol concentrations in fish compared to sediment (Singh et al., 2015). HCBD, heptachlor and trans-heptachlor epoxide showed a very low detection rate in all three matrices. We did, however, have a very small sample size for HCBD and trans-heptachlor epoxide measurements in water. In general, it should be made clear that the variation in magnitude of LOQs (*Appendix D: Table D.9*) can significantly impact the detection in different matrices. In a monitoring study reporting data collected between 2000 and 2006 in two tributaries of the Nete basin in Flanders, the frequency of detection of HCB and ∑PCB in the environment (water and sediment) was even lower than the present study, despite lower LOQ values (Belpaire et al., 2008). This might indicate an increased presence of these compounds in the environment. However, in the present study, different large water basins were included, and the ∑PCB was interpreted instead of individual congeners. Van Ael et al. (2012) found a high detection rate of PBDEs and PCBs in the Scheldt basin sediment.

Furthermore, high detection rates of PCBs and organochlorine pesticides (such as HCB) have been reported in eel (Belpaire et al., 2008; Belpaire and Goemans, 2007a; Weltens et al., 2002) and in perch (Bremle and Ewald, 1995) compared to environmental matrices. Furthermore, in line with the results of the present study, a 100% detection rate in eel has been found in previous Flemish studies for mercury and PBDEs (Belpaire and Goemans, 2007a). General low detection rates for hexachlorobutadiene in fish, as was the case for the present study, have been reported before (Macgregor et al., 2010; Roose et al., 2003). To our knowledge, no publications on dicofol concentrations in perch or eel are available.

The only compounds with a high detection frequency in water in the present study were mercury, PFOS and PAHs. However, both for mercury and PAHs a large seasonal variation was observed with a noticeable amount of concentrations at each location below LOQ. In sediment, mercury, PFOS, ∑PBDE, ∑PCB and PAHs were often detected. As stated before, mercury and PFOS show a high affinity for proteins and are less hydrophobic (Amlund et al., 2007; Zhong et al., 2019) than the other priority compounds, which have a pronounced lipophilic character. The water solubility of perfluoroalkyl

substances (PFAS) is inversely proportional to the carbon chain length (Labadie and Chevreuil, 2011). Short-chain PFAS and PFOS, previously showed a high detection frequency in water and biota, whereas longer chain compounds are only to partition to sediment particles, with PFOS being the predominant compound in sediment as well (Loi et al., 2011; Xu et al., 2014). Due to the very low water solubility of PCBs and PBDEs, on the other hand, sediment particles are considered a sink for these pollutants (Kuosmanen et al., 2001; Watanabe and Sakai, 2003).

5.4.3 Relation between biota and environmental samples

In the present study, a direct relationship to accumulated concentrations in biota was identified for water concentrations of PFOS and benzo(a)pyrene and sediment concentrations of HCB, ∑PBDE and ∑PCB (*Table 5.3*). As stated before, PFOS shows a larger solubility in water compared to the other priority compounds. Therefore, a more direct relationship between dissolved concentrations in water and bioaccumulated concentrations was to be expected. However, Houde et al. (2008) reported a relationship between PFOS concentrations in invertebrates and sediment rather than between invertebrates and water concentrations. Mussels are exposed mainly to the water column as filter feeders, and therefore a direct relationship for accumulated benzo(a)pyrene to dissolved concentrations is logical. However, kinetic models based on uptake and elimination variability proved to describe the relation between PAH concentrations in zebra mussels and environmental concentrations better than simple equilibrium partitioning (Bourgeault and Gourlay-Francé, 2013). Sediment concentrations of flame retardants (∑PBDE and HBCD) showed a comparable pattern to the concentrations measured in eel in a monitoring study on 18 sampling locations in the Scheldt basin and three reference locations between 2000 and 2001 (Belpaire et al., 2003). However, Van Ael et al. (2012) found that concentrations of PCBs and PBDEs in sediment were poorly correlated to accumulated concentrations in species from different trophic levels and sediment in the Scheldt estuary. The strongest relations were found for organisms on lower trophic levels, which is expected because they are more likely to ingest sediment or particle-bound hydrophobic compounds. A correlation between eel concentrations and the

sum of sediment and dissolved concentrations of PCBs was reported in a pilot study for water quality assessment in Flanders (Weltens et al., 2002). Furthermore, less seasonal fluctuation in PCB concentrations was observed in eel compared to sediment. Both Hg concentrations in fish and fluoranthene in mussels were not significantly affected by abiotic compartments. The absence of a relationship for Hg might be explained by the fact that even at lower trophic levels Hg, in its organic form (methylmercury), is primarily ingested via food rather than absorbed from the water and is strongly bioaccumulative (Bradley et al., 2017). Accordingly, no relationship was found between environmental concentrations in water or sediment and the trophic magnification slope (TMS) of mercury (Lavoie et al., 2013). In contrast to the present study, De Jonge et al. (2014) did not find a significant relationship between accumulated HCB concentrations in eel and water or sediment concentrations, although it should be taken into account that their sample size was much smaller. For Hg, on the other hand, they found a relationship between perch and water concentrations. However, this might have been due to very high tissue concentrations at one of the sampling locations.

As a measure for hydrophobicity, the octanol-1-water partition coefficient (K_{ow}) does not provide a straightforward explanation for relationships between biota and environmental matrices. Compounds with a $\log K_{ow} < 5$ are considered to biomagnify less (Kim and Kang, 2019). Although, the $\log K_{ow}$ for PFOS is relatively low (4.49; https://pubchem.ncbi.nlm.nih.gov/). It should, however, be taken into account that this is an estimated value. Due to the surface-active properties of PFOS, the $\log K_{ow}$ cannot be measured accurately (http://www.atsdr.cdc.gov/toxprofiles/index.asp). For benzo(a)-pyrene, the $\log K_{ow}$ value is considerably higher (6.13; De Maagd et al., 1998). This is in contrast to the relationship found between water and accumulated concentrations for benzo(a)pyrene in the present study. On the other hand, the $\log K_{ow}$ for HCB (5.5), PCBs (5.6-6.6) and PBDEs (5.9-7.9) do explain the relationship with the sediment compartment due to a higher hydrophobicity (Braekevelt et al., 2003; Larsen et al., 1992; Mackay et al., 1992). PCBs and PBDEs typically show a parabolic relationship between TMF and $\log K_{ow}$ of different congeners, with TMF increasing until $\log K_{ow} = 7$, before a depression for higher hydrophobicity (Bremle and Ewald, 1995; Wu et al., 2009). This decrease in

biomagnification potential is probably due to the large molecular size slowing down transport and subsequent fast elimination (Fisk et al., 1998). However, other studies found a linear increase, even for PCBs with $\log K_{ow} > 7$ (Van Ael et al., 2013). The $\log K_{ow}$ values for PCBs and PBDEs generally increase with degree of halogenation. However, a stronger biomagnification effect for PCBs than PBDEs with the same halogenation degree has been described (Van Ael et al., 2013; Wu et al., 2009).

5.4.4 PCB and PBDE profiles

In the present study, PCB profiles in biota were comparable to those in the environmental matrices, with PCB 153 contributing the most to the ∑PCB, followed by PCB 138 and PCB 180. Belpaire et al. (2008) stated that contamination profiles are location specific, suggesting a different input of pollutants. Furthermore, they found that higher-chlorinated PCBs had a higher detection rate in sediment. Bremle and Ewald (1995) reported PCB patterns in water and sediment being comparable, with perch accumulating more higher-chlorinated PCBs than the abiotic compartments. Accordingly, higher-chlorinated PCBs show a stronger biomagnification, resulting in the largest contribution of PCB 153 in eel and PCB 28 being more prominent in sediment than in biota (Van Ael et al., 2012; Weltens et al., 2002). Similar patterns were found in eel from the North Rhine-Westphalian basin (Guhl et al., 2014) and the Scheldt river (Roosens et al., 2008). Eels from different European countries show the highest contribution for PCB138 and PCB153, with Belgian eels typically characterised by the highest PCB153 contribution followed by PCB 138 (Belpaire et al., 2011; Malarvannan et al., 2014).

The largest PBDE portion in the present study consisted of BDE 47, followed by BDE 99 in perch and BDE 100 in eel. In sediment, BDE 99 and BDE 47 respectively contributed to the total sum the most. BDE 47 is considered the main congener in biota (Roosens et al., 2008; Van Ael et al., 2012), mainly due to the previous elaborate use of pentaBDE in many countries. On the other hand, carp has been shown to metabolise BDE 99 to lower brominated congeners (e.g. BDE 47) (Stapleton et al., 2004). To a lesser extent, this was also seen for American eel (*Anguilla rostrata*; Ashley et al., 2007). Roberts et al. (2011) reported a species-dependent metabolization rate, with carp metabolizing 10-100 times

faster than salmonid fish. This slow metabolization effect might also be true for perch, leading to a larger contribution of BDE 99 to the ∑PBDE. Comparable patterns to the present study were found in eel from Belgium (Malarvannan et al., 2014; Roosens et al., 2008) and Germany (Guhl et al., 2014). A large contribution of BDE 47 and BDE 100 respectively was also reported in perch from the Scheldt, although BDE 99 contribution to the total ∑PBDE was comparable to BDE 100 (Roosens et al., 2008). Voorspoels et al. (2004) indicated that profiles of PBDE, observed in fish samples, did not correspond to profiles in sediment from the Scheldt estuary. Their sediment samples collected from the Scheldt basin showed a comparable PBDE profile to the present study (BDE 99 > BDE 47 > BDE 154 > BDE 100 > BDE 153 > BDE 28). BDE 209 has been identified as an important congener in sediments due to its widespread use of decaBDE as a fire protection surfactant (Van Ael et al., 2012; Voorspoels et al., 2004). However, it was not included in the present study as uptake by aquatic invertebrates or fish is hindered by its physical properties (i.e. high molecular mass, high $\log K_{ow}$), slow uptake, and rapid metabolization (Kierkegaard et al., 1999; Stapleton, 2003).

5.4.5 Effects of abiotic characteristics on bioaccumulation

No significant effect of environmental characteristics could be linked to bioaccumulation of mercury or fluoranthene. Lavoie et al. (2013) found an increase in the TMS of mercury with increasing DOC levels. Other studies, however, showed that DOC could limit the uptake of Hg and lipophilic compounds by reducing bioavailability due to complexation (Dittman and Driscoll, 2009; Li et al., 2015b). This effect was found in the present study for some HOCs, with a negative relationship of DOC or TOC relative to accumulated HCB, ∑PBDE and ∑PCB concentrations in biota being identified. Furthermore, negative effects were found of pH on the accumulation of HCB in eel, of nitrite on PFOS accumulation in eel and of clay on ∑PBDE accumulation in eel. Although extreme pHs have been shown to be toxic to fish (Wood, 2001), no direct relationships to bioaccumulation have been identified. Watras et al. (1998) stated that low pH potentially slows down the growth rate and, therefore, biodilution, resulting in higher accumulated concentrations at a low pH and thus a negative relation between accumulated

concentrations and pH. In the present study, however, pH values ranged from neutral to more basic (6.8-9.1). Furthermore, clay content in the sediment was correlated to TOC levels and is generally considered a sorbent for immobilization and detoxification of hazardous substances (Kowalska et al., 1994; as reviewed by Awad et al., 2019). High concentrations of nitrate, internally converted to nitrite, are toxic to wildlife and humans by reducing the oxygen-binding capacity of haemoglobin and decreasing of total haemoglobin (Monsees et al., 2017; Yang et al., 2019). However, no clear explanation could be found for the negative relation with PFOS accumulation. High nitrite content might even be expected to reduce the fish metabolism, potentially reducing the elimination rate of pollutants. Other factors not included in the present study, might influence bioaccumulation and biomagnification efficiency (e.g. food web structure, food availability, overall quality of ecosystems). For example, biomagnification of Hg has been shown to be the highest in cold and low productivity environments (Lavoie et al., 2013). On the other hand, due to the spread of sampling locations within the relatively small area of Flanders, the abiotic environmental characteristics did not show extreme situations. Therefore, studies in more variable and extreme environments might reveal stronger effects of these factors.

5.4.6 Extrapolation between perch and eel concentrations

Finally, our results showed an extrapolation possibility for bioaccumulated concentrations of PFOS, Hg, HBCD, ∑PBDE and ∑PCB between perch and eel. Weltens et al. (2002) also found a positive relationship between PCB concentrations in eel and other biota (including predator fish). Extrapolation between species can have important implications for future monitoring studies. Monitoring studies on a regular basis as e.g. demanded for the WFD (EC, 2008b) require significant efforts in field work in order to collect the required specimens for analysis. Practical constraints and limits in the distribution and abundance of the targeted species often impede the collection of sufficient suited fish samples. In our study in respectively 25 and 7% of the sites perch and eel could not be sampled (sufficiently). Extrapolation from one species to another can now complement the missing gaps. Our equations may also be useful when comparing and intercalibrating

datasets from different monitoring networks (e.g. different European countries). On the other hand, European eel stocks have been declining over the last decades, probably due to high pollutant levels (Palstra et al., 2006; ICES, 2020), resulting in an IUCN red list status 'critically endangered' (Jackoby and Gollock, 2014). Due to their high fat content, eels tend to accumulate very high levels of lipophilic compounds, facilitating detection and analysis. The present study revealed the European perch (IUCN red list status: 'low concern'; Freyhof and Kottelat, 2008) as a valid alternative.

However, in order to extrapolate accumulation between multiple species from different (European) monitoring programs, species-specific, lifestyle-based traits should be taken into account (e.g. lipid content, trophic level). In the guidance document of the EU on biota monitoring, a standardization based on lipid content, trophic level (TL) and dry weight was proposed (EC, 2014). Concentrations of hydrophobic compounds should be standardized for an individual with a TL of 3-4, containing 5% lipid content. For mercury and PFOS, on the other hand, a standardization for TL and 26% dry weight is recommended, due to their affinity for proteins.

Trophic levels of perch and eel in the Flemish water bodies were not significantly different (Teunen et al., 2021a: *Chapter 4*). Higher concentrations of HOCs in eel compared to perch, on the other hand, were probably a result of the high lipid content in eel (*Appendix D: Table D.1*).

5.5 Conclusions

Accumulated HOCs concentrations in aquatic biota, especially high trophic levels, are generally much higher than concentrations measured in abiotic environmental matrices, allowing for easier detection and analysis of these compounds. Bioaccumulated concentrations are not related merely to environmental concentrations. Frequently, other abiotic parameters (such as organic content) can affect bioavailability and metabolization processes. In the present study, we found a positive relation between bioaccumulated concentrations and dissolved water concentrations for PFOS and benzo(a)pyrene and between bioaccumulated concentrations and sediment concentrations for HCB, ∑PBDE

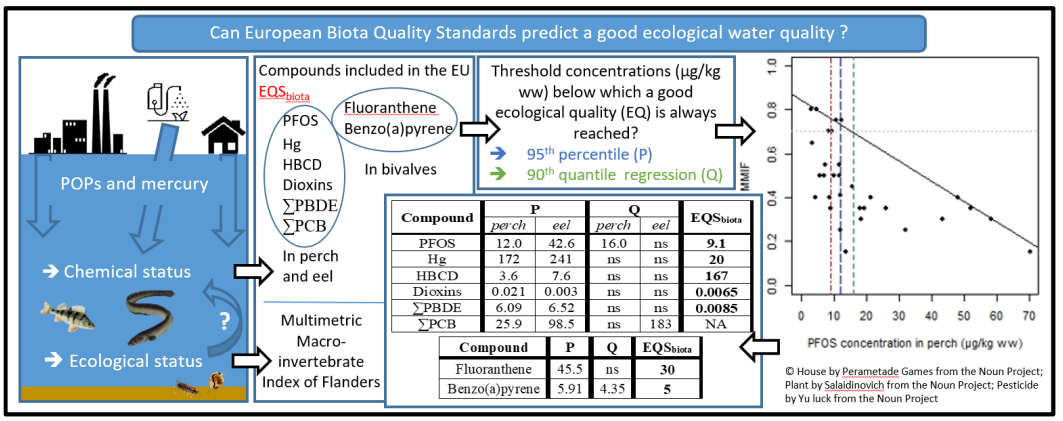
and ∑PCB. Furthermore, an additional negative effect of TOC or DOC was detected for the latter group. PCB profiles between all matrices were comparable, while PBDE profiles showed evidence of metabolization of higher halogenated congeners by the fish. In general, we advise using biota over environmental sampling for monitoring purposes since bioaccumulation and magnification of hydrophobic compounds is a complex process with numerous mediating factors at play. Therefore, modelling concentrations in top predators based on environmental measurements are likely to underestimate or misinterpret effective body burdens. Furthermore, due to seasonal variation, especially for water concentrations, elaborate environmental sampling is needed to predict accumulated concentrations in biota. Finally, we observed an extrapolation potential between perch and eel for PFOS, Hg, HBCD, ∑PBDE, and ∑PCB concentrations. This allows for implementing missing gaps in datasets when field surveys failed to collect suitable fish samples. Equations in bioaccumulation between species may offer new opportunities in calibration exercises between monitoring frameworks of different European countries.

Acknowledgements

This study was partially funded by the Flanders Environment Agency. The technical crew of INBO Linkebeek is acknowledged for their help in fish sampling. We would like to thank Annelies Rogge (VMM) and the VMM laboratory for their aid with the methodology description. We thank Tim Willems for the PFOS analysis in biota and Dr. Valentine Mubiana for the Hg analysis in biota (both University of Antwerp).

Chapter 6

The ecological relevance of European Biota Quality Standards on the macro-invertebrate community



Compound	P		Q		EQSbiota
	perch	*eel*	*perch*	*eel*	
PFOS	12.0	42.6	16.0	ns	**9.1**
Hg	172	241	ns	ns	**20**
HBCD	3.6	7.6	ns	ns	**167**
Dioxins	0.021	0.003	ns	ns	**0.0065**
∑PBDE	6.09	6.52	ns	ns	**0.0085**
∑PCB	25.9	98.5	ns	183	NA

Compound	P	Q	EQSbiota
Fluoranthene	45.5	ns	**30**
Benzo(a)pyrene	5.91	4.35	**5**

Published as:

Lies Teunen, Maarten De Jonge, Govindan Malarvannan, Adrian Covaci, Claude Belpaire, Jean-François Focant, Ronny Blust, Lieven Bervoets. The relevance of European Biota Quality Standards on the ecological quality as determined by the multimetric macro-invertebrate index: a Flemish case study. *Ecotoxicology and Environmental Safety 231,* 113222.

Abstract

European Biota Quality Standards (EQS_{biota}), for compounds with low water solubility and high biomagnification, were derived to sustain water quality and protect top predators and humans from secondary poisoning. In reality, for multiple compounds, an exceedance of these standards is often reported in literature without a decrease in ecological water quality determined by biotic indices. In the present study, threshold concentrations were defined in biota (from 44 sampling locations throughout Flanders (Belgium)), above which a good ecological water quality, assessed by the Multimetric Macroinvertebrate Index Flanders (MMIF), was never reached. Threshold values were compared to current $EQS_{biota.}$ Accumulated perfluorooctane sulfonate (PFOS), mercury (Hg), hexabromocyclododecane (HBCD), polybrominated diphenyl ethers (PBDEs), dioxins and polychlorinated biphenyls (PCBs) concentrations were measured in muscle tissue of European yellow eel (*Anguilla anguilla*) and perch (*Perca fluviatilis*). Fluoranthene and benzo(a)pyrene were also analysed in translocated mussels (*Dreissena bugensis, D. polymorpha* and *Corbicula fluminea*). Threshold values could only be calculated using a 90^{th} quantile regression model for PFOS (in perch; 12 µg kg^{-1} ww), PCBs (in eel; 328 µg kg^{-1} ww) and benzo(a)pyrene in mussels (4.35 µg kg^{-1} ww). The lack of a significant regression model for the other compounds indicated an effective threshold value higher than the concentrations measured in the present study. Alternatively, the 95^{th} percentile of concentrations measured in locations with a good ecological quality (MMIF ≥ 0.7), was calculated for all compounds as an additional threshold value. Finally, fish concentrations were standardized for 5% lipid content (or 26% dry weight content for PFOS and Hg). Threshold values for PFOS and benzo(a)pyrene and the 95^{th} percentiles for dioxins and fluoranthene were comparable to the existing standards. For all other compounds, the 95^{th} percentile was higher than the current EQS_{biota}, while for HBCD it was lower. These results strongly advise revising and fine-tuning of the current EQS_{biota}, especially for ∑PBDE and HBCD.

Keywords: POPs, mercury, biomonitoring, fish, bivalves

6.1 Introduction

Persistent organic pollutants (POPs) and metals in the aquatic environment, mainly anthropogenically introduced, might lead to chronic and acute toxicity in organisms and biodiversity loss (EC, 2008b). Since 2000, the EU implied that a 'good water quality' should be reached and maintained for all water bodies by their member states within the Water Framework Directive (WFD), originally by 2015 (EC, 2000), currently postponed to 2027. Consequently, Environmental Quality Standards were set for a selection of priority substances in order to protect aquatic environments against the adverse effects of chemical pollution (EC, 2008b). However, a specific set of hydrophobic/proteonophilic priority compounds needs to be measured in biota because of their low solubility in water (EU, 2013). Due to their biomagnification ability, these compounds may reach high bioaccumulated concentrations in higher tropic levels. Therefore, they are to be monitored in fish to avoid the risk of secondary poisoning of top predators (such as fish-eating birds and mammals), including for humans (EC, 2014). An exception was made for polyaromatic hydrocarbons (PAHs), benzo(a)pyrene and fluoranthene, because of their fast metabolization and elimination by fish (EC, 2014; Van der Oost et al., 1994). Instead, these PAHs are to be measured in bivalves or crustaceans.

Besides the chemical status and hydromorphological, the ecological status also determines the water quality. Among other anthropogenic pressures possibly affecting aquatic ecosystems (e.g. fishing, climate change, habitat deterioration), pollution also directly affects the general ecosystem health (Bervoets et al., 2005a, Burdon et al., 2019). Their community structure will reflect healthy ecosystems since they can only be maintained by a well-balanced and adaptive community (Costanza, 1992; Van Ael et al., 2015). To allow for comparison between European member states, the ecological water status assessment is to be presented using a harmonized tool, i.e. the Ecological Quality Ratio (EQR), comparing the ecological quality to reference locations (EC, 2000). The EQR score ranges between 0 and 1, reflecting a very poor to very good ecological quality. For rivers and lakes, the EQR monitoring should be based on the status of multiple relevant biological quality elements, including phytoplankton, macrophytes and phytobenthos, benthic

invertebrates and fish fauna. The ecological quality of aquatic environments is most often assessed using biotic indices.

Macroinvertebrate presence and abundance is considered a longstanding standard monitoring tool for evaluating the general ecosystem health. Biotic indices based on macroinvertebrate communities have been widely used and adapted to local conditions (Moya et al., 2011; Pond et al., 2013; Woodiwiss, 1964). The Multimetric Macroinvertebrate Index for Flanders, the northern part of Belgium (MMIF) was updated by Gabriels et al. (2010) in order to comply with the WFD guidelines and take into account the typology of the sampling site.

The relationship between accumulated concentrations in fish and the ecological water quality might have been used to determine threshold values before (Awrahman et al., 2016; Bashnin et al., 2019; De Jonge et al., 2013; Rainbow et al., 2012; Van Ael et al., 2014, 2015). However, to our knowledge, this has never been done on an elaborate dataset including the priority substances enclosed in the European Biota Quality Standards. The present study aimed to (1) determine threshold values for bioaccumulated concentrations of POPs and mercury above which the ecological water quality was never good and (2) compare these threshold values to existing EQS_{biota} and evaluate their suitability as a protective measure for the aquatic ecosystem quality.

6.2 Materials and Methods

6.2.1 Sampling locations and species

Biotic samples were collected and analysed as part of an extensive monitoring study on the European Biota Quality Standards in Flanders (the northern part of Belgium) between 2015 and 2018 (Teunen et al., 2020b: *Chapter 2*). Sampling locations (N = 44) were selected from the existing monitoring network used to implement the WFD by the Flanders Environment Agency, with water bodies characterized as canals, rivers and streams. For a detailed view of sampling locations, we refer to *Figure 5.1* (*Chapter 5*) and *Appendix D: Table D.1*.

6.2.2 Passive biomonitoring using indigenous fish species

Fish collection was performed by the Research Institute for Nature and Forest (INBO). Two predatory fish species, perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*), were collected using electrofishing (Fishtronics Rudd and Smith Root type VVP 15 C) and/or fyke nets. European eels were targeted in their juvenile yellow eel stage, ranging between 45 and 55 cm total length. Unfortunately, both species could not be caught at all sampling locations. In total, 515 perches and 132 eels were collected. The numbers and species collected per location and sampling years are given in *Appendix D: Table D.1*. Fish were sacrificed using MS-222 (Acros Organics, Geel, Belgium), chilled on ice for transport and subsequently frozen at -24°C.

Using European eels might raise concerns due to their critically endangered status. However, to understand the effects of pollutants on its population decline, it is imperative to continue monitoring and studying this species. Furthermore, the bioaccumulation of lipophilic compounds, as those included in the EQS_{biota}, is incomparable to any other fish species due to the high fat content in eel. In order to minimize the effect on the population, however, accumulated concentrations collected from these samples were used in multiple studies (present, Teunen et al., 2020b; Teunen et al., 2021b: *Chapter 5*; ICES reports Belgium: e.g. Belpaire et al., 2017). Finally, as previously reported by Belpaire and Goemans (2007a), eels used for monitoring purposes are only a negligible portion compared to annual catch and consumption of eel by anglers in Belgium.

6.2.3 Active biomonitoring using translocated bivalves

In order to have sufficient individuals of the same species to compare among locations, active biomonitoring was performed for measuring PAHs in bivalves. This technique implies the translocation of certain species, preferably collected from a reference location with low background concentrations, to the study sites. The accumulated pollutant concentrations will then reflect the local pollution load after a sufficient exposure time. In the present study, freshwater bivalves of the *Dreissena* genus were used. However, in brackish waters (mean EC20 > 2.4 mS cm^{-1}; mean salinity: > 1.2 g L^{-1}), Asian clams (*Corbicula fluminea*) were used instead, a species able to cope with higher salinity levels.

Mussels were exposed for six weeks at the same locations and in the same period that the fish sampling took place (*Appendix D: Table D.1*).

Reference locations were selected based on low background concentrations of organic micropollutants (polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs)) previously measured in indigenous mussels (Bervoets et al., 2005b). Zebra mussels (*Dreissena polymorpha*) were collected from the recreational lake Blaarmeerse in Gent in 2015 and from the drinking water reservoir of the Antwerp Drinking Water Company (Water-link) in Duffel in 2016. From 2017 onward, quagga mussels (*Dreissena bugensis*) collected from the recreational lake the Nekker in Mechelen were exposed, due to the declining population of zebra mussels. In 2016, both *D. polymorpha* and *D. bugensis* were exposed in 5 locations simultaneously in order to compare bioaccumulation between the two species. All Asian clams were collected from the Blaarmeerse in Ghent.

At least two weeks prior to exposure, the mussels were acclimated to ambient temperature in a semi-natural pond filled with dechlorinated tap water (mesocosm structure, University of Antwerp, Belgium). A subset of 30-60 individuals was kept separate for analysis of background concentrations. Mussels of comparable size were exposed at each site during six weeks in the water column in two cages made of polyethylene pond baskets (11 x 11 x 22 cm; mesh size 2 x 4 cm) to allow free water circulation (Bashnin et al., 2019; Bervoets et al., 2005b; Smolders et al., 2002). The cages were positioned approximately 1 m below the water surface and were attached to bridges or other solid structures on the river banks using metal chains and locks. Anticipating possible mortality and ensuring sufficient tissue for analysis, 70-75 *Dreissena sp.* or 25-30 *Corbicula fluminea* specimens were exposed per location. To reduce the risk of spreading these alien species to the sampling locations, exposure was performed during autumn and winter since water temperatures below 12°C reduce mussel reproduction (Wong et al., 2012). Furthermore, a previous Flemish study has shown that *Dreissena* species are already widespread through locations providing the adequate environment for this species to survive and reproduce (i.e. hard substrates to attach to) (Bervoets et al., 2004) After recollection,

particle-free water from the respective sampling sites was used to depurate the mussels for at least 15h at 15-20°C before dissection.

6.2.4 Sample preparation and analysis

Before dissection, fish were measured (up to 1 mm) and weighted (Sartorius CP4202S, up to 0.01 g, Göttingen, Germany) (*Appendix D: Table D.1*). Fish muscle tissue was collected, while for mussels, the whole soft tissue was removed from the shell and weighted. In the case of *Dreissena sp.,* byssus threads (i.e. the filament bundle used for attachment to substrates) were removed because they complicate digestion and homogenization. Samples were pooled and homogenized (fish: stainless steel kitchen mixer, Bosch, MSM65PER; mussels: Qiagen TissueRuptor, Qiagen, Hilden, Germany) per species per location and frozen at -20°C until further analysis.

Analytical methods for the bioaccumulated concentrations of the persistent organic compounds and mercury used in the present study have been reported in *Appendix D1*. In addition to the compounds for which the European Commission (EU, 2013) defined specific EQS_{biota} (i.e. hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), mercury (Hg), perfluorooctane sulfonate (PFOS), hexabromocyclododecane (HBCD), polybrominated diphenyl ethers (PBDEs), dicofol, dioxins, heptachlor and –epoxide, and PAHs fluoranthene and benzo(a)pyrene) also polychlorinated biphenyls (PCBs) were measured in fish muscle tissue, due to their high biomagnification potential.

Total PCB, further referred to as ∑PCB, was calculated as the sum of congeners PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180 (PCB ICES 7). The total of the polybrominated diphenyl ethers (PBDE), further referred to as ∑PBDE, was calculated as the sum of BDE 28, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154 (PBDE ICES 6). HBCD existed of the sum of α-, β- and γ-HBCD. Dioxins were considered the sum of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDF's) and dioxin-like PCBs (PCB-DL). If the concentration of at least one of the congeners of a specific compound was above the limit of quantification (LOQ), ½ LOQ was used for the congeners with concentrations <LOQ to calculate the sum of that compound (Bervoets et al., 2004; Custer et al., 2000). Of all compounds included in the

present study, only those with more than 50% of the measurements above the LOQ were further included in the statistical analysis and determination of threshold values (*Appendix E: Table E.1*). This was the case for PFOS (100% > LOQ), Hg (100%), HBCD (perch: 61%, eel: 95%), dioxins (100%), ∑PBDE (perch: 85%, eel: 100%), ∑PCB (100%), fluoranthene (98%) and benzo(a)pyrene (86%) (*Table 5.2, Chapter 5*).

Except for dioxins, all compounds were calculated in µg kg^{-1} wet weight (ww). Concentrations of dioxins were expressed in µg WHO_{2005} toxic equivalent (WHO_{2005}-TEQ) kg^{-1} ww (Van den Berg et al., 2006).

6.2.5 Ecological quality assessment

The ecological quality assessment of the aquatic environment was performed using the Multimetric Macroinvertebrate Index of Flanders (MMIF), taking into account water body type. The MMIF has been specifically created for rivers and lakes. However, according to the WFD (EC, 2000), artificial and heavily modified water bodies are assigned the "most similar type". The MMIF is assessed according to that type with a class boundary adjusted according to the ecological potential of that water body. All ecological quality data were available from a long-term monitoring program of the Flanders Environment Agency (http://geoloket.vmm.be/Geoviews/). Only ecological assessments performed no longer than two years before or after sample collection were used. We believe the use of this large time frame to be appropriate since very limited variation in MMIF scores was detected within sites over multiple years (data between 2013-2019; *Appendix E: Table E.3*).

The MMIF was calculated as described by Gabriels et al. (2010). This index is in line with the WFD definitions for invertebrate assemblages assessment. The metrics included for the calculation of the MMIF were taxa richness, number of Ephemeroptera, Plecoptera and Trichoptera taxa (EPT), number of other (non-EPT) sensitive taxa, the Shannon-Wiener Diversity (SWD; Shannon and Weaver, 1949) index and the mean tolerance score. Gabriels et al. (2010) published reference values for each metric for all lake and river types. Based on threshold values, sampling locations were scored between 0 and 4 for each metric. Here, a value of 4 indicated a value closest to the reference condition. The

sum of these scores was divided by 20. This resulted in an overall EQR between 0 and 1, with 0 referring to a very poor ecological quality and 1 representing a location with a very high ecological quality. Macroinvertebrates were collected using a standard handnet (200 x 300 mm frame, 300-500 µm mesh) or deploying artificial substrates for at least three weeks (when the previous method was not possible due to high depth of the water body) as described by De Pauw and Vanhooren (1983) and Van Ael et al. (2015).

6.2.6 Statistical analysis

Statistical analyses were performed using the software package R (R version 4.0.4; R Core Team, 2021). Relationships between bioaccumulated concentrations and ecological water quality were investigated with two methods (i.e. 95th percentiles, 90th quantile regression model). For both approaches, a MMIF score of 0.7, indicating a good ecological quality, was considered as a threshold value (Gabriels et al., 2010). Significant outliers of individual parameters were removed using the Grubbs' test in Graphpad. Adjusted datasets were then used for statistics and figures, while original datasets are presented in *Appendix E: Table E.1*. The significance level was set at a p-value < 0.05. All calculations and statistics were performed for the compounds of interest individually.

Two different approaches were used for assessing threshold values for pollutant concentrations in perch and eel (or mussels), above which a good ecological quality with respect to the macroinvertebrate community was never achieved. This was done by calculating the 95th percentile of the accumulated concentrations measured in locations with a MMIF value of at least 0.7 or constructing a 90th quantile regression model of accumulated concentrations against the EQR value (Bervoets et al., 2016; Van Ael et al., 2015).

6.2.6.1 90th quantile regression model

A quantile regression model is often used when an ecological response in the field is expected to be caused by multiple environmental factors, besides the stressors of interest (e.g. habitat, species interactions, abiotic conditions) (De Jonge et al., 2013; Iwasaki and Ormerod, 2012; Van Ael et al., 2015). In the present study, also effects and interactions of other pollutants, which are location-specific, might result in a low ecological quality

even when the contaminants of interest show low concentrations. A 90th quantile regression model (quantreg package, R) only considers the highest (90th quantile) ecological responses (EQR) as a function of the accumulation of a specific pollutant, compensating for these unmodelled factors. In the case of a significant model, a threshold concentration for a MMIF score of 0.7 could be calculated. This allows to determine the highest concentrations in the sampled biota (fish and/or bivalves) at which a good ecological quality is still achieved. In the case of a significant 90th quantile regression model, a threshold accumulation level for the compound could be derived.

6.2.6.2 Normalization of fish data based on lipid or dry weight content

Due to the lipophilic characteristics of the priority compounds in the present study, differences in lipid content between species might lead to a variation in accumulation. Eel showed higher and more variable lipid concentrations (12% ± 6.7%) compared to perch (0.84% ± 0.13%) (Teunen et al., 2021b: *Chapter 5*), leading to higher concentrations in eel than in perch in general. A standardization based on a lipid content of 5% was proposed in the Guidance document No. 32 of the European Commission on biota monitoring and the implementation of the EQS_{biota} (EC, 2014). An exception was made for Hg and PFOS, which are, because of their high affinity for proteins rather than for lipids (Amlund et al., 2007; Jones et al., 2003; Zhong et al., 2019), normalized based on a dry weight content of 26%. Furthermore, the calculations of the relationships between bioaccumulated concentrations and ecological water quality were repeated for the normalized concentrations to compare results for perch and eel in a more standardized manner.

This normalisation was not performed for PAHs in mussels since lipid concentrations were within range (1.2% ± 0.38%) of the 1% that was proposed in the Guidance document (EC, 2014) and multiple species were not exposed at the same locations.

6.3 Results and discussion

6.3.1 Accumulated concentrations in biota and ecological quality

Accumulated concentrations of the priority compounds included in the present study (*Table 6.1*) have previously been reported, including a comprehensive literature review, in Teunen et al. (2021b: *Chapter 5*). Therefore, these were not discussed in the present study. Mean concentrations per location and per species are given in *Appendix E: Table E.1*.

Table 6.1: Ranges (and median) of measured concentrations in biota (µg kg^{-1} ww), including standardized fish concentrations for a 5% lipid content or 26% dry weight content (for PFOS and Hg).

Parameter	Accumulated concentrations		Standardized concentrations	
	Perch	Eel	Perch	Eel
PFOS	2.4-54 (10)	1.5-65 (8.3)	3-70 (12)	1.1-56 (6.7)
Hg	32-148 (58)	32-332 (132)	41-192 (74)	21-389 (104)
HBCD	<LOQ-1.1 (0.29)	<LOQ-412 (9.0)	0.68-5.7 (1.7)	0.06-290 (4)
Dioxins[a]	0.0003-0.004 (0.001)	0.001-0.04 (0.008)	0.002-0.03 (0.009)	0.002-0.02 (0.004)
∑PBDE	<LOQ-1.4 (0.73)	0.25-106 (7.4)	0.91-9.7 (4.6)	0.13-75 (3.5)
∑PCB	0.75-140 (18)	5.3-1320 (385)	5-769 (117)	7.1-1143 (159)
Fluoranthene[b]	<LOQ-107 (22)	NA	NA	NA
Benzo(a)pyrene[b]	<LOQ-27 (3.0)	NA	NA	NA

[a] concentrations in µg WHO-TEQ$_{2005}$ kg^{-1} ww. [b] measured in mussels instead of fish. LOQs are indicated in *Appendix E: Table E.1*.

Zebra mussels collected from the drinking water basin (2016) showed high fluoranthene background concentrations (21 µg kg^{-1} ww). However, in some locations the measured accumulated concentrations in mussels after field deployment were lower than the background concentrations (Teunen et al., 2021b: *Chapter 5*). This reveals a high elimination rate for fluoranthene (Thorsen et al., 2004). Therefore, we believe that concentrations of this compound measured in mussel tissue after exposure might still reflect the local pollution profile. On the other hand, a possible overestimation of the situation in the field should be taken into account, specifically for fluoranthene. However, no significant difference in accumulated fluoranthene concentrations was detected between sampling years ($H_{(3)} = 2.34$; $p = 0.51$). For benzo(a)pyrene, all reference locations showed background concentrations below the LOQ of 1 µg kg^{-1} ww.

In the present study, the EQR based on the MMIF ranged between 0.15 and 0.80 with a mean score of 0.45 (*Appendix E: Table E.1*). According to the criteria used, only 20% of the locations showed a good ecological quality (MMIF ≥ 0.7).

6.3.2 Ecological threshold values

Threshold values for pollutant concentrations in perch and eel (or mussels) above which a good ecological quality was never achieved were calculated using the 95th percentile and 90th quantile regression model. Threshold values, significant regression models and visualization of the results can be found in *Tables 6.2-6.3* and *Appendix E: Figure E.1*, respectively. For all compounds, the eel threshold values on a wet weight basis were much higher than those in perch (*Table 6.2*). The threshold values for PAHs were the same when calculated for all mussel species or only including the *Dreissena* spec. (*Appendix E: Table E.4*) and were thus reported combining the different mussel species (*Figure 6.1*).

Table 6.2: Threshold values (µg kg^{-1} ww) for different compounds based on the 95th percentile and 90th quantile regression approaches.

Compound	95th percentile		90th quantile regression	
	perch	*eel*	*perch*	*eel*
PFOS	9.50	48.8	12.0	ns
Hg	133	228	ns	ns
HBCD	0.64	35.2	ns	ns
Dioxins[a]	0.0038	0.011	ns	ns
∑PBDE	1.10	18.5	ns	ns
∑PCB	4.80	312	ns	328
Fluoranthene[b]	45.5		ns	
Benzo(a)pyrene[b]	5.91		4.35	

[a] concentrations in µg WHO-TEQ_{2005} kg^{-1} ww. [b] PAHs were measured in bivalves instead of fish. ns: no threshold value could be calculated because no significant ($p < 0.05$) quantile regression model was found.

Table 6.3: Results of the 90th quantile regression models. In case of a significant model (p < 0.05) an equation was constructed.

Compound	90th quantile regression model	
	perch	***eel***
PFOS	EQR = -0.012[PFOS] + 0.844 (p<0.001)	ns (p=0.85)
Hg	ns (p=0.81)	ns (p=0.65)
HBCD	ns (p=0.49)	ns (p=0.77)
Dioxins[a]	ns (p=0.83)	ns (p=0.30)
∑PBDE	ns (p=0.45)	ns (p=0.33)
∑PCB	ns (p=0.10)	EQR = -0.0004 [PCB] + 0.831 (p<0.001)
Fluoranthene[b]	ns (p=0.25)	
Benzo(a)pyrene[b]	EQR = -0.026[benzo(a)pyrene] + 0.813 (p=0.005)	

[a] concentrations in µg WHO-TEQ_{2005} kg^{-1} ww. [b] PAHs were measured in bivalves instead of fish. ns: the quantile regression model was not significant ($p > 0.05$).

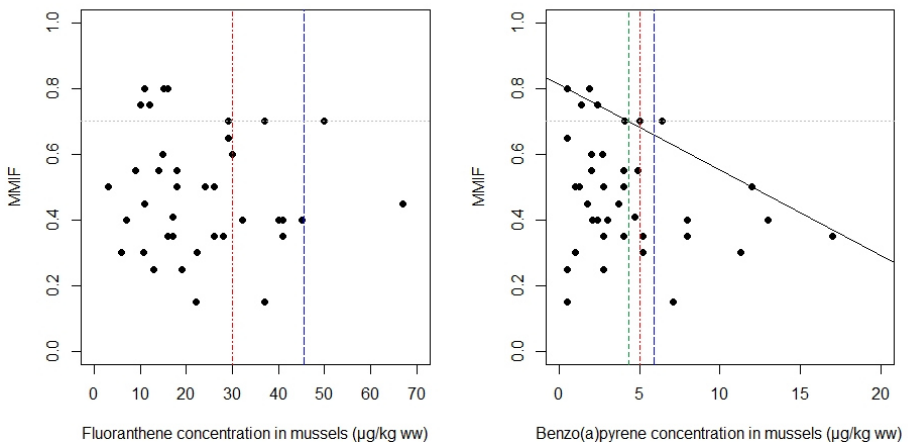


Figure 6.2: Scatterplots of the relationship between accumulated concentrations of PAHs in mussels and the ecological quality calculated as the MMIF. The (blue) 'longdashed' line — — indicates the threshold concentration calculated with the 95th percentile, the green 'dashed' line - - - indicates the threshold concentration based on the 90th quantile regression model and the (red) 'dotdash' line — · — indicates the current EQS_{biota} (Table 6.2). The horizontal dotted line indicates an MMIF (EQR) value of 0.7, the threshold for a good ecological quality. In the case of fluoranthene, a significant 90th quantile regression model could not be constructed (Table 6.3).

A robust threshold concentration could only be derived when a significant quantile regression model was found. This was the case for PFOS in perch ($p < 0.001$), ∑PCB in eel ($p < 0.001$) and benzo(a)pyrene in mussels ($p < 0.01$), where higher accumulated concentrations resulted in a significantly lower ecological quality. In the other cases where no significant quantile regression could be found, a threshold value was only calculated using the 95th percentile approach. As this value is merely determined by the highest concentrations measured in field situations in the present study, the threshold value may

lay even higher in reality. Therefore, it should be stressed that when only the 95th percentile approach could be used, the calculated concentrations were used to compare to the current EQS_{biota} rather than dictate a robust threshold value.

Macro-invertebrate communities can reflect direct effects of contaminant pollution on population size and growth. Exposure to PFOS has been shown to result in decreased fitness and reproduction of *Daphnia magna* (Jeong et al., 2016; Ji et al., 2008). Furthermore, community composition can be altered. Cox and Clements (2013) found that PAH-contaminated sites showed a significantly lower abundance of sensitive amphipods (*Diporeia* spp.) than reference sites. Mercury contamination from mining resulted in a decreased EPT richness and abundance (Costas et al., 2018). In the present study, a negative relationship was found between the accumulated concentrations of PFOS in perch and benzo(a)pyrene in mussels (reflecting the local pollution load) and ecological quality assessed by the MMIF. However, for mercury no such relationship was found.

Multiple studies investigated the relationship between dissolved or accumulated pollutant concentrations and ecological quality. A lower IBI score (the Index of Biotic Integrity, aka. Fish Index) was found for a pesticide-contaminated river than for a reference site (Mayon et al., 2006). In the Arkansas River in Wichita, Kansas (USA), no relationship was found between the total IBI score and accumulated organochlorine pesticides in fish (Eaton and Lydy, 2000). However, some individual metrics did show a relationship. The same was found along a PCB gradient in Indiana (USA) by Simon et al. (2013). Bashnin et al., (2019), determined threshold values (95th percentile method) of accumulated pesticides in transplanted *Dreissena polymorpha,* reflecting a decrease in macro-invertebrate community quality, calculated using the MMIF. However, no significant relationships were found using the quantile regression approach.

In contrast to the present study, a significant 90th quantile regression model could be found for total and dissolved Hg concentrations in the water column and MMIF values in a survey of 185 locations in Flanders (Van Ael et al., 2015). However, in that study, critical concentrations determined for Hg were well below the standards for the water column

(EC, 2008b). In another Flemish study, bioaccumulated concentrations in eel were compared to the IBI based on fish community (Van Ael et al., 2014). That study did not find a significant decrease in the EQR score, even after exceeding the EQS_{biota} of 20 µg kg^{-1} ww for Hg. For ∑PCB, however, they found a threshold concentration in eel of 431 µg kg^{-1} ww above which a good ecological quality (EQR ≥ 0.6) was never reached, which is even higher than the threshold value suggested by the present study (328 µg kg^{-1} ww), although they were all derived without lipid normalization. Besides lipid normalization, another important difference between both studies is the specific biotic index used. The IBI is based on fish community, while MMIF is based on macroinvertebrate community. Since invertebrates are more sensitive for many compounds than fish (Buckler et al., 2005; Xin et al., 2015), this might result in lower EQR values for invertebrate-based indices and thus in lower threshold values. However, community health at different trophic levels is thought to be interconnected since the disappearance of keystone species both at low (e.g. invertebrates) or high trophic levels (e.g. top predators) are known to affect entire ecosystem structures (Collier et al., 2016; Rodríguez-Lozano et al., 2015). On the other hand, it has been shown that, apart from chemical pollutant influences, fish-based indices are strongly affected by physical habitat quality of water bodies (e.g. channel or riffle quality) compared to invertebrate-based indices (Pilière et al., 2014).

As previously mentioned, the dataset of accumulated concentrations in biota used in the present study was already published before (Teunen et al., 2021b: *Chapter 5*). In that study, a positive relationship was found between PFOS accumulation in perch and eel and water concentrations and between benzo(a)pyrene concentrations in mussel tissue and water. Furthermore, a positive relationship was also found between fish and sediment for PBDEs and PCBs. In the present study, on the other hand, a significant effect of pollution (measured in fish) on the MMIF was only detected for PFOS (in perch), PCBs (in eel) and benzo(a)pyrene. Although we found some agreements, the absence of relationships might be due to concentrations in the lower trophic levels being sufficiently low not to cause any changes to the macro-invertebrate community. Since the compounds of interest are known to biomagnify, the highest concentrations are to be expected in the higher trophic levels.

The quantile regression approach allows for investigating the relationship between a specific pollutant and the ecological quality and health. However, this does not necessarily reflect a clear causal relationship. In reality, ecosystems are not affected by a single pollutant but rather by a complex mixture of multiple compounds interacting with other environmental stressors (e.g. increasing temperatures, food availability and quality, habitat deterioration). This was reflected in locations with a low MMIF score, despite low accumulated concentrations. Furthermore, to a large extent, ecological quality can be explained by water characteristics (Van Ael et al., 2014, 2015). Nonetheless, the present study, covering different aquatic ecosystems in a temperate climate, allows for the derivation of safe threshold values and evaluation of existing standards for biota, at least for field situations comparable to those in Flanders.

It is important to note that, to a certain degree, the results may depend on the number of selected sampling locations and their characteristics. Apart from pollution load, the macro-invertebrate community might be affected by general environmental characteristics of the location (e.g. structures in and on the sediment, pH, salinity) (Rezende et al., 2014). Furthermore, the calculated threshold values depended on the accumulated concentration in the targeted fish species. Since we merely focussed on lipophilic compounds, lipid content in the fish can strongly affect these concentrations. Eel, for example, is known to accumulate high concentrations due to its high lipid level (Belpaire & Goemans, 2007a). This was partly solved by standardization of the concentrations based on lipid content. However, a difference between perch and eel was still visible. This might be caused by differences in exposure routes, lifestyles, age or internal metabolization and elimination pathways. The species used in the present study are generally used monitoring species with a broad distribution throughout Europe (Bashnin et al., 2019; Bervoets et al. 2005b; Fliedner et al., 2018; Foekema et al., 2016; Jürgens et al., 2013; Hendriks et al., 1998; Poma et al., 2014) and therefore our findings could be extrapolated to other European regions. To identify a narrower, more robust threshold value, and counter the above effects, the study should thus be repeated for a broad range of location types and monitoring (fish) species.

6.3.3 Are the EQS_{biota} protective of the ecological quality of aquatic ecosystems?

For comparison of the derived threshold concentrations with the existing EQS_{biota}, standardized concentrations (on lipid or dry weight basis) as proposed in the Guidance document (EC, 2014) were used for fish (*Tables 6.1 and 6.4; Appendix E: Table E.2*). As stated before, all compounds measured in fish, except for PFOS and Hg, were standardized to 5% lipid content. A normalization to 26% dry weight content was performed for PFOS and Hg. After normalization of the fish data, threshold values in general increased for perch and decreased for eel (except for Hg in eel; *Table 6.4; Figures 6.2 and 6.3*), as did the accumulated concentrations (*Table 6.1*).

Table 6.4: Threshold values (µg kg^{-1} ww) for different compounds based on the 95th percentile and 90th quantile regression approaches for data normalized for lipid content (HBCD, dioxins, ∑PBDE and ∑PCB) or dry weight (PFOS and Hg). For each compound the European environmental quality standard for biota (EQS_{biota}) is also given (EU, 2013).

Compound	95th percentile		90th quantile regression		EQS_{biota}
	perch	*eel*	*perch*	*eel*	
PFOS	12.0	42.6	16.0	ns	**9.1**
Hg	172	241	ns	ns	**20**
HBCD	3.6	7.6	ns	ns	**167**
Dioxins[a]	0.021	0.003	ns	ns	**0.0065**
∑PBDE	6.09	6.52	ns	ns	**0.0085**
∑PCB	25.9	98.5	ns	183	NA

[a] concentrations in µg $WHO\text{-}TEQ_{2005}$ kg^{-1} ww. ns: no threshold value could be calculated because no significant ($p < 0.05$) quantile regression model was found. Significant regression models can be found in *Appendix E: Table E.5*. NA: no EQS_{biota} exists up to date.

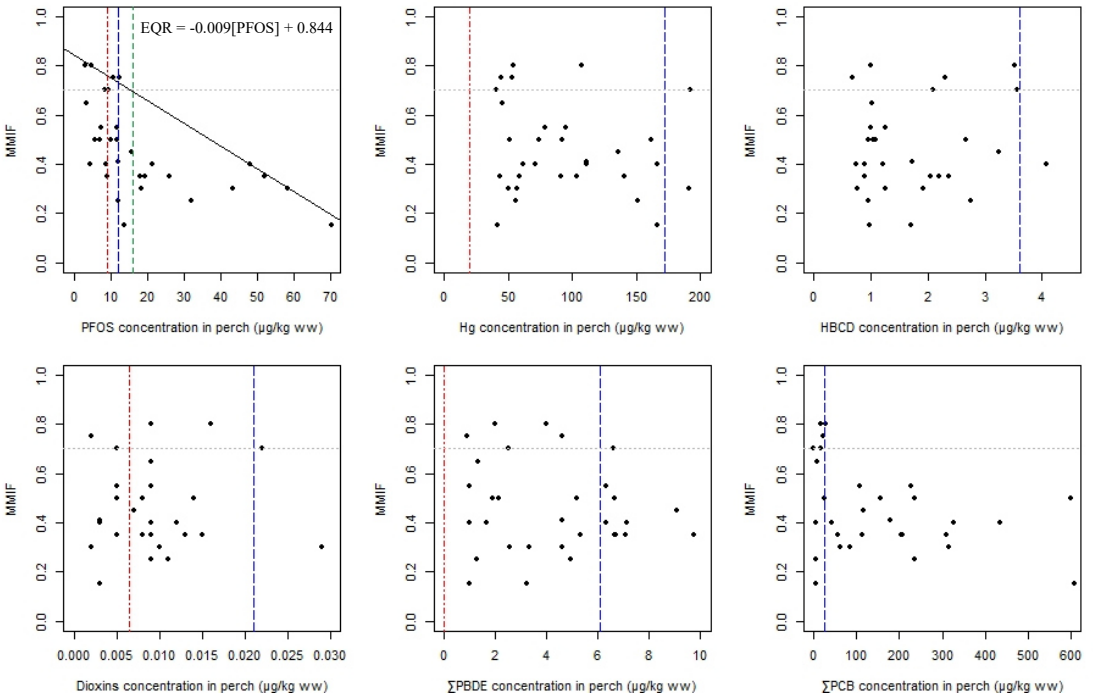


Figure 6.3: Scatterplots of the relationship between (standardized) accumulated concentrations of priority compounds in perch and the ecological quality calculated as the MMIF. The (blue) 'longdashed' line — — indicates the threshold concentration calculated with the 95th percentile, the green 'dashed' line - - - indicates the threshold concentration based on the 90th quantile regression model and the (red) 'dotdash' line — · — indicates the current EQS_{biota} (Table 6.4). The horizontal dotted line indicates an MMIF (EQR) value of 0.7, the threshold for a good ecological quality. Regression lines were only indicated when the quantile regression model was significant (Table E.5).

Both PFOS threshold concentrations (95th percentile: 16 µg kg^{-1} ww, and quantile regression: 12 µg kg^{-1} ww) calculated for perch were comparable to the existing EQS_{biota} for PFOS of 9.1 µg kg^{-1} ww (EU, 2013). Contrastingly, for eel, the 95th percentile concentration of 42.6 µg kg^{-1} ww was 4.7 times higher than the current EQS_{biota}. The threshold concentrations for Hg calculated with the 95th percentile concentrations were between 8.6 and 12 times higher than the existing EQS_{biota} of 20 µg kg^{-1} ww (EU, 2013) for perch (172 µg kg^{-1} ww) and eel (241 µg kg^{-1} ww), respectively. For HBCD, on the other hand, the 95th percentiles concentrations were between 46 (perch: 3.6 µg kg^{-1} ww) and 22 (eel: 7.6 µg kg^{-1} ww) times lower than the existing EQS_{biota} (167 µg kg^{-1} ww; EU, 2013). The threshold values (95th percentile) for dioxins in perch and eel ranged from 0.003 to 0.021 µg $TEQ_{WHO-2005}$ kg^{-1} ww, which included the existing standard of 0.0065

µg $TEQ_{WHO-2005}$ kg^{-1} ww (EU, 2013). For ∑PBDE these values were comparable between perch and eel (6.09 and 6.52 µg kg^{-1} ww respectively, but were between 716 and 767 times higher than 0.0085 µg kg^{-1} ww, the current biota standard (EU, 2013). The estimated threshold values for PAHs in the present study were comparable to EQS_{biota} (*Table 6.4*). As discussed before, PAH concentrations were not standardised for lipid content. A 95th percentile concentration of 45.5 µg kg^{-1} ww was found for fluoranthene, similar to the EQS_{biota} of 30 µg kg^{-1} ww (EU, 2013). Finally, both threshold values for benzo(a)pyrene (95th percentile: 5.91 µg kg^{-1} ww, and quantile regression: 4.35 µg kg^{-1} ww) were around the existing standard of 5 µg kg^{-1} ww (EU, 2013).

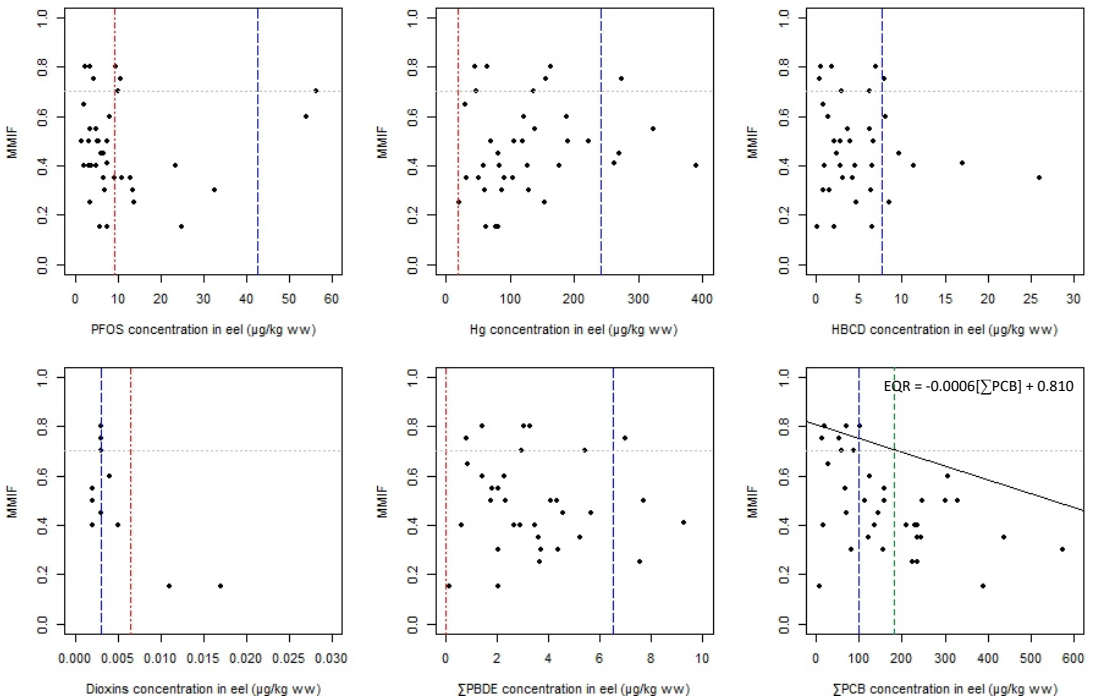


Figure 6.4: Scatterplots of the relationship between (standardized) accumulated concentrations of priority compounds in eel and the ecological quality calculated as the MMIF. The (blue) 'longdashed' line — — indicates the threshold concentration calculated with the 95th percentile, the green 'dashed' line - - - indicates the threshold concentration based on the 90th quantile regression model and the(red) 'dotdash' line — · — indicates the current EQS_{biota} (Table 6.4). The horizontal dotted line indicates an MMIF (EQR) value of 0.7, the threshold for a good ecological quality. Regression lines were only indicated when the quantile regression model was significant (Table E.5).

From the above results, it can be observed that the current EQS_{biota} for HBCD is exceptionally high, even though effects to ecosystem health were detected at much lower

concentrations and thus might need to be revised. In the present study, one outlier in eel contained 412 µg kg^{-1} ww, exceeding the standard. This is in line with the study published by Eljarrat and Barceló (2018), stating that the EQS_{biota} for HBCD is exceeded rarely on a global scale. In contrast to HBCD, the current EQS_{biota} for ∑PBDE is extremely low, even below LOQ of current analysis methods. Effects on ecosystem health are only detected at concentrations more than 700 times higher than the current standard. Thus the EQS_{biota} might need revising to a higher threshold concentration. This vast exceedance of the standard was also found by Ejarrat and Barceló (2018), who found that 25% of fish samples from studies over the world even showed exceedances up to ten thousand times. The EQS_{biota} for PBDEs has previously been criticized by multiple authors since it was based on the observed effects of one congener (BDE 99) on mice and was determined, including very large safety factors (EU, 2011a; Eljarrat and Barceló; 2018; Jürgens et al., 2013).

Based on our results, the current EQS_{biota} seems to be sufficiently protective of aquatic ecosystem quality for benzo(a)pyrene and PFOS (measured in perch). Furthermore, we found a strong indication that an EQS_{biota} for ∑PCB should range between 98.5 and 183 µg kg^{-1} ww (as was measured for both approaches in eel; *Table 6.4*). For PCBs, no EQS_{biota} exists to date. However, the consumption limit for muscle tissue of wild eel or product thereof specifically (EU, 2011a) is set at 300 µg kg^{-1} ww (although calculated for the sum of PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180). This value is comparable to the threshold derived for eel (without standardization) of 328 µg kg^{-1} ww (*Table 6.2*). The 95^{th} percentiles for fluoranthene and dioxins were also close to the existing EQS_{biota}, but could not be confirmed with a 90^{th} percentile regression model (as was indicated before).

Besides the general protection of ecosystem health, the EQS_{biota} were set with a specific double purpose in mind (EC, 2014). Firstly, these standards are meant to protect the aquatic ecosystems and prevent secondary poisoning of top predators ($EQS_{biota,\ secpois}$). Secondly, human health risks were taken into account ($EQS_{biota,\ hh}$). However, any statements on the protective value of the current EQS_{biota} on the protection of ecosystem integrity, with the focus on macro-invertebrate community, are relevant. The main focus

of the present study was on investigating whether we could define threshold values that guaranteed protection of the ecological quality as assessed by the macro-invertebrate community. As previously discussed, macro-invertebrates, occupying relatively low levels in the food chain, can reflect the general health of the ecosystem and therefore also higher trophic levels. However, to further investigate the relevance of the current EQS_{biota}, we recommend repeating the current study using a fish-based index. On the other hand, since no effects of secondary poisoning or human health risk were investigated in the present study, no conclusive statements can be made on the effectiveness of EQS_{biota} as originally proposed by the EU. Therefore, the results should be merely interpreted as an indication for further investigation.

Between the $EQS_{biota, secpois}$ and $EQS_{biota, hh}$, the most sensitive was selected as EQS_{biota}. As in the present study, however, the main focus is on the ecological risk of the ecosystem health; secondary poisoning seems like a more relevant endpoint than the human health risk. Therefore, threshold values were further compared to the $EQS_{biota, secpois}$ specifically. This latter differed from the used EQS_{biota} for ∑PBDEs (44 µg kg^{-1} ww), fluoranthene (11522 µg kg^{-1} ww), hexachlorobenzene (16.7 µg kg^{-1} ww), PFOS (33 µg kg^{-1} ww), dioxins (0.0012 µg $TEQ_{WHO-2005}$ kg^{-1} ww) and heptachlor (epoxide) (33 µg kg^{-1} ww). For benzo(a)pyrene, included in the PAHs, no separate $EQS_{biota, secpois}$ was available.

The biota quality standards specified for the risk of secondary poisoning resulted in values much higher than the calculated threshold values in the present study for ∑PBDE (6.09-6.52 µg kg^{-1} ww), fluoranthene (45.5 µg kg^{-1} ww). For dioxins, the $EQS_{biota, secpois}$ were lower than the calculated threshold values (0.003-0.021 µg $TEQ_{WHO-2005}$ kg^{-1} ww) and for PFOS they were comparable to the threshold concentration calculated for eel (42.6 µg kg^{-1} ww). However, as stated before, since no significant quantile regression model could be derived for ∑PBDE, PFOS (in eel), dioxins or fluoranthene, it might be possible that, in reality, the threshold values are higher and closer to the current $EQS_{biota, secpois}$. To further investigate this, ecosystems with a higher pollution load need to be studied in order to determine the actual threshold concentration resulting in a decrease in ecological quality.

Although the findings of the current study might indicate that some of the current EQS_{biota} might be too strict or too high, this only serves as an indication for revision of the current standards. The alteration of current standards should be investigated with care and more research on this topic is needed. Firstly, the present study should be repeated using higher trophic levels for ecological quality assessment (e.g. fish-based indices), since these show a more direct relationship with the accumulated concentrations in fish. However, the selection of sampling location in the current study did not allow for this approach. Furthermore, these studies should be replicated in other European member states in order to verify and strengthen our findings. Especially, for the lowering of current standards, their effects both at the individual and ecosystem level should be extensively investigated.

6.4 Conclusions

Threshold concentrations based on the 90^{th} quantile regression model could only be calculated for PFOS (in perch), ∑PCB (in eel) and benzo(a)pyrene. The present study revealed that for PFOS (in perch) and benzo(a)pyrene, the EQS_{biota} is sufficiently protective of the aquatic ecosystem quality. For dioxins and fluoranthene, the calculated 95^{th} percentile thresholds were comparable to the existing standards. However, no significant quantile regression model could be derived for these compounds. Thus, since the threshold values were calculated on the contamination load and ranges of the locations targeted in the present study, they might be even higher in reality.

As a consequence, the threshold values of the present study should be validated in other aquatic ecosystems. For all other compounds, the current EQS_{biota} was too strict to protect the ecosystem quality with respect to macroinvertebrate community structure and needs re-evaluation. For HBCD, on the other hand, the EQS_{biota} was not sensitive enough. Furthermore, since fish concentrations were standardized based on lipid (or dry weight) content, threshold concentration ranges can be extrapolated to other fish species. Our findings should be taken into account for revision and fine-tuning of the current $EQS_{biota.}$

Acknowledgements

This study was partially funded by the Flanders Environment Agency. The technical crew of INBO Linkebeek is acknowledged for their help in fish sampling. We thank Tim Willems for the PFOS analysis and dr. Valentine Mubiana for the Hg analysis (both University of Antwerp).

Chapter 7

General discussion

7.1 EQS_{biota} monitoring in Flanders (Belgium)

Multiple monitoring networks on accumulation of pollutants in biota, conform the Water Framework Directive, were put in place throughout Europe (Fliedner et al., 2018; Foekema et al., 2016; Wolfram et al., 2021). Bioaccumulated concentrations of POPs and mercury in perch and eel from Flanders (Belgium) showed to be comparable to other European monitoring studies targeting these species in *Chapter 5*. For all compounds, except for PFOS, wet weight concentrations were higher for eel than for perch, predominantly caused by the higher lipid content in eel muscle (*Chapter 2*). Polyaromatic hydrocarbons fluoranthene and benzo(a)pyrene were measured in transplanted bivalves (*Dreissena* spp. and *Corbicula fluminea*). These concentrations were comparable to other studies as well (*Chapter 5*). European literature on PAH monitoring in freshwater mussels, however, is scarce. Throughout this thesis it became apparent that PBDEs, Hg, PFOS, HBCD, fluoranthene, benzo(a)pyrene and dioxins were omnipresent in Flanders, despite their restrictions in use and emission. For HCB and cis-heptachlor epoxide this was only true in eel.

Besides the existing European restrictions (e.g. Stockholm Convention), specific Belgian efforts have been made to reduce the pollution load in the aquatic environment. In 1996, the first Manure Action Plan (MAP) was introduced in Flanders to reduce and regulate the amount of phosphates and nitrates used in agriculture (Deketelaere et al., 1997). The National Action Plan (NAPAN) was then drafted by the Belgian Government to reduce the risks and impacts of pesticides (Belgian Federal Government, 2014). All this resulted in Belgium being the only European country that succeeded in lowering the average ecological risk of organic compounds on aquatic invertebrates between 2001 and 2015 (Wolfram et al., 2021). In February 2020, a PFAS action plan was created by the Flemish Government in response to high accumulated PFAS concentrations measured in monitoring of humans and the known endocrine disruptive effects (https://omgeving.vlaanderen.be/pfas-actieplan). The European Commission published

the ‘Chemicals Strategy for Sustainability’ in October 2020, including actions for phasing out PFAS in the EU (EC, 2020).

In *Chapter 2*, where possible, concentrations of the micropollutants in eel were specifically compared to other Flemish studies including some of the same sample locations or water bodies (Maes et al., 2005, 2008; Malarvannan et al., 2014; Roosens et al., 2010; Van Ael et al., 2014), allowing for the investigation of general trends over time in the long-term eel monitoring network. A notable decrease was found for HCB, HBCD and PCBs in the last decades. For Hg and PBDEs, however, the decrease was less pronounced or even absent between studies. This clear decreasing trend for HCB and the absence of a trend for mercury in eel were previously found by De Jonge et al. (2014). However, to be able to make a substantiated statement on time trends of pollutants in the environment, long-term studies (over several decades) should be performed on the exact same locations. The EQS_{biota} monitoring network will lend itself to this purpose as the monitoring campaign will be repeated for the coming years, coordinated by the Flanders Environment Agency. Currently, the first repetition cycle of sample collection has been completed (2019-2021).

Frequent exceedances of the respective EQS_{biota} were found for Hg, ∑PBDE and PFOS in both fish species and for dioxins and cis-heptachlor epoxide in eel (*Chapter 2*). An explicit statement on the exceedance of the EQS_{biota} can, however, only be made after correcting for the varying lipid content (or dry weight content as a proxy for protein content for Hg and PFOS) between monitoring species (EC, 2014). This technique was already applied in *Chapter 6*, calculating threshold concentrations to guarantee a good ecological quality (based on the MMIF). Standardized concentrations were given in *Appendix E*. Overall standardized exceedances of the EQS_{biota} (*Table 7.1*) confirmed that Hg, ∑PBDE, PFOS, dioxins and cis-heptachlor epoxide concentrations could possibly pose a health risk to the environment according to European environmental standards. For the PAHs in mussels, no standardization was needed, because of the limited variation in lipid content. They showed exceedances in one third of the sample locations.

Table 7.1: Percentage of sample locations with exceedances of the EQS_{biota} after standardization for 5% lipid content (or 26% dry weight content for Hg and PFOS).

Compound	Locations with exceedances of EQS_{biota} (%)	
	Perch	***Eel***
Hexachlorobenzene	0	0
Hexachlorobutadiene	0	0
Brominated diphenyl ethers	100	100
Mercury	100	100
Perfluorooctane sulfonate	73	46
Hexabromocyclododecane	0	2.4
Dicofol	0	0
Dioxins and dioxin-like compounds	58	10
Cis- heptachlor epoxide	21	90

The frequencies of EQS_{biota} exceedance found in this PhD were comparable to other European studies. In France, Babut et al. (2020) reported that 80% of locations showed an exceedance for PFOS, measured in barbel (*Barbus barbus*), chub (*Squalius cephalus*) and roach, and adjusted for a 26% dry residue. Normalized concentrations in chub, bream and perch from the German Danube, exceeded the EQS_{biota} in all locations for Hg, PBDEs and PFOS (except for 1) and in none of the locations for HBCD and HCB (Fliedner et al., 2018). For dioxins, they only found an exceedance for the larger individuals. Furthermore, since concentrations in the present study were comparable to other European studies on perch and eel (*Chapter 5*), we could expect the same patterns to be found in general.

7.1.1 Monitoring techniques

Within the scope of this thesis, multiple monitoring techniques were used (i.e. active biomonitoring, passive biomonitoring and passive samplers). In general, PAHs were analysed in bivalves (ABM) and the other compounds in fish (PBM) as recommended by the EQS guidelines (EC, 2014). However, for PFAS (*Chapter 4*), we compared both techniques. This revealed different pollution profiles between the monitoring methods (ABM vs. PBM) and trophic levels (predatory fish vs. bivalves). The main finding was that PFOS contributed the most in the fish, while PFOA contributed the most in bivalves. It is therefore important to take into account that different monitoring species can reflect

a different image of the situation. In general, PFOS is considered the predominant perfluorinated compound in the aquatic environment due to its high biomagnification potential (Houde et al., 2011). However, when interpreting the risk for the entire ecosystem, PFOA should also be focussed on, especially for lower trophic levels which form the basis of the ecosystem.

Even though multiple bivalve species were used and compared in this thesis, no significant differences were detected after exposure between sample years or based on their reference locations (*Chapter 5*). In agreement, Evariste et al. (2018) did not find a significant difference between accumulated PAH concentrations in zebra and quagga mussels collected from the same locations. Furthermore, in *Chapter 4*, no difference in isotopic niche was observed between quagga mussels and Asian clams. Therefore, *Dreissena* spp. and *Corbicula fluminea* can be appropriately used interchangeably as monitoring species and based on the local conditions (e.g. salinity, sediment texture, presence as indigenous species). Although, for *Mytilus edulis* a slightly different PFAS profile was found compared to the other mussel species, no conclusive statement could be formed due to its small dataset (N = 1 location). Further investigation is needed to compare blue mussels with fresh water mussels, as well as to determine the isotopic niche.

The broader isotopic niche for eel compared to perch was explained by the more diverse and flexible diet of the former (Belpaire et al., 1992). However, lipids are also known to be depleted in ^{13}C, relative to proteins and carbohydrates (Post et al., 2007). Therefore higher lipid contents tend to create a bias towards a lower $\delta^{13}C$. Thus, the large lipid variation in eel might lead to a broader $\delta^{13}C$ variation, which could therefore decrease after lipid normalisation.

In *Chapter 2*, biomonitoring was compared to passive samplers for HCB, ∑PBDE, benzo(a)pyrene, fluoranthene and ∑PCB. In fish, we only found a relationship between passive samplers and HCB concentrations in perch and between passive samplers and ∑PCB concentrations in eel. Benzo(a)pyrene concentrations in passive samplers were also correlated with concentrations in freshwater mussels, but for fluoranthene, no correlation

was found. Literature shows this relationship especially for lower trophic levels. Bivalves, for example, show a more direct exposure to these compounds in the water column and sediment and less complex metabolization processes.

Thus, the use of passive samplers can currently provide additional information on the bioavailability and bioaccumulation of pollutants directly from the water column. Measurements in biota, on the other hand, present the in-situ situation of bioaccumulation after complex metabolization and elimination process and trophic transfer. In time, passive samplers might offer a less invasive alternative for biota monitoring. However, more research in this area is needed to fully understand the processes of bioaccumulation and -magnification and how to translate this to the use of passive samplers. Eventually, the goal is to identify a reference technique, independent of water body and biota type, which can be used in every EU member state in the context of the WFD.

When comparing the two biomonitoring techniques, the first differences can be seen in the species that were used and their exposure time. While the fish (passive biomonitoring) are generally exposed for multiple years to the local conditions, exposed mussels (active biomonitoring) only reflect the condition over several weeks. Therefore, using active biomonitoring, the seasonal variation that might be present in the measurement has to be taken into account. In order to minimize this effect between years, we always exposed the mussels during the same season (late autumn). Furthermore, the small size of mussels requests a larger sample size to have sufficient tissue for the required analyses. On the other hand, the largest disadvantage of passive biomonitoring is that you cannot guarantee that the target species will be present at all sampling sites and in the specific numbers and size ranges you intended. In this way, active biomonitoring allows for a more standardized way of sampling. Unfortunately, this technique is not appropriate for larger individuals, such as fish.

7.2 Human health risk

One of the objectives of the EQS_{biota} is protection against the risk of secondary poisoning for piscivorous predators, which includes humans. However, safe pollutant concentrations in food, with a specific focus on human health, have been established as well. A remark should be made that, for the specific case of Flanders, wild caught fish are generally not consumed by the average population. Recreational fishermen, taking home their 'catch of the day' for personal consumption, are therefore the main focus group for human health risk assessment. In *Chapter 3* and *Chapter 4* human health risks were already calculated for mercury and PFOS respectively. For methylmercury, multiple safe consumption amounts on a daily basis are available based on body weight, ranging from 0.1 - 0.3 µg kg^{-1} body weight day^{-1} (ATSDR, 2018; FAO/WHO, 2010; UNEP, 2008). Although, these values differed, they all focussed on neurodevelopmental issues after maternal exposure as a critical endpoint. Therefore, the most strict one will provide the most complete protection level. For PFAS, the most recent standard was determined on the sum of PFOA, PFOS, PFHxS and PFNA (EFSA, 2020) and is 4.4 ng kg^{-1} body weight $week^{-1}$ (0.63 ng kg^{-1} body weight day^{-1}), after a reduced immunoresponse after vaccination was observed in children. We found that for Hg, only frequent eel consumption (> 71 g day^{-1}) of the most polluted individuals included in this thesis posed a possible human health risk. For PFAS, with a main focus on PFOS, however, it was found that eel posed a general health risk, while perch might only be a problem in highly contaminated sites.

Provisional tolerable weekly (PTWI) or daily intake (TDI) levels are available for HCB (0.17 µg kg^{-1} body weight day^{-1}; IPCS/WHO, 1997), HCBD (0.2 µg kg^{-1} body weight day^{-1}; WHO, 2017), PBDEs (0.7 µg kg^{-1} body weight $week^{-1}$; EFSA, 2005), dicofol (2.2 µg kg^{-1} body weight day^{-1}; EFSA, 2011), dioxins (14 pg WHO-TEQ kg^{-1} body weight $week^{-1}$; EFSA, 2005) and the sum of heptachlor and heptachlor epoxide (0.1 µg kg^{-1} body weight day^{-1}; JMPR, 1994). Analogous to *Chapter 3*, estimated daily intake (EDI) levels were calculated for an adult of 70 kg and based on known consumption rates of perch and eel by anglers. A hazard quotient (HQ) was then determined by dividing the EDI by the TDI

values. An HQ>1 revealed a potential health risk. The human health risk was assessed on the complete dataset of 44 locations as presented in *Chapter 5*, with mean pollutant concentrations per location. An HQ>1 was found for none of the compounds mentioned above, except for dioxins. Maximum concentrations in perch were all less than 1% (<0.004 - 0.3%) of the allowed levels, for eel these ranged between <0.02% (dicofol) and 27% (PBDEs) of the tolerable levels. For dioxins in eel, on the other hand, 56% of locations showed a possible health risk, with the highest measured concentration resulting in an EDI almost five times the TDI. The maximum concentration in perch, however, were only 8% of what is tolerated.

On a European scale, maximum levels (ML) which can be present in foodstuff were established for dioxins (0.0065 µg WHO-TEQ kg^{-1} ww for perch, 0.010 WHO-TEQ kg^{-1} ww for eel) and the sum of 6 indicator PCBs, excluding PCB118 (75 µg kg^{-1} ww for perch, 300 µg kg^{-1} ww for eel) (EC 1259/2011). However, it should be noted that for these safe concentrations, the effect of weight/age of the consumer were not taken into account as they were for the tolerable intake concentrations above. Furthermore, for mercury, this was set at 0.5 µg g^{-1} ww for perch and 1 µg g^{-1} ww for eel (*Chapter 3*). Analogous with the PTWI, the ML for dioxins was only exceeded for eel (37.5% of locations). For PCBs, the ML was exceeded in 9% of the locations for perch and in 56% of the locations for eel. Belpaire et al. (2011), previously, strongly discouraged the consumption of wild eel from Flemish water bodies due to high PCB concentrations.

For HBCD, no human health risk concentrations are currently available due to limited toxicological data. Furthermore, for PAHs no human health risk was assessed because fresh water bivalves are not usually consumed in Belgium.

The mercury standards for human consumption were never exceeded. It was apparent that a discrepancy between the ML for human consumption and EQS_{biota} (including risk for secondary poisoning) exists for mercury. The ML is 25-50 higher than the EQS_{biota} for perch and eel respectively. For dioxins, on the other hand, both values are comparable. In the updated version of the WFD guidelines (EC, 2018), a calculation method was

introduced that can be used to translate TDI-values to threshold concentrations for human health (i.e. ML). This latter is then determined for a 70 kg person, taking into account the mean European consumption rate of 115 g day^{-1} and an allocation factor of 20%. This resulted in a ML-value lower than the current EQS_{biota} for PFAS (77 ng kg^{-1} ww; 118 times lower) and HCBD (24 µg kg^{-1} ww; ½ of the EQS_{biota}). For HCB an ML of 21 µg kg^{-1} ww was calculated (2.1 times higher than the EQS_{biota}) and for dicofol of 268 µg kg^{-1} ww (8.1 times higher). Extreme differences were found for PBDEs and heptachlor (both 165 µg kg^{-1} ww), with the ML respectively being more than 19,000 and 24,000 times higher than the current EQS_{biota}. A side note should be made that the 115 g daily consumption is much higher than the known consumption rate in Flanders (by anglers; i.e. 2.7 g of perch day^{-1} and 18 g of eel day^{-1}). When included in the WFD calculation, this would result in ML values 42.6 and 6.4 times higher than reported above, for perch and eel respectively.

An important side-note on the assessment of human health risk are that in reality fish will be exposed to a mixture of pollutants, that might possible lead to additive effects. The larger image should therefore always be taken into account. On the other hand, most 'safe' concentrations already take into account long-term exposure. Thus, a one-time exposure to higher concentrations might not directly result in health effects.

Another large shortcoming of the current human health risk assessment is the failure to account for pregnant woman. As explained in the introduction, humans in their early developmental stages, can be very sensitive to pollution, through maternal transfer. This can cause developmental disorders (e.g. immune system, nervous system, growth). Therefore, even more locations and/or pollutants in the present study could pose a risk in this specific situation and stricter standards should be considered.

7.3 Specific cases of compounds with protein affinity

As described before, mercury and PFOS gained some special attention in this thesis due to their high affinity to proteins, in contrast to the other lipophilic POPs included in the EQS_{biota}. Additionally, in the ECOSPHERE laboratory (University of Antwerp, Belgium)

we have years of expertise in analysing metals and PFAS. Accumulation and distribution differences between biota were investigated for mercury and PFOS, with an important focus on the consequences for monitoring implementations.

Mercury was analysed in perch and eel, comparing muscle and liver concentrations and investigating the relationship with size/weight (as a proxy for age), taking into account the effect of location (*Chapter 3*). It was found that only for perch concentrations increased with size, indicating that standardized size range sampling is very important for this species. For eel, on the other hand, this relation with size was very location dependent and not unidirectional. Therefore, the most representative pollution image in this species is probably achieved by collecting different sizes. Mercury concentrations in general were higher in eel than in perch, mainly due to its high affinity for muscle proteins (i.e. eels are very muscular) and the bottom-dwelling lifestyle of eel. The lower mercury concentration in perch, on the other hand, might also have been caused by the predominant amount of small (juvenile) individuals caught. For perch, concentrations were higher in muscle tissue than in liver tissue, for eel the opposite was true. A novel approach (Kahilainen et al., 2016) was used to correct for the effect on varying lipid content on the tissue weight and thus mercury concentrations. This resulted in the same conclusions, although the difference between muscle and liver tissue for eel was no longer present. These finding confirmed the recommendation for using muscle tissue as a monitoring tool for mercury (EC, 2014).

In *Chapter 4*, besides PFOS, 14 other PFAS have been analysed in fish and mussels. It was apparent that PFAS profiles differed between these trophic levels, a result of the different bioaccumulation efficiencies depending on the carbon chain length and functional group (i.e. carboxyl vs. sulfonic acid). In general, short-chained PFAS and PFOA showed a high contribution in mussels (low trophic levels), whereas long-chained PFAS and PFOS contributed the most in fish (high trophic levels). Furthermore, the method of exposure (indigenous fish vs. transplanted mussels in the water column) was believed to affect accumulation patterns. We also found that concentrations of PFTrDA,

PFTeDA, PFOS, PFDoDA in mussels could predict those in fish. Both PFOS and PFDA concentrations were higher in perch than in eel, in contrast to PFOA, PFDoDA, PFTrDA and PFTeDA for which the opposite was true.

Overall, it was remarkable that even though both EQS_{biota} compounds are considered to have a high protein affinity, accumulation patterns between perch and eel differed for these pollutants. While PFOS clearly showed a higher accumulation in perch compared to eel, for Hg the opposite was true (analogous to lipophilic compounds). Besides the fact that the study on mercury was performed on a smaller amount of sample sites and potentially smaller perch compared to the PFAS study, also the specific structures and compounds to which the pollutants show affinity might affect these accumulation patterns. While mercury is known to bind specifically to sulphur and thiol containing proteins in the muscle tissue (Amlund et al., 2007; Bradley et al., 2017), PFAS are prone to bind to blood serum albumin and fatty acid proteins (Forsthuber et al., 2020; Ng and Hungerbühler, 2013). Furthermore, a species-specific biotransformation of PFAS has been previously observed (Babut et al., 2017; Galatius et al., 2013).

7.4 Influence of the environment

In *Chapter 5*, we investigated the relationship between bioaccumulated concentrations and environmental concentrations (in sediment and water) and studied the effect of abiotic conditions (pH, oxygen, conductivity, nitrate/nitrite, DOC, TOC, clay content) on this relationship. Firstly, we found that environmental concentrations were often below the LOQ, potentially underestimating the risk for biota that accumulated rather high concentrations due to biomagnification (with the exception of dicofol, for which the opposite was true). Furthermore, water concentrations for mercury and PAHs showed large seasonal variations. These results confirmed that monitoring POPs and mercury in biota is recommended and will result in a more standardized and reliable risk assessment. Secondly, we found that PFOS and benzo(a)pyrene water concentrations could predict concentrations of these compounds in fish and mussels respectively. This could be explained by the relatively high solubility of PFOS in water and the more direct exposure

of bivalves to the water column. Sediment concentrations of HCB, PBDEs and PCBs could predict accumulated concentrations in fish. Indeed, these hydrophobic compounds (low $\log K_{ow}$) tend to bind to organic particles being directly ingested by fish and sediment creating a more direct exposure medium for bottom-dwelling species. However, bioaccumulation of these compounds was negatively affected by organic material (DOC or TOC), due to complexation reducing their bioavailability (Dittman and Driscoll, 2009; Li et al., 2015). Furthermore pH showed to have a negative effect on accumulation of HCB, nitrite on PFOS and clay on ∑PBDE in eel. Both low pH and high nitrite/nitrate are known to negatively affect general fish condition rather than bioavailability of pollutants, possibly leading to a reduced elimination by the fish (Watras et al., 1998; Wood, 2001). However, this could not directly explain the negative relationship for nitrite. Clay content, on the other hand, is known to immobilize pollutants.

In general, it should be stressed that modelling accumulation of a specific pollutant and effects of environmental concentrations and conditions in a field situation is tricky and an approximation of the situation. We should take into account that the natural environment is complex and the condition and accumulation potential (including uptake and elimination) of biota is never unidirectional but influenced by a complex combination of factors. It is already known that a mixture of pollutants can cause additive affects and enhance toxicity (Jiang et al., 2021; Megharaj and Naidu, 2008). Furthermore, pollutants and other factors not included in the current models could also play a role.

Additionally, PCB and PBDE profiles were compared between fish species and environmental media, with PCB 153 being the predominant PCB congener, followed by PCB 138 and PCB 180 in all media. However, higher-chlorinated PCBs contributed more to the ∑PCB in fish, due to their higher biomagnification potential. For PBDEs, on the other hand, only fish and sediment could be compared. Although BDE 99 contributed the most to the ∑PBDE in sediment (followed by BDE 47), for fish BDE 47 was the predominant congener, possibly caused by metabolization of higher brominated congeners. For eel BDE 47 was followed by BDE 99, for perch this was BDE 100.

Another important finding of this chapter was the extrapolation potential between bioaccumulated concentration in perch and eel for Hg, PFOS, HBCD, ∑PBDE and ∑PCB. These results for mercury and PFOS were previously found in *Chapter 3 and 4*. This has important implications for monitoring, allowing for a reduction of species needing to be collected as it became clear from the Flemish monitoring campaign, that it was not possible to collect sufficient individuals of both species at all locations. Furthermore, European eel currently has an IUCN red list status of 'critically endangered' (Jacoby and Gollock, 2014) and could therefore partly or completely be replaced by perch as alternative indicator fish species.

7.5 Ecological relevance of EQS_{biota}

The main objective we addressed in this thesis was the evaluation of the general (ecological) relevance of the current European EQS_{biota}. In *Chapter 2*, we found a first indication that the standards were unachievable or even unrealistic for specific compounds.

As discussed before, Hg, ∑PBDE, PFOS, dioxins and cis-heptachlor epoxide showed frequent exceedances of their respective standards. Mercury and PFOS, on the one hand, showed regular exceedances up until a factor 10. For PBDEs and cis-heptachlor epoxide, on the other hand, this even reached a factor 100-1000. Mercury, brominated diphenyl ethers and PFOS concentrations previously have been identified as potentially problematic in a Flemish pilot study on biota monitoring, as their standards were found to be unachievable (De Jonge et al., 2014). Contrastingly, the overall physicochemical and biotic water quality in Flanders has strongly improved over the last three decades, although, mainly caused by improvement of oxygen levels (Flanders Environment Agency; www.vmm.be). Furthermore, no drastic effects on biota (e.g. lowered condition, mortality), which are to be expected with extreme exceedances of environmental standards, are observed. This potentially indicates that the current EQS_{biota} are too strict and not realistic. The risk of standards that are too low and thus exceeded on a large scale, is that governments and executive institutions might neglect to take action since the

situation seems overwhelming and insurmountable, when in reality only some of the highly contaminated sites should be tackled.

An evaluation of the literature used as a basis for EQS_{biota} (*Chapter 1, section 1.2)* revealed that updating of the current standards might be necessary for some compounds, since they are often based on outdated studies of irrelevant species for the aquatic food chain, with small sample sizes and incorporating large safety factors. Therefore, we recommend to review the current EQS_{biota} , taking into account more elaborate, recent toxicity studies, focussing on relevant aquatic species and a dietary exposure route. Preferably, the focus should be on species of higher trophic levels, since these accumulate the highest concentrations (due to biomagnification) and can give the opportunity to effectively calculate the risk for secondary poisoning.

In *Chapter 6*, on the other hand, the relevance of biota quality standards on ecological quality was evaluated using macro-invertebrate communities. Macro-invertebrates are considered keystone-species in the aquatic environment, providing a primary indication of the local ecological water quality (Collier et al., 2016; Rodríguez-Lozano et al., 2015). Accumulated threshold concentrations were determined above which the ecological quality was always insufficient, using 90^{th} quantile regression and the 95^{th} percentile approaches. The first method was more robust and could only be calculated in case of a decreasing trend of water quality with increasing concentrations. This was only possible for PFOS in perch, PCBs in eel and benzo(a)pyrene in mussels.

First of all, it was clear that threshold values differed strongly between fish species. In order to reduce this variation, accumulated concentrations were corrected according to the recommendations for EQS_{biota} monitoring extrapolation (i.e. 5% lipid content; or 26% dry content for PFOS and Hg). This resulted in a smaller difference of threshold values between perch and eel. These threshold values were then compared with the current EQS_{biota}. It was found that for PFOS and benzo(a)pyrene the current standard was sufficiently protective.

For all other compounds, only the 95th percentile method could be performed. This means that even the highest concentrations found in Flanders, still reflected a good ecological quality. Therefore, it is possible that the effective threshold value is even higher. This resulted in a threshold value higher than the current standard for Hg (around 10 times) and ∑PBDE (> 700 times). For HBCD, on the other hand, the threshold value was 22-46 times lower than the standard and all concentrations (except for one outlier) were below the current standard. The threshold values for dioxins and PAHs were comparable to the current standard. For PCBs a threshold value was proposed between 98.5 and 183 µg kg^{-1} ww (based on eel).

Although, using macro-invertebrates to assess the ecological quality can already give a first indication, the EQS_{biota} focus on the risk of secondary poising of top predators. In order to fully assess the relevance of the current EQS_{biota}, higher trophic levels should be implemented (e.g. fish based indices). In the setting of this thesis, however, more than half of the sampling locations were tidal waters and canals, or insufficient data was collected. Therefore, the IBI could not be used to determine ecological quality based on fish community composition. Furthermore, available results did not contain 'good water quality' locations.

7.6 General conclusions and future perspectives

The monitoring of biota in Flanders revealed that Hg, ∑PBDE, PFOS, dioxins and cis-heptachlor epoxide show frequent exceedances of the standards. These results have brought to light that a comprehensive revision of the current EQS_{biota} is recommended, evaluating existing standards and adding new ones (e.g. emerging compounds, PCBs). However, based on human health risk assessment, PFOS, dioxins and PCBs pose a possible health risk, especially when consuming eel. Additional efforts might be needed to further reduce environmental concentrations of these compounds. Considering mercury, only high consumption of eel (> 71 g day^{-1}) might potentially be harmful. Based on these results, we would like to reinforce the current advice to not consume wild caught fish from Flemish rivers on a regular basis. Furthermore, the assessment of the ecological

relevance of EQS_{biota} on the macro-invertebrate community pointed out that the current standards for PBDEs (and to a lesser extent Hg) might be unrealistically low and for HBCD extremely low and need revising. For all other compounds, the current EQS_{biota} were considered relevant. A standardized threshold value based on eel concentrations was proposed (98.5-183 $\mu g\ kg^{-1}$ ww).

Although, due to regional efforts, a decrease in the aquatic pollution over the last decades can be seen, it remains essential to continue monitoring the evolution of the Flemish water quality. The persistence of the compounds of interest in this thesis still results in high accumulated concentrations and their omnipresence in Flanders. The results in *Chapter 5* strengthened the importance of using biota monitoring in assessing concentrations of POPs and mercury in the aquatic environment. Passive samplers seem to be a promising alternative or supplementary technique, allowing for a reduction of biota sample size. However, more research is needed to fully model the relationship and extrapolation possibilities between these methods, taking into account species-specific metabolization and elimination processes.

The field-based evidence from *Chapter 3* and *4*, confirmed that biota monitoring should be performed as standardized as possible, since monitoring species, size or age, trophic levels and methods of exposure could have important implications on accumulated concentration and pollution profiles. In this thesis, total mercury was used as a proxy for methylmercury, as subscribed according to the WFD. However, for the specific case of internal distribution of mercury, it might be interesting to distinguish between methylmercury and inorganic mercury. We would expect this to give a slightly different image for liver tissue and between different sizes/ages of fish (i.e. higher proportion of inorganic mercury in liver compared to muscle and in younger fish compared to older ones). Furthermore, for PFAS, currently PFOS is considered the target molecule for biota monitoring due to its predominance in higher trophic levels. However, in the bigger picture, PFOA was found to largely contribute to PFAS pollution in lower trophic levels and should be taken into account for the evaluation of the entire ecosystem health as it is

currently already included for human health assessment. Furthermore, recent alternatives for PFOA (Hopkins et al., 2018), such as GenX (the ammonium salt of hexafluoropropylene oxide-dimer acid), ADONA (4,8-dioxa-3H-perfluorononanoic acid) and F-53B (2-[(6-chloro-1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexyl)oxyl]-1,1,2,2-tetra-fluoroethanesulfonic acid, potassium salt), should also be included in future studies, as well as their ecological and human health risks.

The extrapolation potential for Hg, PFOS, HBCD, PBDEs and PCBs found between perch and eel in this thesis has some very promising implications. This should therefore be further investigated using more monitoring fish species from multiple (international) water bodies. The possibility of comparing different species will strongly decrease the amount of individuals that need to be sacrificed and unify international monitoring efforts, creating a uniform pollution image throughout Europe. In order to compare between species and monitoring studies, we would like to stress the importance of standardization for lipid content variation (or dry weight for PFOS and Hg).

To further investigate the ecological relevance of the current EQS_{biota} (*Chapter 6*), a larger dataset with higher accumulated concentrations should be used, so the quantile regression method could be performed for more compounds. Furthermore, this study should be repeated using a fish based index, on a selection of sample locations allowing to determine the IBI and ranging to 'good ecological water quality'.

Although we believe this thesis provides an elaborate first indication of EQS_{biota} relevance, all studies performed in this thesis were restricted to the Flemish environment. In order to extrapolate and verify our finding on a European scale, international (cross-country) studies could be performed to provide a wider range of conditions and species.

Chapter 8

Nederlandstalige samenvatting

8.1 Milieukwaliteitsnormen voor biota

Wereldwijd hebben oppervlaktewateren en aquatische ecosystemen te lijden onder de schadelijke effecten van chemische vervuiling (bv. verlies van biodiversiteit; EC, 2008b; Malaj et al., 2014). Deze stoffen komen voornamelijk in het milieu terecht als afval- of bijproducten van industrie, landbouw en/of huishoudens. Vanuit de Europese Unie werd in 2000 de Kaderrichtlijn Water opgestart als legaal actiekader om een algemene 'goede waterkwaliteit' te bereiken in alle lidstaten (EC, 2000). Om hieraan te voldoen, dienen prioritaire schadelijke stoffen gemonitord en gerapporteerd te worden. In 2008, werd een lijst gepubliceerd met milieukwaliteitsnormen (MKN = EQS) voor 33 prioritaire stoffen die in het water (of sediment) gemeten moeten worden (EC, 2008b).

Alhoewel het gebruik van de meeste gekende schadelijke stoffen ondertussen verboden of sterk beperkt is, blijven vele organische polluenten en metalen door hun persistent karakter vaak nog decennia in de omgeving aanwezig (Schwarzenbach et al., 2006). Daarnaast staan ze erom bekend om gemakkelijk in dierlijke weefsel op te slaan (bioaccumulatie) en hoge concentraties te bereiken in hogere niveaus van de voedselketen (biomagnificatie) (Deribe et al., 2011; Lavoie et al., 2013; Mackay and Fraser, 2000). Zo kunnen toppredatoren en mensen uiteindelijk blootgesteld worden aan zeer hoge, mogelijk toxische concentraties. Binnen de Kaderrichtlijn Water werd de lijst met prioritaire stoffen in 2013 aangevuld met 11 (groepen van) componenten die, omwille van hun hydrofoob karakter en biomagnificatie, niet of moeilijk meetbaar zijn in water en daarom in biota gemonitord dienen te worden: de MKN_{biota} (EQS_{biota}: *Table 1.1*; EU, 2013). Het betreft hexachloorbenzeen (HCB), hexachloorbutadieen (HCBD), kwik (Hg), gebromeerde difenyl ethers (PBDE's), poly- en perfluoralkylstoffen (PFAS) en specifiek PFOS, hexabroomcyclododecaan (HBCD), dicofol, dioxines, heptachloor (en heptachloor epoxide), benzo(a)pyreen en fluorantheen. Door te meten in biota (liefst hogere trofische niveaus, bv. vis), kan het risico op secundaire vergiftiging van top-predatoren – waaronder visetende vogels, zoogdieren en zelfs de mens – ingeschat worden (d.i. aan welke

concentraties de hoogste trofische niveaus worden blootgesteld door het eten van sterk gecontamineerde dieren uit het aquatisch milieu) (EU, 2013; EC, 2014).

Monitoringscampagnes op biota, hebben echter reeds aangetoond dat voor sommige componenten (bv. kwik en PBDEs) globaal grote overschrijdingen van de normen worden waargenomen. Men zou verwachten dat dit zich vertaalt in een enorme daling in waterkwaliteit en biodiversiteit. Dit is echter niet direct het geval, hetgeen een indicatie zou kunnen zijn voor te strikte normen. Het risico hierbij is, dat het aantal 'probleemlocaties' disproportioneel groot wordt waardoor overheden niet meer willen ingrijpen omdat het probleem onoverkomelijk lijkt. Dit terwijl er in realiteit enkel zeer sterk vervuilde locaties aangepakt dienen te worden.

8.2 Doelstellingen van het onderzoek

De hoofddoelstelling van deze thesis was het onderzoeken van de relevantie van de huidige Europese biota normen (MKN_{biota}) – omvat in de Kaderrichtlijn Water – voor de evaluatie van de ecologische kwaliteit van zowel zoet als brak water ecosystemen. In het kader van deze thesis werd de bioaccumulatie van de 11 prioritaire stoffen nagegaan met behulp van passieve en actieve biomonitoring. Voor de passieve biomonitoring werden twee inheemse vissoorten verzameld (Europese baars *Perca fluviatilis* en paling *Anguilla anguilla* – in het juveniele 'gele aal' stadium) uit verschillende Vlaamse waterlopen. Deze soorten werden voornamelijk geselecteerd door hun hoge trofische niveau en sedentaire karakter, waardoor ze een goed beeld geven van de lokale vervuiling. Actieve biomonitoring werd uitgevoerd door het blootstellen van zoetwatermosselen (driehoeksmossel *Dreissena polymorpha*, quagga mossel *Dreissena bugensis* en Aziatische korfmossel *Corbicula fluminea*) in kooien. Over het algemeen werden alle polluenten enkel in vis gemeten (tenzij anders vermeld), met de uitzondering van de polyaromatische koolwaterstoffen (PAK's) benzo(a)pyreen en fluorantheen. Deze werden omwille van hun snelle metabolisatie in vis, gemeten in de mosselen. Daarnaast werden ook polychloorbifenyls (PCB's) gemeten in vis, alhoewel deze momenteel nog niet behoren tot de huidige prioritaire stoffen voor biota. Met hun lipofiele eigenschappen en

bioaccumulatie potentieel, vertonen deze componenten echter wel veel gelijkenissen met de prioritaire stoffen voor biota.

8.3 Biota monitoring in Vlaanderen

In de eerste plaats werden deze concentraties in biota getoetst aan hun desbetreffende normen. **Hoofdstuk 2** bevat de samenvatting van de Vlaamse veldcampagne biotamonitoring uitgevoerd op 44 locaties tussen 2015 en 2018 in het kader van de Kaderrichtlijn Water en in opdracht van de Vlaamse Milieumaatschappij en dient als een algemene rapportage van de huidige toestand van de Vlaamse waterlopen. De uitgebreide dataset verkregen uit dit project, diende daarnaast ook als uitgangspunt voor verschillende studies die verder in deze thesis werden opgenomen. Naast eventuele overschrijdingen van de norm en vergelijking van accumulatie tussen soorten, werd in dit hoofdstuk ook gekeken naar het gebruik passieve samplers voor een subset (HCB, PBDE's, PAK's en PCB's) van stoffen en de relatie met bioaccumulatie.

In de eerste plaats was het duidelijk dat voor alle polluenten de concentraties hoger waren in paling dan in baars, met de uitzondering van PFOS waar het omgekeerde waar was. Verder werden er frequente overschrijdingen van de huidige normen gemeten voor Hg (100% van de locaties), ∑PBDE (≥ 85%) en PFOS (≥ 58%) in beide vissoorten en voor dioxines (69%) en cis-heptachloor (90%) epoxide in paling. Voor kwik en PFOS waren deze overschrijdingen maximaal met een factor 10, terwijl de normen voor PBDE's en cis-heptachloor epoxide zelfs met een factor 100-1000 werden overschreden. Een vergelijking met bestaande (Vlaamse) literatuur bracht voor HCB, HBCD and PCB's een daling over de laatste decennia aan het licht in paling. Voor Hg en PBDE's werd deze trend niet waargenomen.

Ten slotte, konden concentraties op passieve samplers voornamelijk geaccumuleerde concentraties voorspellen voor PAK's in mosselen. Deze relatie valt te verklaren door de meer rechtstreekse blootstelling van mosselen aan polluenten in de waterkolom. De andere polluenten werden gemeten in vissen, dewelke blootgesteld via een complex

metabolisatie proces en blootstelling via het dieet. Ondanks het veelbelovende gebruik van passieve samplers als alternatieve niet-invasieve methode, is meer onderzoek nog vereist alvorens dit de biota monitoring zou kunnen vervangen.

8.4 Specifieke studies van kwik en PFAS

In **hoofdstukken 3 en 4** werd vervolgens gefocust op bioaccumulatie van respectievelijk kwik en PFAS. Deze prioritaire stoffen onderscheiden zich van de andere doordat ze een hoge affiniteit hebben voor proteïnen i.p.v. lipiden (Amlund et al., 2007; Bradley et al., 2017; Forsthuber et al., 2020; Ng and Hungerbühler, 2013). Daarnaast bestaat er voor deze componenten een jarenlange expertise in ons laboratorium (ECOSPHERE, Universiteit Antwerpen). De richtlijnen rond de MKN_{biota} geven geen expliciete specificaties rond welke soorten of monitoring methoden gebruikt moeten worden. Daarom is het onderling vergelijken van accumulatie in verschillende soorten aan te raden om een beter idee te krijgen van accumulatiepatronen en zodat resultaten tussen verschillende (Europese) studies geëxtrapoleerd kunnen worden, zeker in contrast met de andere lipofiele prioritaire stoffen. Daarnaast werd voor beide (groepen van) stoffen het gezondheidsrisico voor de mens bepaald bij eventuele consumptie van de vissen. Dit werd berekend o.b.v. gekende gegevens voor hengelaars die eigen vangst consumeren, aangezien wilde vis uit eigen waterlopen over het algemeen niet wordt gegeten in België

Kwikconcentraties werden vergeleken tussen baars en paling en tussen lever- en spierweefsel (**Hoofdstuk 3**). Daarnaast werd er nagegaan of deze concentraties toenamen met lengte, als maat voor leeftijd. In totaal werden 26 locaties gesampled, die een verscheidenheid aan verschillende omgevingssituaties reflecteerden. Met effecten van de omgeving werd rekening gehouden door te werken met ‘mixed models’. Vervolgens werden geaccumuleerde concentraties in deze studie eveneens gestandaardiseerd voor vetgehalte, aangezien grote verschillen in vetgehalte tussen seizoenen of soorten een effect kunnen hebben op totale weefselgewichten. Kwik wordt echter niet opgeslagen in vet, maar in proteïnen. Daarom kan dit een vertekend beeld geven van de effectieve concentraties en vergelijkingen tussen soorten en weefsels bemoeilijken.

Analoog aan hoofdstuk 2 werden in paling hogere kwikconcentraties gevonden dan in baars. In baars waren concentraties hoger in het spierweefsel, in paling in het leverweefsel. Daarnaast namen concentraties enkel voor baars toe met de lengte, onafhankelijk van de locatie. Voor paling was deze relatie erg locatie-afhankelijk een niet éénduidig. Deze resultaten tonen aan dat het belangrijk is om gestandaardiseerd (soort, weefsel, lengte) te werk te gaan bij het monitoren van kwik in biota. De concentraties gestandaardiseerd voor vetgehalte, gaven dezelfde resultaten met uitzondering van paling, waar beide weefsels niet langer een verschil toonden. Ten slotte, werd gevonden dat enkel frequente consumptie van paling mogelijk humane gezondheidsproblemen zouden kunnen veroorzaken.

In **hoofdstuk 4** werden geaccumuleerde PFAS concentraties (4 perfluoroalkyl sulfonaten en 11 perfluoroalkyl carboxylaten) gemeten in biota. Hiervoor werden concentraties gemeten in vis en gekooide mosselen. Op deze manier konden PFAS accumulatieprofielen vergeleken worden tussen passieve (vis) en actieve (mossel) biomonitoring en tussen de verschillende trofische niveaus.

We vonden dat accumulatieprofielen wel degelijk verschilden tussen beide groepen. Over het algemeen hadden korte-keten PFAS en PFOA het grootste aandeel in mosselen, terwijl lange-keten PFAS en PFOS het grootste aandeel uitmaakten in vis. Een mogelijke verklaring was de hogere kans op biomagnificatie van deze laatsten. Verder vormden voornamelijk palingen op veel locaties een gezondheidsrisico bij humane consumptie, en baarzen in mindere mate. Ondanks het feit dat er hogere concentraties werden gemeten in baars dan in paling, wordt er algemeen meer paling gegeten dan baars, wat leidde tot het hogere risico voor paling.

Ondanks dat zowel kwik als PFOS een hoge affiniteit hebben voor proteïnen, is het opvallend dat kwik het hoogst is in paling, terwijl PFOS dat is in baars. Dit is hoogstwaarschijnlijk te wijten aan de specifieke structuren waaraan ze binden. Kwik bindt voornamelijk aan zwavel- en thiolhoudende proteïnen in spierweefsel (Amlund et al., 2007; Bradley et al., 2017). Paling heeft dan ook meer spierweefsel dan baars. PFOS, aan

de andere kant, bindt aan bloed serum albumine en vetzuurproteïnen (Forsthuber et al., 2020; Ng and Hungerbühler, 2013).

Aanvullend op de gezondheidsrisico's die bepaald werden voor humane consumptie van deze stoffen, werd dit in **hoofdstuk 7** voor alle overige stoffen nagagaan. Hieruit bleek dat enkel dioxines in palingen en PCB's in paling (en mindere mate in baars) een mogelijk risico konden geven. Opvallend was de discrepantie tussen grenswaarden voor humane consumptie en biotanormen. Voor PBDE's en heptachloor waren de waarden voor humane consumptie veel hoger dan de biotanormen, voor PFAS was het omgekeerde waar.

8.5 Effect van de omgeving op accumulatie in biota

Als volgende stap in het verhaal werd in **hoofdstuk 5** nagegaan of geaccumuleerde concentraties van de prioritaire stoffen in biota voorspeld konden worden door omgevingsconcentraties (in water en sediment) en wat het effect van water- en sedimenteigenschappen (pH, zuurstof, conductiviteit, nitraat, nitriet, kleigehalte, TOC en DOC) hierop is. Daarnaast werd ook nagegaan of er een duidelijk lagere detectie van deze typisch hydrofobe stoffen in water werd gevonden en werden PBDE en PCB profielen vergeleken tussen de verschillende media.

In de eerste plaats vonden we een algemeen lagere detectie in de omgeving. Bovendien werd er een grote seizoenale variatie waargenomen voor o.a. kwik en PAK's. Daarnaast konden opgeloste PFOS en benzo(a)pyreen concentraties de concentraties in biota (in vis en mossel respectievelijk) voorspellen. Een mogelijke verklaring hiervoor was dat PFOS beter oplost in water dan de andere prioritaire stoffen en dat mosselen een meer directe blootstelling aan water ondervinden. Anderzijds konden sedimentconcentraties van HCB, PBDE's en PCB's de respectievelijke concentraties in vis voorspellen. Sterk hydrofobe stoffen hebben de neiging om gemakkelijk aan organische deeltjes, die ook in sediment aanwezig, zijn te binden. Door rechtstreeks contact met de bodem tijdens het foerageren, kunnen de vissen deze polluenten gemakkelijk opnemen of indirect via ongewervelden

die in het sediment leven. Er werd echter wel een negatief effect gevonden met de hoeveelheid organisch materiaal (DOC of TOC). Te veel organisch materiaal zal zorgen voor complexvorming waardoor de biobeschikbaarheid vermindert (Dittman and Driscoll, 2009; Li et al., 2015). Daarnaast werd er ook een negatief effect gevonden van pH op HCB accumulatie in paling en van nitriet op PFOS in paling. Een lage pH kan mogelijk de algemene conditie van de vissen negatief beïnvloeden waardoor de eliminatie efficiëntie achteruitgaat (Watras et al., 1998; Wood, 2001). Voor het effect van nitriet kon er geen directe verklaring gevonden worden. Het negatieve effect van kleigehalte op PBDE's in paling kan net als DOC/TOC gehalten te verklaren zijn door immobilisatie van de polluenten.

Vervolgens bleek PCB 153 de overheersende PCB congeneer in alle media, gevolgd door PCB 138 en PCB 180. Algemeen hadden hoger gechloreerde PCBs door hun hogere biomagnificatie een groter aandeel in de totale PCB som in vis. PBDEs werden enkel vergeleken tussen vis en sediment. Hierbij was BDE 99 overheersend in sediment en BDE 47 in vis. Dit laatste is waarschijnlijk het resultaat van de metabolisatie van hoger gebromeerde congeneren in vis. Congeneer BDE 47 werd dan gevolgd door BDE 99 in baars en BDE 100 in paling.

Ten slotte werd gevonden dat geaccumuleerde concentraties geëxtrapoleerd konden worden tussen baars en paling voor Hg, PFOS, HBCD, ∑PBDE en ∑PCB. Dit impliceert dat in de toekomst enkel baars gebruikt kan worden om concentraties in paling (een 'kritisch bedreigde' soort) te voorspellen.

8.6 Relevantie van de MKN_{biota} op ecologische kwaliteit

Uiteindelijk werd in **hoofdstuk 6** een antwoord gezocht op de vraag of de huidige MKN_{biota} voldoende bescherming bieden voor de ecologische kwaliteit van aquatische ecosystemen. De ecologische kwaliteit werd hiervoor gescoord o.b.v. Multimetrische Macroinvertebraten Index voor Vlaanderen (MMIF). Vervolgens konden er drempelwaarden bepaald worden voor concentraties in biota waarboven een goede

ecologische kwaliteit nooit werd bereikt. Op basis van deze drempelwaarden konden de huidige normen dan geëvalueerd worden.

Drempelwaarden werden berekend m.b.v. een 90^{e} kwantiel regressie model en het 95^{e} percentiel. Deze eerste methode is meer robuust en kan enkel bepaald worden indien er daadwerkelijk een dalende trend van water kwaliteit met toenemende concentraties werd waargenomen. Dit was enkel het geval voor PFOS in baars, PCB's in paling en benzo(a)pyreen in mosselen. De grote variatie in drempelwaarden tussen vissoorten werd gecorrigeerd o.b.v. 5% vetgehalte (of 26% droogrest voor PFOS en Hg). Een vergelijking met de huidige normen gaf aan dat deze voor PFOS en benzo(a)pyreen voldoende bescherming boden.

Voor alle andere stoffen, kon enkel het 95^{e} percentiel berekend worden, aangezien zelfs locaties met hoogste gemeten concentraties voor deze stoffen nog een goede ecologische kwaliteit hadden. Met deze minder robuuste methode is het mogelijk dat de effectieve drempelwaarden in realiteit nog hoger liggen. De gevonden drempelwaarden lagen in dit geval hoger dan de huidige normen voor Hg (ca. 10 keer) en ∑PBDE (> 700 keer). Voor HBCD lag de drempelwaarde dan weer 22-46 keer lager dan de huidige norm. De drempelwaarden voor dioxines en PAK's waren vergelijkbaar met huidige normen. Ten slotte werd voor PCB's een drempelwaarde en mogelijke norm gevonden tussen 98.5 en 183 µg kg^{-1} versgewicht (gebaseerd op paling).

8.7 Algemene bevindingen en conclusies

Uit de monitoring van biota in Vlaanderen is gebleken dat Hg, ∑PBDE, PFOS, dioxines en cis-heptachloor epoxide de normen frequent overschrijden, wat de nood voor een algehele herziening van de huidige MKN_{biota} naar boven brengt. Consumptie van de vissen vormde echter voor PFOS, dioxines en PCB's een mogelijk gezondheidsrisico voor mensen, met name voor paling. Extra inspanningen kunnen nodig zijn om de milieuconcentraties van deze verbindingen verder te verlagen. Wat kwik betreft, zou alleen een hoge consumptie van paling (> 71 g dag^{-1}) mogelijk schadelijk kunnen zijn. Op

basis van deze resultaten willen we het huidige advies om geen wilde vis uit Vlaamse rivieren op regelmatige basis te consumeren, versterken. Verder toonde de beoordeling van de ecologische relevantie van MKN_{biota} voor de macroinvertebraten gemeenschap aan dat de huidige normen voor PBDE's (en in mindere mate Hg) wellicht onrealistisch laag zijn en voor HBCD extreem hoog en moeten worden herzien. Voor alle andere verbindingen werden de huidige MKN_{biota} relevant geacht. Er werd een gestandaardiseerde drempelwaarde op basis van palingconcentraties voorgesteld (98.5-183 µg kg^{-1} versgewicht).

Hoewel, dankzij regionale inspanningen, een afname van de waterverontreiniging gedurende de laatste decennia kan worden waargenomen, blijft het van essentieel belang de evolutie van de Vlaamse waterkwaliteit te blijven volgen. De persistentie van de verbindingen die in deze thesis werden opgenomen, resulteert nog steeds in hoge geaccumuleerde concentraties en hun alomtegenwoordigheid in Vlaanderen. De resultaten in *hoofdstuk 5* hebben het belang van de monitoring van biota voor de beoordeling van de concentraties van POP's en kwik in het aquatisch milieu nog eens bevestigd. Passieve samplers lijken een veelbelovend alternatief of aanvullende techniek te zijn, die het mogelijk maakt om het aantal biotamonsters te beperken. Er is echter meer onderzoek nodig om een volledig inzicht te krijgen in het verband en de extrapolatiemogelijkheden tussen deze methoden.

De veldstudies uit *hoofdstuk 3 en 4* hebben bevestigd dat de monitoring van biota zo gestandaardiseerd mogelijk moet worden uitgevoerd, aangezien de monitoring van soorten, grootte of leeftijd, trofische niveaus en blootstellingsmethoden belangrijke implicaties kunnen hebben op de geaccumuleerde concentraties en verontreinigings-profielen. Wat PFAS betreft, wordt PFOS momenteel beschouwd als de doelmolecule voor de monitoring van biota, omdat het overheerst in hogere trofische niveaus. In een groter geheel bleek PFOA echter in hoge mate bij te dragen tot PFAS-verontreiniging in lagere trofische niveaus en moet het in aanmerking worden genomen bij de beoordeling van de gezondheid van het gehele ecosysteem, zoals het momenteel al wordt meegenomen

bij de beoordeling van de menselijke gezondheidsrisico's. Bovendien zal in de toekomst ook rekening gehouden moeten worden met alternatieven voor PFOS en PFOA, zoals GenX, ADONA en F-53B.

Het gevonden extrapolatiepotentieel voor Hg, PFOS, HBCD, PBDE's en PCB's tussen baars en paling heeft een aantal veelbelovende implicaties, maar dient verder onderzocht te worden. De mogelijkheid om verschillende soorten te vergelijken kan het aantal individuen dat moet worden opgeofferd sterk verminderen en de internationale monitoringinspanningen verenigen, waardoor een uniform beeld van de verontreiniging in heel Europa kan ontstaan. Om vergelijkingen tussen soorten en monitoringstudies mogelijk te maken, willen we het belang benadrukken van standaardisatie voor variatie in vetgehalte (of drooggewicht voor PFOS en Hg).

Hoewel we van mening zijn dat deze thesis een uitgebreide eerste indicatie geeft van de relevantie van de MKN_{biota}, waren alle studies die in deze thesis werden uitgevoerd beperkt tot het Vlaamse milieu. Om onze bevindingen te extrapoleren en te verifiëren op Europese schaal, zouden internationale, grensoverschrijdende studies uitgevoerd kunnen worden om een bredere waaier van omstandigheden en soorten aan te bieden.

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Supplementary Information

Appendix A: Chapter 2

A1. Sampling locations

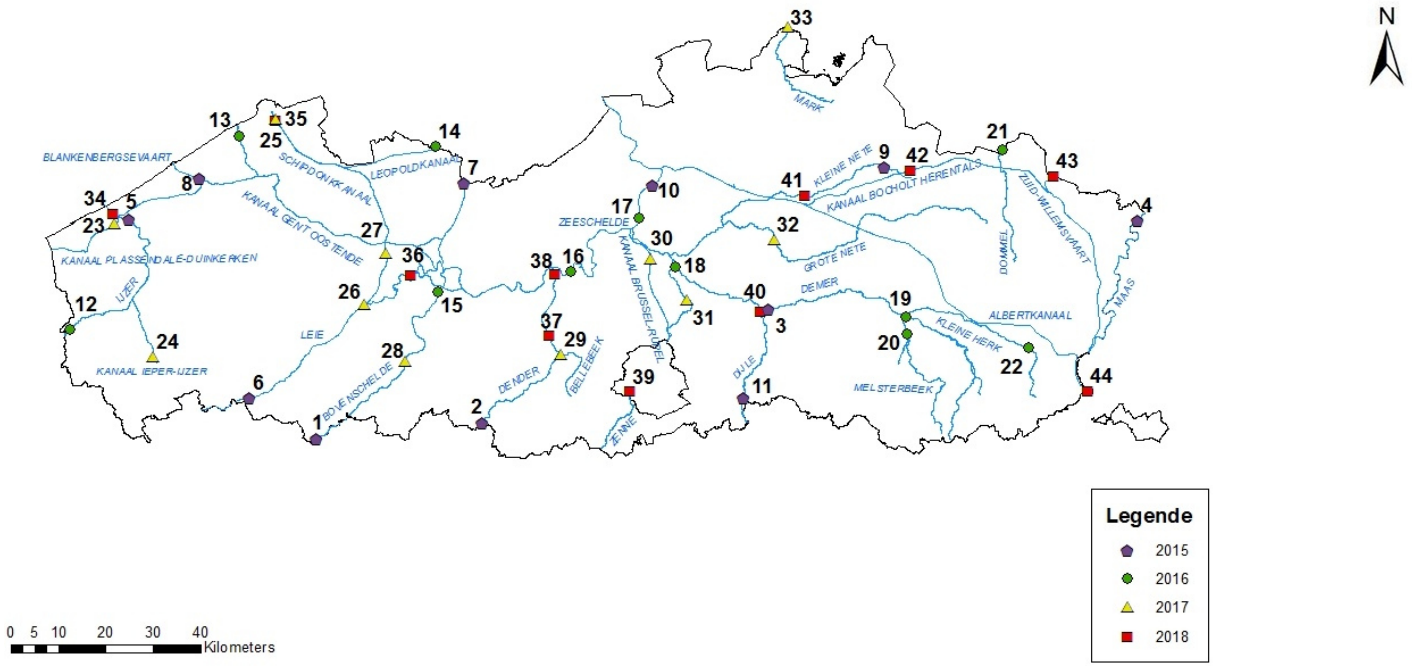

Figure A1.1: Map with an overview of the sampling locations and sampling years.

Table A1.2: Overview of sampling locations and coordinates.

No.	Sampling year	Code waterbody	VMM Location code	Waterbody	Basin	City	X-coordinate (Lambert)	Y-coordinate (Lambert)
1	2015	VL08_55	179000	BOVEN-SCHELDE I	Boven-Schelde	Pecq	79181	157135
2	2015	VL08_67	511000	DENDER I	Dender	Geraardsbergen	114132	160631
3	2015	VL05_104	390000	DEMER VII	Demer	Werchter	174581	184472
4	2015	VL11_203	122050	MAAS I+II+III	Maas	Kinrooi	252525	203301
5	2015	VL05_9	910000	IJZER III	IJzer	Nieuwpoort	39617	203488
6	2015	VL08_48	581000	LEIE I	Leie	Wevelgem	65139	165773
7	2015	VL08_165	30000	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	Gentse Kanalen	Zelzate	110399	211142
8	2015	VL08_164	770000	KANAAL GENT-OOSTENDE III	Brugse Polders	Oostende	54608	212041
9	2015	VL11_126	276700	KLEINE NETE I	Nete	Retie	198974	214563
10	2015	VL05_43	154100	ZEESCHELDE IV	Beneden-Schelde	Antwerpen	150151	210616
11	2015	VL05_77	221000	DIJLE I	Dijle Zenne	Sint-Joris-Weert	169300	165850
12	2016	VL08_7	916000	IJZER I	IJzer	Poperinge	27250	180320
13	2016	VL08_16	877000	BLANKENBERGSE VAART + NOORDEDE	Brugse Polders	Blankenberge	62799	220991
14	2016	VL08_172	12000	LEOPOLDKANAAL I	Gentse Kanalen	Oostburg	104330	218850
15	2016	VL05_58	172100	BOVEN-SCHELDE IV	Boven-Schelde	Gent	104745	188127
16	2016	VL08_41	164000	ZEESCHELDE II	Beneden-Schelde	Dendermonde	132788	192322
17	2016	VL11_42	162000	ZEESCHELDE III + RUPEL	Beneden-Schelde	Hemiksem	147328	203675
18	2016	VL08_82	212000	GETIJDEDIJLE-GETIJDEZENNE	Dijle Zenne	Mechelen	155010	193500
19	2016	VL05_108	446000	HERK + KLEINE HERK	Demer	Herk-de-Stad	203500	182930
20	2016	VL11_207	433900	MELSTERBEEK I+II	Demer	Herk-de-Stad	203850	179330
21	2016	VL05_136	91000	DOMMEL	Maas	Neerpelt	223950	218080
22	2016	VL05_98	401000	DEMER I	Demer	Bilzen	229423	176366
23	2017	VL05_161	680000	KANAAL DUINKERKE-NIEUWPOORT	IJzer	Koksijde	36550	202500
24	2017	VL05_166	946000	KANAAL IEPER-IJZER	IJzer	Ieper	44800	174550

Table A1.2 (continued)

25	2017	VL08_173	6000	LEOPOLDKANAAL II	Brugse Polders	Brugge	70580	224570
26	2017	VL05_50	573300	LEIE III	Leie	Deinze	89248	185468
27	2017	VL05_150	768000	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	Gentse Kanalen	Nevele	93948	196249
28	2017	VL11_204	174000	BOVEN-SCHELDE II+III	Boven-Schelde	Oudenaarde	97860	173600
29	2017	VL05_66	523000	BELLEBEEK	Dender	Liedekerke	130847	175040
30	2017	VL11_181	351000	ZEEKANAAL BRUSSEL-SCHELDE	Beneden-Schelde	Willebroek	149744	195080
31	2017	VL05_93	341560	ZENNE II	Dijle Zenne	Zemst	157305	186511
32	2017	VL08_132	253000	GROTE NETE III	Nete	Heist-op-den-Berg	175730	199242
33	2017	VL11_145	72000	MARK (Maas)	Maas	Hoogstraten	178630	244024
34	2018	VL05_15	122	HAVENGEUL IJZER	IJzer	Nieuwpoort	36342	204544
35	2018	VL05_149	765007	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	Brugse Polders	Zeebrugge	70611	224389
36	2018	VL05_54	571900	TOERISTISCHE LEIE	Leie	Gent	99220	191690
37	2018	VL05_70	503500	DENDER IV	Dender	Aalst	128300	178917
38	2018	VL08_71	499500	DENDER V	Beneden-Schelde	Dendermonde	129551	191944
39	2018	VL08_92	347000	ZENNE I	Dijle Zenne	Anderlecht	145348	167154
40	2018	VL08_80	216000	DIJLE IV	Dijle Zenne	Werchter	172866	184039
41	2018	VL11_127	274000	KLEINE NETE II	Nete	Herentals	182382	208594
42	2018	VL05_160	848200	KANAAL BOCHOLT-HERENTALS	Nete	Dessel	204501	213799
43	2018	VL05_183	851700	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	Maas	Bocholt	234841	212548
44	2018	VL05_151	824000	ALBERTKANAAL	Maas	Kanne	241872	167342

A2. Fish pools and sampling results

Table A2.1.Nnumbers of eel and perch caught during sampling campaigns 2015-2019.

No.	Waterbody	City	VMM code	# Perch	# Eel
1	BOVEN-SCHELDE I	Spiere-Helkijn	179000	20	3
2	DENDER I	Geraardsbergen	511000	20	3
3	DEMER VII	Werchter	390000	9	3
4	MAAS I+II+III	Kinrooi	122050	21	4
5	IJZER III	Nieuwpoort	910000	20	3
6	LEIE I	Wevelgem	581000	17	3
7	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	Zelzate	30000	20	2
8	KANAAL GENT-OOSTENDE III	Oostende	770000	20	3
9	KLEINE NETE I	Grobbendonk	272000	5	3
	KLEINE NETE I	Retie	276700	1	0
	KLEINE NETE I	Dessel		19	3
10	ZEESCHELDE IV	Antwerpen	154100	4	11
11	DIJLE I	Oud-Heverlee	221000	0	3
12	IJZER I	Poperinge	916000	20	1
13	BLANKENBERGSE VAART + NOORDEDE	Blankenberge	877000	6	4
14	LEOPOLDKANAAL I	Oostburg	12000	20	3
15	BOVEN-SCHELDE IV	Gent	172100	12	3
	BOVEN-SCHELDE IV	De Pinte		8	0
16	ZEESCHELDE II	Kastel	164000	3	4
17	ZEESCHELDE III + RUPEL	Niel	162000	3	3
18	GETIJDEDIJLE-GETIJDEZENNE	Mechelen	212000	4	3
19	HERK + KLEINE HERK	Herk-de-Stad	446000	0	2
20	MELSTERBEEK I+II	Herk-de-Stad	433900	1	2
21	DOMMEL	Neerpelt	91000	1	0
	DOMMEL	Overpelt	401000	15	2
22	DEMER I	Bilzen	916000	4	1
23	KANAAL DUINKERKE-NIEUWPOORT	Koksijde	680000	20	3
24	KANAAL IEPER-IJZER	Ieper	946000	1	3
25	LEOPOLDKANAAL II	Brugge	6000	14	3
26	LEIE III	Deinze	573300	10	4
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	Nevele	768000	9	4
28	BOVEN-SCHELDE II+III	Oudenaarde	174000	0	3
29	BELLEBEEK	Liedekerke	523000	3	3
30	ZEEKANAAL BRUSSEL-SCHELDE	Willebroek	351000	20	3
31	ZENNE II	Zemst	341560	2	0
	ZENNE II	Zemst		4	4
	ZENNE II	Zemst		1	0
32	GROTE NETE III	Heist-op-den-Berg	253000	16	4
33	MARK (Maas)	Hoogstraten	72000	19	4
34	HAVENGEUL IJZER	Nieuwpoort	122	0	4
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	Brugge	765007	20	3
36	TOERISTISCHE LEIE	Gent	571900	20	3
37	DENDER IV	Aalst	503500	20	3
38	DENDER V	Dendermonde	499500	0	3
	DENDER V	Dendermonde		20	3
39	ZENNE I	Beersel	347000	20	0
40	DIJLE IV	Wijgmaal	216000	0	3

Table A2.1 (continued)

41	KLEINE NETE II	Herentals	274000	2	3
42	KANAAL BOCHOLT-HERENTALS	Mol	848200	20	0
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	Bocholt	851700	20	3
44	ALBERTKANAAL	Kanne, Riemst	824000	20	2

Table A2.2. Overview of different pools.

No.	Waterbody	VMM code	Poolnumber	Length class (mm)	Weight class (g)	Species	# individuals
1	BOVEN-SCHELDE I	179000	21	86-107	6.7-15.3	Perch	18
1	BOVEN-SCHELDE I	179000	22	173-207	76.3-121	Perch	2
1	BOVEN-SCHELDE I	179000	23	318-634	60.7-538.7	Eel	3
2	DENDER I	511000	14	140-165	30.5-51.4	Perch	8
2	DENDER I	511000	15	179-213	78.7-124	Perch	5
2	DENDER I	511000	16	75-92	4.5-8.3	Perch	9
2	DENDER I	511000	17	489-720	227.9-707.6	Eel	3
3	DEMER VII	390000	8	98-169	22.6-55.8	Perch	5
3	DEMER VII	390000	9	174-190	66.3-79.3	Perch	3
3	DEMER VII	390000	10	502-651	216.9-763.2	Eel	3
4	MAAS I+II+III	122050	31	99-122	9.2-26.1	Perch	9
4	MAAS I+II+III	122050	32	149-195	44.6-96.7	Perch	7
4	MAAS I+II+III	122050	30	209-228	123-160	Perch	5
4	MAAS I+II+III	122050	33	365-534	85-234.2	Eel	4
5	IJZER III	910000	26	88-112	8.3-17.9	Perch	14
5	IJZER III	910000	25	127-220	23.3-150	Perch	5
5	IJZER III	910000	24	385-494	134.2-234.7	Eel	3
6	LEIE I	581000	18	91-114	8.9-18.5	Perch	10
6	LEIE I	581000	19	168-222	59.4-145	Perch	4
6	LEIE I	581000	20	673-840	572.8-978	Eel	3
7	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	30000	11F	111-137	14.6-31.3	Perch	11 females
7	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	30000	11M	111-129	16.9-26.4	Perch	7 males
7	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	30000	12	198-214	96.9-128	Perch	3
8	KANAAL GENT-OOSTENDE III	770000	6	90-116	8-18.6	Perch	15
8	KANAAL GENT-OOSTENDE III	770000	5	148-194	41.8-88.6	Perch	5
8	KANAAL GENT-OOSTENDE III	770000	7	447-700	183.1-790.1	Eel	3
9	KLEINE NETE I	276700	28	140-157	33.7-48	Perch	8
9	KLEINE NETE I	276700	27	161-187	51.1-90.5	Perch	9
9	KLEINE NETE I	276700	29	468-527	208.9-344	Eel	3
10	ZEESCHELDE IV	154100	3	281-388	45.8-107.7	Eel	5
10	ZEESCHELDE IV	154100	2	411-479	124.8-256.7	Eel	4
10	ZEESCHELDE IV	154100	1	625-645	444.2-633.2	Eel	2
11	DIJLE I	221000	4	450-485	196.3-242.8	Eel	3
12	IJZER I	916000	1	75-95	4.9-9.1	Perch	14
12	IJZER I	916000	2	95-103	9.6-11.8	Perch	6

Table A2.2 (continued)

12	IJZER I	916000	3	622	350.5	Eel	1
13	BLANKENBERGSE VAART + NOORDEDE	877000	4	87-125	6.7-23	Perch	5
13	BLANKENBERGSE VAART + NOORDEDE	877000	5	239	201.3	Perch	1
13	BLANKENBERGSE VAART + NOORDEDE	877000	6	449-542	165.1-275.3	Eel	3
14	LEOPOLDKANAAL I	12000	7	70-92	3.3-8.1	Perch	20
14	LEOPOLDKANAAL I	12000	8	465-482	174.8-215	Eel	2
14	LEOPOLDKANAAL I	12000	9	662	517	Eel	1
15	BOVEN-SCHELDE IV	172100	10	107-155	10.7-40.8	Perch	8
15	BOVEN-SCHELDE IV	172100	11	105-137	13.4-30.9	Perch	8
15	BOVEN-SCHELDE IV	172100	12	175-203	77.9-106.4	Perch	2
15	BOVEN-SCHELDE IV	172100	13	462-495	177-239.2	Eel	3
16	ZEESCHELDE II	164000	14	100-120	13.5-21.2	Perch	3
16	ZEESCHELDE II	164000	15	362-415	78.3-117	Eel	3
16	ZEESCHELDE II	164000	16	431	137.5	Eel	1
17	ZEESCHELDE III + RUPEL	162000	17	411	97.7	Eel	1
17	ZEESCHELDE III + RUPEL	162000	18	429	100	Eel	1
17	ZEESCHELDE III + RUPEL	162000	19	444	154.1	Eel	1
18	GETIJDEDIJLE-GETIJDEZENNE	212000	20	93-115	11-19.3	Perch	4
18	GETIJDEDIJLE-GETIJDEZENNE	212000	21	395-432	107.1-151.3	Eel	2
18	GETIJDEDIJLE-GETIJDEZENNE	212000	22	425	163	Eel	1
19	HERK + KLEINE HERK	446000	23	586	295	Eel	1
19	HERK + KLEINE HERK	446000	24	611	443	Eel	1
20	MELSTERBEEK I+II	433900	25	447	156	Eel	1
20	MELSTERBEEK I+II	433900	26	594	432	Eel	1
21	DOMMEL	91000	27	143-165	42.4-64.9	Perch	7 males
21	DOMMEL	91000	28	135-171	31.5-74.8	Perch	8 females
21	DOMMEL	91000	29	738-823	820.6-1079.6	Eel	2
22	DEMER I	401000	30	86-178	9-77.7	Perch	4
22	DEMER I	401000	31	352	82.7	Eel	1
23	KANAAL DUINKERKE-NIEUWPOORT	680000	1	81-129	6.1-23.6	Perch	12
23	KANAAL DUINKERKE-NIEUWPOORT	680000	2	133-167	32.6-59	Perch	8
23	KANAAL DUINKERKE-NIEUWPOORT	680000	3	424-491	150.9-189.2	Eel	3
24	KANAAL IEPER-IJZER	946000	4	422	136.4	Eel	1
24	KANAAL IEPER-IJZER	946000	5	455	167.3	Eel	1
24	KANAAL IEPER-IJZER	946000	6	592	383.7	Eel	1
25	LEOPOLDKANAAL II	6000	7	137-188	34.6-77.4	Perch	3
25	LEOPOLDKANAAL II	6000	8	189-205	96.8-120.6	Perch	3
25	LEOPOLDKANAAL II	6000	9	435-510	170.7-293.8	Eel	3
26	LEIE III	573300	10	106-190	17.3-95.2	Perch	10
26	LEIE III	573300	11	360-445	11.6-178.7	Eel	2
26	LEIE III	573300	12	446-583	195.9-367.5	Eel	2
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	768000	13	120-200	24.4-116.9	Perch	9

Table A2.2 (continued)

27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	768000	14	392-443	112.7-168	Eel	2
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	768000	15	520-537	294.3-306.1	Eel	2
28	BOVEN-SCHELDE II+III	174000	16	465	215.4	Eel	1
28	BOVEN-SCHELDE II+III	174000	17	483	213.6	Eel	1
28	BOVEN-SCHELDE II+III	174000	18	507	237.9	Eel	1
29	BELLEBEEK	523000	19	494	163.2	Eel	1
29	BELLEBEEK	523000	20	500	197.3	Eel	1
29	BELLEBEEK	523000	21	490	205.2	Eel	1
30	ZEEKANAAL BRUSSEL-SCHELDE	351000	22	97-112	11.5-17	Perch	10
30	ZEEKANAAL BRUSSEL-SCHELDE	351000	23	114-132	17.9-29.1	Perch	10
30	ZEEKANAAL BRUSSEL-SCHELDE	351000	24	442-522	160.5-294.6	Eel	3
31	ZENNE II	341560	25	96-120	8.2-21.5	Perch	7
31	ZENNE II	341560	26	467-506	160.1-232.4	Eel	2
31	ZENNE II	341560	27	518-528	319.4-254	Eel	2
32	GROTE NETE III	253000	28	92-101	10.4-13.9	Perch	7
32	GROTE NETE III	253000	29	146-169	45.2-60.8	Perch	9
32	GROTE NETE III	253000	30	432-449	113.1-129	Eel	3
32	GROTE NETE III	253000	31	541	277.1	Eel	1
33	MARK (Maas)	72000	32	80-123	5.7-18.9	Perch	14
33	MARK (Maas)	72000	33	131-142	28.4-36.4	Perch	4
33	MARK (Maas)	72000	34	372-383	87.7-119.6	Eel	2
33	MARK (Maas)	72000	35	420-452	159.8-211.2	Eel	2
34	HAVENGEUL IJZER	122	1	557	332	Eel	1
34	HAVENGEUL IJZER	122	2	522	299	Eel	1
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	765007	3	80-119	6.2-24	Perch	12
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	765007	4	164-211	57-142	Perch	8
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	765007	5	191-270	190-268	Eel	3
36	TOERISTISCHE LEIE	571900	6	75-90	4.5-8.7	Perch	13
36	TOERISTISCHE LEIE	571900	7	120-148	20-31	Perch	7
36	TOERISTISCHE LEIE	571900	8	480-541	205-274	Eel	3
37	DENDER IV	503500	9	88-127	8.2-28	Perch	18
37	DENDER IV	503500	10	162-173	54-61	Perch	2
37	DENDER IV	503500	11	445-518	166-289	Eel	3
38	DENDER V	499500	12	84-122	6.8-20	Perch	18
38	DENDER V	499500	13	175-180	81-79	Perch	2
38	DENDER V	499500	14	420-450	161-216	Eel	3
38	DENDER V	499500	15	482-543	183-295	Eel	3
39	ZENNE I	347000	16	106-131	13-25	Perch	14
39	ZENNE I	347000	17	204-225	126-191	Perch	3
39	ZENNE I	347000	18	237-240	247-280	Perch	2
40	DIJLE IV	216000	19	468	186	Eel	1
40	DIJLE IV	216000	20	520	248	Eel	1

Table A2.2 (continued)

40	DIJLE IV	216000	21	568	308	Eel	1
41	KLEINE NETE II	274000	22	418	120	Eel	1
41	KLEINE NETE II	274000	23	625	452	Eel	1
41	KLEINE NETE II	274000	24	687	697	Eel	1
42	KANAAL BOCHOLT-HERENTALS	848200	25	91-145	9.6-35	Perch	10
42	KANAAL BOCHOLT-HERENTALS	848200	26	143-158	37.9-47	Perch	6
42	KANAAL BOCHOLT-HERENTALS	848200	27	159-168	51-69	Perch	4
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	851700	28	84-95	6.4-9.8	Perch	11
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	851700	29	96-135	9.6-28	Perch	9
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	851700	30	365-515	75-252	Eel	3
44	ALBERTKANAAL	824000	31	100-123	8.2-19	Perch	18
44	ALBERTKANAAL	824000	32	149-160	34-50	Perch	2
44	ALBERTKANAAL	824000	33	587-748	275-856	Eel	2

A3. Mussel pools

Table A3.1: Overview of mussel species used, survival percentages and number of individuals used for PAHs analyses.

No.	Waterbody	VMM code	Species	Survival (%)	# individuals
1	BOVEN-SCHELDE I	179000	*Dreissena polymorpha*	64	43
1	BOVEN-SCHELDE I	179000	*Anodonta cygnea*	100	3
2	DENDER I	511000	*Dreissena polymorpha*	93	69
2	DENDER I	511000	*Anodonta cygnea*	67	2
3	DEMER VII	390000	*Dreissena polymorpha*	50	30
3	DEMER VII	390000	*Anodonta cygnea*	100	3
4	MAAS I+II+III	122050	*Dreissena polymorpha*	94	70
4	MAAS I+II+III	122050	*Anodonta cygnea*	67	2
5	IJZER III	910000	*Dreissena polymorpha*	54	34
5	IJZER III	910000	*Anodonta cygnea*	100	3
6	LEIE I	581000	*Dreissena polymorpha*	97	72
6	LEIE I	581000	*Anodonta cygnea*	67	2
7	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	30000	*Dreissena polymorpha*	80	57
7	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	30000	*Anodonta cygnea*	67	2
8	KANAAL GENT-OOSTENDE III	770000	*Dreissena polymorpha*	94	70
8	KANAAL GENT-OOSTENDE III	770000	*Anodonta cygnea*	67	2
9	KLEINE NETE I	276700	*Dreissena polymorpha*	92	68
9	KLEINE NETE I	276700	*Anodonta cygnea*	67	3
10	ZEESCHELDE IV	154100	*Corbicula fluminea*	87	26

Table A3.1 (continued)

11	DIJLE I	221000	*Dreissena polymorpha*	88	38
11	DIJLE I	221000	*Anodonta cygnea*	0	0
12	IJZER I	916000	*Dreissena polymorpha*	100	65
13	BLANKENBERGSE VAART + NOORDEDE	877000	*Corbicula fluminea*	80	17
14	LEOPOLDKANAAL I	12000	*Corbicula fluminea*	90	20
15	BOVEN-SCHELDE IV	172100	*Dreissena polymorpha*	99	64
15	BOVEN-SCHELDE IV	172100	*Dreissena bugensis*	96	62
16	ZEESCHELDE II	164000	*Dreissena polymorpha*	99	64
16	ZEESCHELDE II	164000	*Dreissena bugensis*	97	63
17	ZEESCHELDE III + RUPEL	162000	*Dreissena polymorpha*	97	63
17	ZEESCHELDE III + RUPEL	162000	*Dreissena bugensis*	97	63
18	GETIJDEDIJLE-GETIJDEZENNE	212000	*Dreissena polymorpha*	100	66
19	HERK + KLEINE HERK	446000	*Dreissena polymorpha*	98	34
20	MELSTERBEEK I+II	433900	*Dreissena polymorpha*	100	66
21	DOMMEL [a]	91000	*Dreissena polymorpha*	0	63
21	DOMMEL [a]	91000	*Dreissena bugensis*	0	66
22	DEMER I	401000	*Dreissena polymorpha*	100	66
23	KANAAL DUINKERKE-NIEUWPOORT	680000	*Corbicula fluminea*	97	20
24	KANAAL IEPER-IJZER	946000	*Dreissena bugensis*	10	7
25	LEOPOLDKANAAL II	6000	*Corbicula fluminea*	53	11
26	LEIE III	573300	*Dreissena bugensis*	94	29
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	768000	*Dreissena bugensis*	100	62
28	BOVEN-SCHELDE II+III	174000	*Dreissena bugensis*	97	58
29	BELLEBEEK	523000	*Dreissena bugensis*	100	62
30	ZEEKANAAL BRUSSEL-SCHELDE	351000	*Dreissena bugensis*	97	58
31	ZENNE II	341560	*Dreissena bugensis*	99	59
32	GROTE NETE III	253000	*Dreissena bugensis*	97	58
33	MARK (Maas)	72000	*Dreissena bugensis*	97	57
34	HAVENGEUL IJZER[b]	122	*Mytilus edulis*	NA	10
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	765007	*Dreissena bugensis*	88	48
36	TOERISTISCHE LEIE	571900	*Dreissena bugensis*	94	54
37	DENDER IV	503500	*Dreissena bugensis*	90	50
38	DENDER V	499500	*Dreissena bugensis*	95	52
39	ZENNE I	347000	*Dreissena bugensis*	95	52
40	DIJLE IV	216000	*Dreissena bugensis*	98	53
41	KLEINE NETE II	274000	*Dreissena bugensis*	58	29
42	KANAAL BOCHOLT-HERENTALS	848200	*Dreissena bugensis*	77	42
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	851700	*Dreissena bugensis*	93	50
44	ALBERTKANAAL	824000	*Dreissena bugensis*	63	39

[a] For analysis in the Dommel, tissue was used that could be recuperated from dead mussels. [b] In the harbour channel of the IJzer, indigenous blue mussels were used because of the high salinity.

A4. Pollutant concentrations in biota

Table A4.1. Overview of individual results for HCB, HCBD, Hg, ∑PBDE, HBCD, PFOS and ∑PCB concentrations per wet weight (µg kg^{-1} ww).

No.	Pool-number	VMM code	species	HCB	HCBD	Hg	∑PBDE	HBCD	PFOS	∑PCB
1	21	179000	Perch	<0.1	<0.5	36	1.2	0.50	8.4	44
1	22	179000	Perch	<0.1	<0.5	118	1.5	1.6	7.1	35
1	23	179000	Eel	3.6	<0.5	74	106	412	9.5	624
2	16	511000	Perch	<0.1	<0.5	156	0.48	0.40	5.3	37
2	14	511000	Perch	<0.1	<0.5	248	1.1	0.40	5.1	102
2	15	511000	Perch	<0.1	<0.5	24	1.4	0.45	5.2	130
2	17	511000	Eel	6.3	<0.5	292	11	10	7.0	858
3	8	390000	Perch	0.10	<0.5	96	1.4	0.53	12	17
3	9	390000	Perch	0.10	<0.5	92	1.4	0.54	9.2	19
3	10	390000	Eel	7.8	<0.5	332	11	19	8.4	282
4	31	122050	Perch	0.10	<0.5	37	0.71	0.28	16	27
4	32	122050	Perch	0.10	<0.5	98	0.67	0.30	11	30
4	30	122050	Perch	0.10	<0.5	199	0.87	0.36	10	36
4	33	122050	Eel	1.1	<0.5	252	5.0	9.2	7.1	385
5	26	910000	Perch	<0.1	<0.5	75	0.34	<0.3	30	10
5	25	910000	Perch	<0.1	<0.5	190	0.60	<0.3	29	13
5	24	910000	Eel	1.1	<0.5	145	1.9	0.74	15	76
6	18	581000	Perch	0.10	<0.5	30	1.7	0.95	17	76
6	19	581000	Perch	<0.1	<0.5	91	1.1	0.69	18	52
6	20	581000	Eel	10.0	<0.5	238	16	21	5.9	1088
7	11F	30000	Perch	<0.1	<0.5	94	1.3	0.54	48	62
7	11M	30000	Perch	0.10	<0.5	92	1.5	0.23	42	73
7	12	30000	Perch	0.10	<0.5	157	1.1	0.44	35	46
8	6	770000	Perch	<0.1	<0.5	89	0.80	0.44	26	33
8	5	770000	Perch	<0.1	<0.5	154	0.96	0.48	27	53
8	7	770000	Eel	2.7	<0.5	268	7.3	9.3	24	472
9	27	276700	Perch	0.10	<0.5	35	0.80	0.37	7.5	3.9
9	28	276700	Perch	<0.1	<0.5	43	0.71	0.36	8.1	4.9
9	29	276700	Eel	3.2	<0.5	162	13	14	11	96
10	3	154100	Eel	3.3	<0.5	87	13	5.4	33	713
10	2	154100	Eel	5.4	<0.5	156	20	5.9	27	1175
10	1	154100	Eel	6.6	<0.5	189	32	12	27	1442
11	4	221000	Eel	3.1	<0.5	323	4.3	15	3.4	165
12	1	916000	Perch	0.10	<0.5	31	0.19	<0.3	12	8.8
12	2	916000	Perch	<0.1	<0.5	42	0.18	<0.3	8.5	1.5
12	3	916000	Eel	0.20	<0.5	232	0.25	<0.3	3.6	5.3
13	4	877000	Perch	<0.1	<0.5	144	<0.3	<0.3	13	1.7
13	5	877000	Perch	<0.1	<0.5	124	<0.3	0.52	9.9	0.74
13	6	877000	Eel	0.60	<0.5	111	0.33	<0.3	10	17
14	7	12000	Perch	<0.1	<0.5	50	<0.3	<0.3	3.5	0.75
14	8	12000	Eel	0.50	<0.5	116	0.56	0.91	3.5	17
14	9	12000	Eel	0.30	<0.5	147	0.56	0.98	6.8	16
15	10	172100	Perch	0.10	<0.5	62	1.3	0.41	16	7.1
15	11	172100	Perch	0.20	<0.5	56	1.7	0.34	16	11
15	12	172100	Perch	<0.1	<0.5	122	1.1	0.26	14	5.6
15	13	172100	Eel	3.7	<0.5	136	65	73	17	681
16	14	164000	Perch	0.10	<0.5	36	1.0	0.30	26	20
16	15	164000	Eel	1.2	<0.5	98	16	7.0	20	633
16	16	164000	Eel	2.2	<0.5	100	45	19	19	1240
17	17	162000	Eel	2.2	<0.5	64	15	5.7	36	908
17	18	162000	Eel	1.5	<0.5	97	14	13	21	1365
17	19	162000	Eel	4.8	<0.5	72	24	8.3	20	1151

Table A4.1(continued)

18	20	212000	Perch	0.20	<0.5	46	1.4	0.40	11	27
18	21	212000	Eel	2.3	<0.5	51	4.4	7.2	9.4	725
18	22	212000	Eel	5.2	<0.5	29	6.9	13	5.2	816
19	23	446000	Eel	2.9	<0.5	86	9.5	5.8	8.1	146
19	24	446000	Eel	4.8	<0.5	140	10	4.9	8.5	158
20	25	433900	Eel	2.0	<0.5	175	11	5.8	80	194
20	26	433900	Eel	2.5	<0.5	140	10	5.9	50	160
21	27	91000	Perch	<0.1	<0.5	42	0.82	0.40	2.3	2.2
21	28	91000	Perch	<0.1	<0.5	45	0.61	0.83	2.6	3.9
21	29	91000	Eel	5.8	<0.5	85	21	44	6.7	114
22	30	401000	Perch	0.20	<0.5	35	0.61	0.50	8.1	3.5
22	31	401000	Eel	1.4	<0.5	52	4.1	8.6	11	81
23	1	680000	Perch	<0.1	<0.5	30	<0.3	<0.3	3.0	1.7
23	2	680000	Perch	<0.1	<0.5	41	<0.3	<0.3	2.3	0.65
23	3	680000	Eel	1.8	<0.5	34	1.5	1.5	2.4	49
24	4	946000	Eel	0.40	<0.5	116	0.80	0.36	58	47
24	5	946000	Eel	0.70	<0.5	159	1.4	0.91	52	77
24	6	946000	Eel	2.7	<0.5	75	3.0	1.8	47	334
25	7	6000	Perch	<0.1	<0.5	54	<0.3	<0.3	10	1.2
25	8	6000	Perch	<0.1	<0.5	36	<0.3	<0.3	9.3	1.0
25	9	6000	Eel	1.4	<0.5	32	38	42	5.4	1122
26	10	573300	Perch	0.17	<0.5	34	0.73	0.29	20	15
26	11	573300	Eel	6.0	<0.5	53	21	20	11	655
26	12	573300	Eel	7.8	<0.5	41	5.9	3.7	20	267
27	13	768000	Perch	0.25	<0.5	40	0.66	0.29	15	13
27	14	768000	Eel	5.6	<0.5	70	15	16	10	491
27	15	768000	Eel	5.9	<0.5	95	13	24	9.6	506
28	16	174000	Eel	11.8	<0.5	87	71	51	6.3	702
28	17	174000	Eel	5.0	<0.5	81	63	52	6.3	693
28	18	174000	Eel	8.7	<0.5	121	59	61	4.3	566
29	19	523000	Eel	1.9	<0.5	226	6.5	4.0	6.2	765
29	20	523000	Eel	2.1	<0.5	226	4.0	2.4	9.5	653
29	21	523000	Eel	2.4	<0.5	199	4.6	2.6	12	607
30	22	351000	Perch	0.13	<0.5	39	0.43	<0.3	45	40
30	23	351000	Perch	0.29	<0.5	50	0.36	<0.3	45	36
30	24	351000	Eel	9.4	<0.5	94	5.7	2.3	35	885
31	25	341560	Perch	0.08	<0.5	32	0.54	0.15	54	94
31	26	341560	Eel	4.9	<0.5	86	8.0	7.6	9.7	1235
31	27	341560	Eel	4.3	<0.5	81	6.1	6.1	6.2	1406
32	28	253000	Perch	0.36	<0.5	22	0.32	<0.3	8.0	4.1
32	29	253000	Perch	0.69	<0.5	58	0.37	<0.3	11	4.5
32	30	253000	Eel	2.4	<0.5	152	2.7	1.2	6.7	158
32	31	253000	Eel	10.4	<0.5	230	2.8	8.3	4.5	224
33	32	72000	Perch	0.16	<0.5	70	0.33	<0.3	3.6	6.2
33	33	72000	Perch	<0.1	<0.5	93	<0.3	<0.3	3.8	2.2
33	34	72000	Eel	1.1	<0.5	56	1.4	0.54	7.8	68
33	35	72000	Eel	0.8	<0.5	67	1.9	0.63	10	92
34	1	122	Eel	1.2	<0.5	188	2.0	0.98	1.0	98
34	2	122	Eel	1.4	<0.5	160	1.4	0.57	2.1	93
35	3	765007	Perch	0.20	<0.5	64	0.24	<0.3	26	5.8
35	4	765007	Perch	<0.1	<0.5	106	0.32	<0.3	47	8.4
35	5	765007	Eel	0.5	<0.5	314	1.1	2.5	19	87
36	6	571900	Perch	<0.1	<0.5	37	1.1	<0.3	10	33
36	7	571900	Perch	<0.1	<0.5	54	1.3	<0.3	19	37
36	8	571900	Eel	5.1	<0.5	129	22	18	13	992
37	9	503500	Perch	<0.1	<0.5	37	0.42	<0.3	5.6	16
37	10	503500	Perch	0.11	<0.5	78	1.6	0.24	5.5	57
37	11	503500	Eel	4.2	<0.5	175	6.2	11	6.2	478
38	12	499500	Perch	0.16	<0.5	49	1.1	0.20	5.7	32
38	13	499500	Perch	<0.1	<0.5	65	0.62	<0.3	10	21

Table A4.1(continued)

38	14	499500	Eel	1.4	<0.5	72	6.6	6.2	5.9	357
38	15	499500	Eel	1.8	<0.5	80	8.3	3.7	10	542
39	16	347000	Perch	0.10	<0.5	119	1.3	0.40	5.8	148
39	17	347000	Perch	0.22	1.1	113	1.1	0.54	6.6	116
39	18	347000	Perch	0.11	0.71	210	1.3	1.0	7.5	155
40	19	216000	Eel	3.9	<0.5	154	3.4	22	0.50	447
40	20	216000	Eel	3.6	<0.5	154	7.0	21	1.8	302
40	21	216000	Eel	1.1	<0.5	149	5.6	17	3.2	280
41	22	274000	Eel	0.7	<0.5	195	1.6	0.97	2.6	140
41	23	274000	Eel	5.6	<0.5	165	20	13	3.2	571
41	24	274000	Eel	3.1	<0.5	301	10	4.9	4.5	399
42	25	848200	Perch	<0.1	<0.5	57	<0.3	<0.3	8.0	19
42	26	848200	Perch	<0.1	<0.5	80	<0.3	<0.3	11	14
42	27	848200	Perch	<0.1	<0.5	83	<0.3	<0.3	8.5	14
43	28	851700	Perch	<0.1	0.8	63	8.0	<0.3	6.4	312
43	29	851700	Perch	<0.1	0.78	79	0.42	<0.3	4.8	47
43	30	851700	Eel	0.1	2.1	103	0.20	2.2	3.1	21
44	31	824000	Perch	0.40	<0.5	110	1.5	0.23	6.4	97
44	32	824000	Perch	0.12	<0.5	133	0.54	<0.3	6.2	41
44	33	824000	Eel	7.0	<0.5	243	8.5	9.0	5.1	669

Table A4.2. Pooled results per location for lipid content (%), dry weight content (g dry weight g^{-1} wet weight), and accumulated concentrations in perch per wet weight (µg kg^{-1} ww).

No.	VMM code	Lipid content	Dry weight content	HCB	Hg	∑PBDE	HBCD	PFOS	∑PCB	Dioxins
1	179000	0.97	0.22	<0.1	77	1.3	1.1	7.7	40	0.0009
2	511000	0.75	0.23	<0.1	143	0.97	0.41	5.2	90	0.0021
3	390000	0.77	0.18	0.10	94	1.4	0.53	11	18	0.0011
4	122050	0.87	0.26	0.10	111	0.75	0.31	12	31	0.0005
5	910000	0.98	0.18	<0.1	132	0.47	<0.3	30	12	0.0004
6	581000	0.98	0.22	<0.1	60	1.4	0.82	18	64	0.0018
7	30000	0.98	0.21	<0.1	114	1.3	0.40	42	61	0.0016
8	770000	0.91	0.21	<0.1	122	0.88	0.46	26	43	0.0020
9	276700	0.87	0.19	<0.1	39	0.76	0.37	7.8	4.4	0.0003
10	154100									
11	221000									
12	916000	1.10	0.21	<0.1	36	0.18	<0.3	10	5.1	
13	877000	1.00	0.21	<0.1	134	<0.3	0.34	11	1.2	0.0006
14	12000	1.00	0.21	<0.1	50	<0.3	<0.3	3.5	0.75	
15	172100	0.72	0.20	0.12	80	1.4	0.34	15	7.8	0.0021
16	164000	0.70	0.20	0.10	36	1.0	0.30	26	20	
17	162000									
18	212000	0.78	0.17	0.20	46	1.4	0.40	11	27	
19	446000									
20	433900									
21	91000	0.88	0.21	<0.1	44	0.71	0.62	2.4	3.1	0.0028
22	401000	1.20	0.22	0.20	35	0.61	0.50	8.1	3.5	0.0011
23	680000	0.74	0.20	<0.1	35	<0.3	<0.3	2.7	1.2	0.0014
24	946000									
25	6000	0.79	0.21	<0.1	45	<0.3	<0.3	9.7	1.1	0.0015
26	573300	0.66	0.20	0.17	34	0.73	0.29	20	15	0.0017
27	768000	0.76	0.21	0.25	40	0.66	0.29	15	13	0.0015
28	174000									
29	523000									
30	351000	0.60	0.20	0.21	44	0.40	<0.3	45	38	0.0035
31	341560	0.77	0.20	<0.1	32	0.54	<0.3	54	94	
32	253000	0.79	0.21	0.52	41	0.34	<0.3	9.5	4.3	0.0013
33	72000	0.75	0.20	<0.1	82	0.24	<0.3	3.7	4.2	0.0013
34	122									
35	765007	0.84	0.20	0.13	85	0.28	<0.3	37	7.1	0.0005
36	571900	0.85	0.20	<0.1	45	1.2	<0.3	14	35	0.0016
37	503500	0.79	0.19	<0.1	58	1.0	0.20	5.5	36	0.0014
38	499500	0.83	0.20	0.11	57	0.86	0.18	7.8	26	0.0008
39	347000	0.91	0.20	0.14	148	1.2	0.65	6.6	140	0.0040
40	216000									
41	274000									
42	848200	0.75	0.20	<0.1	73	<0.3	<0.3	9.0	16	0.0008
43	851700	0.72	0.20	<0.1	71	0.31	<0.3	5.6	34	0.0011
44	824000	0.79	0.19	0.26	121	1.3	0.19	5.6	69	0.0019

Dioxins are given in µg TEQ-WHO_{2005} kg^{-1} ww. Empty cell refer to locations were insufficient fish could be caught to perform analyses. For results below LOQ, ½ LOQ was used a value.

Table A4.3. Pooled results per location for lipid content (%), dry weight content (g dry weight g^{-1} wet weight), and accumulated concentrations in eel per wet weight (µg kg^{-1} ww).

No.	VMM code	Lipid content	Dry weight content	HCB	Hg	∑PBDE	HBCD	PFOS	∑PCB	Dioxins
1	179000	7.1	0.37	3.6	74	106	412	9.5	624	
2	511000	13	0.34	6.3	292	11	10	7.0	858	
3	390000	9.7	0.32	7.8	332	11	19	8.3	282	
4	122050	2.7	0.25	1.1	252	5.0	9.2	7.1	385	
5	910000	4.6	0.29	1.1	145	1.9	0.74	15	76	
6	581000	23	0.74	10	238	16	21	5.9	1088	
7	30000									
8	770000	10	0.45	2.7	268	7.3	9.3	24	472	
9	276700	9.2	0.27	3.2	162	13	14	11	96	
10	154100	13	0.38	5.1	144	22	7.7	29	1110	0.0379
11	221000	12	0.26	3.1	323	4.3	15	3.4	165	0.0040
12	916000	1.9	0.22	0.20	232	0.25	<0.3	3.6	5.3	0.0013
13	877000	12	0.35	0.60	111	0.33	<0.3	10	17	
14	12000	5.0	0.27	0.40	132	0.56	0.94	5.2	16	0.0015
15	172100	14	0.34	3.7	136	65	73	17	681	
16	164000	4.1	0.30	1.7	99	31	13	20	937	0.0171
17	162000	6.8	0.26	2.8	78	18	8.9	25	1141	0.0226
18	212000	11	0.32	3.8	40	5.7	10	7.3	771	0.0097
19	446000	11	0.36	3.9	113	10	5.3	8.3	152	0.0070
20	433900	10	0.30	2.3	158	11	5.9	65	177	0.0062
21	91000	32	0.48	5.8	85	21	44	6.7	114	
22	401000	6.9	0.28	1.4	52	4.1	8.6	11	81	
23	680000	9.1	0.29	1.8	35	1.5	1.5	2.4	49	
24	946000	6.2	0.25	1.3	117	1.8	1.0	52	153	0.0046
25	6000	25	0.39	1.4	32	38	42	5.4	1122	
26	573300	19	0.38	6.9	47	14	12	16	461	
27	768000	16	0.36	5.7	83	14	20	9.8	498	
28	174000	24	0.43	8.5	97	64	54	5.6	654	0.0241
29	523000	11	0.3	2.2	217	5.0	3.0	9.4	675	0.0085
30	351000	7.7	0.28	9.4	94	5.7	2.3	35	885	
31	341560	17	0.35	4.6	83	7.0	6.9	7.9	1321	0.0361
32	253000	6.0	0.26	6.4	191	2.7	4.8	5.6	191	
33	72000	5.6	0.25	0.94	62	1.6	0.59	9.1	80	
34	122	16	0.35	1.3	174	1.7	0.78	1.5	95	0.0080
35	765007	1.9	0.21	0.48	314	1.1	2.5	19	87	
36	571900	21	0.37	5.1	129	22	18	13	992	
37	503500	15	0.33	4.2	175	6.2	11	6.2	478	
38	499500	9.1	0.28	1.6	76	7.4	5.0	8.1	450	
39	347000									
40	216000	15	0.33	2.9	152	5.3	20	1.8	343	0.0069
41	274000	18	0.35	3.1	220	11	6.4	3.4	370	0.0117
42	848200									
43	851700	5.2	0.25	0.12	103	8.0	2.2	3.1	312	
44	824000	16	0.36	7.0	243	8.5	9.0	6.3	669	

Dioxins are given in µg TEQ-WHO_{2005} kg^{-1} ww. Empty cell refer to locations were insufficient fish could be caught to perform analyses. For results below LOQ, ½ LOQ was used a value.

Table A4.4. Pooled results per location for perch in lipid weight (µg kg^{-1} lw).

No.	VMM code	HCB	Hg	∑PBDE	HBCD	PFOS	∑PCB	Dioxins
1	179000	5.2	7938	134	113	794	4124	0.09
2	511000	6.7	19067	129	55	693	12000	0.28
3	390000	13.0	12208	182	69	1429	2338	0.14
4	122050	11.5	12759	86	36	1379	3563	0.06
5	910000	5.1	13469	48	15	3061	1224	0.04
6	581000	5.1	6122	143	84	1837	6531	0.18
7	30000	5.1	11633	133	41	4286	6224	0.16
8	770000	5.5	13407	97	51	2857	4725	0.22
9	276700	5.7	4483	87	43	897	506	0.03
10	154100							
11	221000							
12	916000	4.5	3273	16	14	909	464	
13	877000	5.0	13400	15	34	1100	120	0.06
14	12000	5.0	5000	15	15	350	75	
15	172100	17	11111	194	47	2083	1083	0.29
16	164000	14	5143	143	43	3714	2857	
17	162000							
18	212000	26	5897	179	51	1410	3462	
19	446000							
20	433900							
21	91000	5.7	5000	81	70	273	352	0.32
22	401000	17	2917	51	42	675	292	0.09
23	680000	6.8	4730	20	20	365	162	0.19
24	946000							
25	6000	6.3	5696	19	19	1228	139	0.19
26	573300	26	5152	111	44	3030	2273	0.26
27	768000	33	5263	87	38	1974	1711	0.20
28	174000							
29	523000							
30	351000	35	7333	67	25	7500	6333	0.58
31	341560	6.5	4156	70	19	7013	12208	
32	253000	66	5190	43	19	1203	544	0.16
33	72000	6.7	10933	32	20	493	560	0.17
34	122							
35	765007	15	10119	33	18	4405	845	0.06
36	571900	5.9	5294	141	18	1647	4118	0.19
37	503500	6.3	7342	127	25	696	4557	0.18
38	499500	13	6867	104	22	940	3133	0.10
39	347000	15	16264	132	71	725	15385	0.44
40	216000							
41	274000							
42	848200	6.7	9733	20	20	1200	2133	0.11
43	851700	6.9	9861	43	21	778	4722	0.15
44	824000	33	15316	165	24	709	8734	0.24

Dioxins are given in µg TEQ-WHO$_{2005}$ kg^{-1} lw. Empty cell refer to locations were insufficient fish could be caught to perform analyses.

Table A4.5. Pooled results per location for eel in lipid weight (µg kg^{-1} lw).

No.	VMM code	HCB	Hg	∑PBDE	HBCD	PFOS	∑PCB	Dioxins
1	179000	51	1042	1493	5803	134	8789	
2	511000	48	2246	85	77	54	6600	
3	390000	80	3423	113	196	86	2907	
4	122050	41	9333	185	341	263	14259	
5	910000	24	3152	41	16	326	1652	
6	581000	43	1035	70	91	26	4730	
7	30000							
8	770000	27	2680	73	93	240	4720	
9	276700	35	1761	141	152	120	1043	
10	154100							0.29
11	221000							0.03
12	916000	11	12211	13	7.9	189	279	0.07
13	877000	5.0	925	2.8	1.3	83	142	
14	12000	8.0	2640	11	19	104	320	0.03
15	172100	26	971	464	521	121	4864	
16	164000	41	2415	756	317	488	22854	0.42
17	162000							0.33
18	212000	35	364	52	91	66	7009	0.09
19	446000							0.06
20	433900							0.06
21	91000	18	266	66	138	21	356	
22	401000	20	754	59	125	159	1174	
23	680000	20	385	16	16	26	538	
24	946000	21	1887	29	16	839	2468	0.07
25	6000	5.6	128	152	168	22	4488	
26	573300	36	247	74	63	84	2426	
27	768000	36	519	88	125	61	3113	
28	174000	35	404	267	225	23	2725	0.10
29	523000	20	1973	45	27	85	6136	0.08
30	351000	122	1221	74	30	455	11494	
31	341560	27	488	41	41	46	7771	0.21
32	253000	107	3183	45	80	93	3183	
33	72000	17	1107	29	11	163	1429	
34	122	8.1	1088	11	4.9	9.4	594	0.05
35	765007	25	16526	58	132	1000	4579	
36	571900	24	614	105	86	62	4724	
37	503500	28	1167	41	73	41	3187	
38	499500	18	835	81	55	89	4945	
39	347000							
40	216000	19	1013	35	133	12	2287	0.05
41	274000	17	1222	61	36	19	2056	0.07
42	848200							
43	851700	2.3	1981	154	42	60	6000	
44	824000	44	1519	53	56	39	4181	

Dioxins are given in µg TEQ-WHO_{2005} kg^{-1} lw. Empty cell refer to locations were insufficient fish could be caught to perform analyses.

Table A4.6. Pooled results per location for perch in dry weight (µg kg^{-1} dw).

No.	VMM code	HCB	Hg	∑PBDE	HBCD	PFOS	∑PCB	Dioxins
1	179000	0.23	341	6.1	4.7	37	182	0.004
2	511000	0.22	604	4.4	1.8	24	407	0.009
3	390000	0.57	531	7.8	3.0	220	103	0.006
4	122050	0.41	474	3.1	1.3	52	128	0.002
5	910000	0.27	698	2.5	0.82	159	63	0.002
6	581000	0.35	277	6.5	3.8	79	293	0.008
7	30000	0.38	532	6.1	1.9	196	282	0.008
8	770000	0.23	564	4.1	2.2	120	199	0.010
9	276700	0.38	201	3.9	1.9	42	23	0.002
10	154100							
11	221000							
12	916000	0.35	170	0.9	0.70	48	24	
13	877000	0.24	651	0.71	1.6	55	6.0	0.003
14	12000	0.24	236	0.71	0.7	17	3.5	
15	172100	0.59	399	6.9	1.7	77	39	0.011
16	164000	0.50	181	5.2	1.5	131	100	
17	162000							
18	212000	1.2	271	8.4	2.4	63	161	
19	446000							
20	433900							
21	91000	0.24	210	3.4	3.0	20	15	0.013
22	401000	0.93	164	2.8	2.3	28	16	0.005
23	680000	0.25	176	0.75	0.75	13	5.9	0.007
24	946000							
25	6000	0.24	216	0.71	0.72	47	5.3	0.007
26	573300	0.85	174	3.7	1.5	100	78	0.009
27	768000	1.2	196	3.2	1.4	71	62	0.007
28	174000							
29	523000							
30	351000	1.0	220	2.0	0.74	223	188	0.018
31	341560	0.40	160	2.7	0.76	270	476	
32	253000	2.5	195	1.7	0.73	46	21	0.007
33	72000	0.54	416	1.2	0.76	19	21	0.006
34	122							
35	765007	0.62	417	1.4	0.74	178	35	0.002
36	571900	0.25	230	6.3	0.76	51	180	0.008
37	503500	0.42	303	5.3	1.0	23	190	0.007
38	499500	0.53	286	4.3	0.89	33	134	0.004
39	347000	0.71	735	6	3.2	33	786	0.020
40	216000							
41	274000							
42	848200	0.26	375	0.75	0.77	46	82	0.004
43	851700	0.26	361	1.4	0.77	29	160	0.005
44	824000	1.4	641	5.7	1.0	29	376	0.010

Dioxins are given in µg TEQ-WHO$_{2005}$ kg^{-1} dw. Empty cell refer to locations were insufficient fish could be caught to perform analyses.

Table A4.7. Pooled results per location for eel in dry weight (µg kg^{-1} dw).

No.	VMM code	HCB	Hg	∑PBDE	HBCD	PFOS	∑PCB	Dioxins
1	179000	9.7	199	285	1106	26	1677	
2	511000	18	850	33	30	20	2501	
3	390000	24	1035	34	58	25	879	
4	122050	4.4	1004	20	36	27	1532	
5	910000	3.8	506	6.6	2.6	51	266	
6	581000	14	322	22	28	8.0	1475	
7	30000							
8	770000	6.0	596	16	21	51	1049	
9	276700	12	605	48	54	45	358	
10	154100	13	374	56	20	78	2889	0.100
11	221000	12	1257	17	56	13	643	0.016
12	916000	0.93	1079	1.2	0.70	17	25	0.006
13	877000	1.7	315	0.90	0.43	29	49	
14	12000	1.5	502	2.1	3.6	27	61	0.006
15	172100	11	407	195	217	51	2030	
16	164000	5.5	333	98	42	67	3038	0.056
17	162000	9.1	276	59	32	94	4001	0.077
18	212000	11	133	18	31	24	2446	0.031
19	446000	11	312	28	15	23	422	0.019
20	433900	7.6	534	37	20	220	602	0.021
21	91000	12	179	44	92	14	240	
22	401000	5.1	188	15	31	41	293	
23	680000	6.3	119	5.3	5.3	8.2	168	
24	946000	4.6	488	6.5	3.7	216	540	0.018
25	6000	3.5	83	98	109	14	2886	
26	573300	18	127	38	33	41	1277	
27	768000	16	231	39	57	28	1397	
28	174000	19	222	149	126	13	1524	0.055
29	523000	7.2	731	17	10	31	2272	0.028
30	351000	33	332	20	8.3	124	3128	
31	341560	13	236	20	19	22	3737	0.103
32	253000	25	735	11	18	22	735	
33	72000	3.8	252	6.7	2.4	37	328	
34	122	3.7	498	5.0	2.2	4.4	273	0.023
35	765007	2.3	1526	5.6	12	94	425	
36	571900	14	349	59	49	34	2692	
37	503500	13	531	19	33	19	1452	
38	499500	5.7	273	27	18	29	1608	
39	347000							
40	216000	8.3	465	17	61	6.1	1023	0.020
41	274000	8.0	661	27	16	10	982	0.033
42	848200							
43	851700	0.48	411	42	8.9	13	1630	
44	824000	19	672	24	25	18	1847	

Dioxins are given in µg TEQ-WHO_{2005} kg^{-1} dw. Empty cell refer to locations were insufficient fish could be caught to perform analyses.

Table A4.8. Results for PAHs in freshwater mussels of the Dreissena *genus in µg kg^{-1} ww.*

No.	VMM code	Benzo(a)pyrene			Fluoranthene		
		D. polymorpha	*D. bugensis*	*Dreissena spec.*	*D. polymorpha*	*D. bugensis*	*Dreissena spec.*
1	179000	5.2		5.2	17		17
2	511000	1.3		1.3	<5		<5
3	390000	1.8		1.8	11		11
4	122050	4.7		4.7	17		17
5	910000	1.0		1.0	5.6		5.6
6	581000	2.1		2.1	6.8		6.8
7	30000	17		17	29		29
8	770000	2.8		2.8	13		13
9	276700	1.4		1.4	10		10
10	154100			<1[a]			28[a]
11	221000	4.9		4.9	14		14
12	916000	2.4		2.4	12		12
13	877000			<1[a]			22[a]
14	12000			2.9[a]			41[a]
15	172100	9.5	6.5	8.0	59	26	43
16	164000	7.7	5.5	6.6	30	20	25
17	162000	8.3	5.8	7.1	53	21	37
18	212000	5.2		5.2	46		46
19	446000	3.7		3.7	67		67
20	433900	6.4		6.4	50		50
21	91000	1.8	2.1	2.0	14	15	15
22	401000	4.1		4.1	37		37
23	680000			<1[a]			29[a]
24	946000		2.7	2.7		30	30
25	6000			<1[a]			19[a]
26	573300		2.8	2.8		16	16
27	768000		5.2	5.2		22	22
28	174000		8.0	8.0		32	32
29	523000		2.0	2.0		15	15
30	351000		11	11		11	11
31	341560		27	27		107	107
32	253000		2.8	2.8		18	18
33	72000		<1	<1		16	16
34	122			1.1[b]			15[b]
35	765007		3.0	3.0		45	45
36	571900		4.4	4.4		26	26
37	503500		1.6	1.6		18	18
38	499500		4.0	4.0		24	24
39	347000		4.9	4.9		29	29
40	216000		1.4	1.4		26	26
41	274000		<1	<1		11	11
42	848200		3.5	3.5		9.0	9.0
43	851700		12	12		18	18
44	824000		13	13		40	40

Empty cells refer to locations where a different species was used for active biomonitoring. For results below LOQ, ½ LOQ was used a value. [a] Azian clam, [b] blue mussel.

Table A4.9. Results for PAHs in freshwater mussels of the Dreissena *genus in µg kg^{-1} dw.*

No.	VMM code	Benzo(a)pyrene			Fluoranthene		
		D. polymorpha	*D. bugensis*	*Dreissena spec.*	*D. polymorpha*	*D. bugensis*	*Dreissena spec.*
1	179000	0.05		0.05	0.15		0.15
2	511000	0.01		0.01	<LOQ		<LOQ
3	390000	0.009		0.009	0.05		0.05
4	122050	0.04		0.04	0.15		0.15
5	910000	0.006		0.006	0.03		0.03
6	581000	0.02		0.02	0.06		0.06
7	30000	0.16		0.16	0.26		0.26
8	770000	0.03		0.03	0.12		0.12
9	276700	0.01		0.01	0.09		0.09
10	154100			0.003[a]			0.21[a]
11	221000	0.04		0.04	0.10		0.10
12	916000	0.02		0.02	0.08		0.08
13	877000			<LOQ [a]			0.13[a]
14	12000			0.01[a]			0.19[a]
15	172100	0.07	0.06	0.06	0.42	0.24	0.33
16	164000	0.05	0.05	0.05	0.20	0.18	0.19
17	162000	0.06	0.05	0.06	0.38	0.19	0.29
18	212000	0.04		0.04	0.33		0.33
19	446000	0.02		0.02	0.39		0.39
20	433900	0.04		0.04	0.28		0.28
21	91000	0.02	0.02	0.02	0.18	0.16	0.17
22	401000	0.03		0.03	0.26		0.26
23	680000			<LOQ [a]			0.17[a]
24	946000		0.03	0.03		0.34	0.34
25	6000			<LOQ [a]			0.12[a]
26	573300		0.02	0.02		0.13	0.13
27	768000		0.04	0.04		0.17	0.17
28	174000		0.06	0.06		0.25	0.25
29	523000		0.01	0.01		0.09	0.09
30	351000		0.09	0.09		0.08	0.08
31	341560		0.27	0.27		1.1	1.1
32	253000		0.02	0.02		0.13	0.13
33	72000		<LOQ	<LOQ		0.11	0.11
34	122			0.01[b]			0.14[b]
35	765007		0.02	0.02		0.35	0.35
36	571900		0.03	0.03		0.20	0.20
37	503500		0.02	0.02		0.16	0.16
38	499500		0.03	0.03		0.20	0.20
39	347000		0.04	0.04		0.21	0.21
40	216000		0.009	0.009		0.17	0.17
41	274000		<LOQ	<LOQ		0.08	0.08
42	848200		0.02	0.02		0.05	0.05
43	851700		0.08	0.08		0.11	0.11
44	824000		0.09	0.09		0.29	0.29

Empty cells refer to locations where a different species was used for active biomonitoring. For results below LOQ, ½ LOQ was used a value. [a] Azian clam, [b] blue mussel.

A5. Pollutant concentrations on passive samplers

Table A5.1. Results of pollutant concentrations (µg kg^{-1} sheet) measured by the Flanders Environment Agency in passive samplers.

No.	VMM code	B(a)p	Flu	HCB	∑PCB	∑PBDE
1	179000	6.9	95	0.7	15	0.3
2	511000					
3	390000	5.6	135	4.4	15	1.1
4	122050	4.9	124	1.8	9.7	0.3
5	910000					
6	581000					
7	30000	16	305	0.8	20	0.4
8	770000					
9	276700	0.50	77	0.4	1.7	0.1
10	154100	5.3	95	1.1	23	1.0
11	221000					
12	916000	0.70	20	5.3	40	0.2
13	877000			0.7	1.3	0.1
14	12000			0.7	1.8	0.2
15	172100	6.0	88	1.7	28	2.2
16	164000	6.8	48	1.5	39	1.6
17	162000			1.4	43	0.6
18	212000	6.3	78	2.4	30	0.8
19	446000	5.5	132	1.2	6.8	0.6
20	433900	2.7	83	1.8	38	0.9
21	91000	2.5	167		7.2	0.3
22	401000	2.5	87	1.1	13	0.3
23	680000	1.8	21	0.8	0.9	0.7
24	946000	2.7	41	1.0	10	0.7
25	6000	0.90	47	0.7	1.7	0.6
26	573300	2.7	59	1.3	35	1.0
27	768000	5.4	163	1.5	49	1.4
28	174000	6.8	103	1.4	21	2.4
29	523000	1.9	27	1.1	27	1.0
30	351000	6.2	28	1.0	22	0.7
31	341560	42	781	1.1	323	1.3
32	253000	4.2	44	11	12	0.9
33	72000	1.8	50	0.7	1.8	0.7
34	122	0.80	23	0.2	2.5	
35	765007	1.8	44	0.6	12	0.3
36	571900	4.2	57	1.0	28	0.4
37	503500	2.7	20	0.4	10	0.2
38	499500	3.3	33	0.6	16	0.6
39	347000	2.9	37	1.2	44	0.3
40	216000	4.0	83	1.9	16	1.0
41	274000	0.80	61	0.9	3.1	0.2
42	848200	5.3	49	0.9	16	0.1
43	851700					
44	824000	3.3	62	1.0	15	0.1

Empty cells represent locations where no passive samplers could be deployed.

A6. Trophic levels and stable N isotopes

Table A6.1. Trophic levels and stable N isotopes ($\delta^{15}N$) in fish.

No.	Waterbody	VMM code	Poolnumber	Species	$\delta^{15}N$ (‰)	Trophic level
12	IJZER I	916000	1	Perch	18.2	4.6
12	IJZER I	916000	2	Perch	20.1	5.2
12	IJZER I	916000	3	Eel	19.5	5
13	BLANKENBERGSE VAART + NOORDEDE	877000	4	Perch	15.7	3.9
13	BLANKENBERGSE VAART + NOORDEDE	877000	5	Perch	18.2	4.7
13	BLANKENBERGSE VAART + NOORDEDE	877000	6	Eel	17.4	4.5
14	LEOPOLDKANAAL I	12000	7	Perch	20.5	5.4
14	LEOPOLDKANAAL I	12000	8	Eel	19	4.9
14	LEOPOLDKANAAL I	12000	9	Eel	19	4.9
15	BOVEN-SCHELDE IV	172100	10	Perch	15.5	3.9
15	BOVEN-SCHELDE IV	172100	11	Perch	14.5	3.7
15	BOVEN-SCHELDE IV	172100	12	Perch	15.2	3.8
15	BOVEN-SCHELDE IV	172100	13	Eel	13.9	3.4
16	ZEESCHELDE II	164000	14	Perch	16.7	4
16	ZEESCHELDE II	164000	15	Eel	20.5	5.1
16	ZEESCHELDE II	164000	16	Eel	20.1	5
17	ZEESCHELDE III + RUPEL	162000	17	Eel	20.6	5.2
17	ZEESCHELDE III + RUPEL	162000	18	Eel	20	5
17	ZEESCHELDE III + RUPEL	162000	19	Eel	20.7	5.3
18	GETIJDEDIJLE-GETIJDEZENNE	212000	20	Perch	16.5	4.2
18	GETIJDEDIJLE-GETIJDEZENNE	212000	21	Eel	15.8	4
18	GETIJDEDIJLE-GETIJDEZENNE	212000	22	Eel	15.3	3.9
19	HERK + KLEINE HERK	446000	23	Eel	15.3	3.9
19	HERK + KLEINE HERK	446000	24	Eel	17.3	4.4
20	MELSTERBEEK I+II	433900	25	Eel	14.2	3.5
20	MELSTERBEEK I+II	433900	26	Eel	15.9	4
21	DOMMEL	91000	27	Perch	14.3	4.2
21	DOMMEL	91000	28	Perch	14.6	4.3
21	DOMMEL	91000	29	Eel	15.1	4.4
22	DEMER I	401000	30	Perch	13.9	3.5
22	DEMER I	401000	31	Eel	11.5	2.8
23	KANAAL DUINKERKE-NIEUWPOORT	680000	1	Perch	21.6	5.7
23	KANAAL DUINKERKE-NIEUWPOORT	680000	2	Perch	23.4	6.2
23	KANAAL DUINKERKE-NIEUWPOORT	680000	3	Eel	23.3	6.2
24	KANAAL IEPER-IJZER	946000	4	Eel	17.8	4.6
24	KANAAL IEPER-IJZER	946000	5	Eel	16.7	4.2
24	KANAAL IEPER-IJZER	946000	6	Eel	14	3.5
25	LEOPOLDKANAAL II	6000	7	Perch	18.8	4.8
25	LEOPOLDKANAAL II	6000	8	Perch	18.6	4.8
25	LEOPOLDKANAAL II	6000	9	Eel	18.2	4.7
26	LEIE III	573300	10	Perch	16	4
26	LEIE III	573300	11	Eel	15.1	3.8
26	LEIE III	573300	12	Eel	13.2	3.2

Table A6.1 (continued)

27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	768000	13	Perch	18	4.6
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	768000	14	Eel	19.3	5
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	768000	15	Eel	17.8	4.6
28	BOVEN-SCHELDE II+III	174000	16	Eel	14.5	3.6
28	BOVEN-SCHELDE II+III	174000	17	Eel	14.7	3.6
28	BOVEN-SCHELDE II+III	174000	18	Eel	15.1	3.8
29	BELLEBEEK	523000	19	Eel	12.6	3
29	BELLEBEEK	523000	20	Eel	11.7	2.8
29	BELLEBEEK	523000	21	Eel	12.1	2.9
30	ZEEKANAAL BRUSSEL-SCHELDE	351000	22	Perch	19	4.9
30	ZEEKANAAL BRUSSEL-SCHELDE	351000	23	Perch	18.7	4.8
30	ZEEKANAAL BRUSSEL-SCHELDE	351000	24	Eel	20.4	5.3
31	ZENNE II	341560	25	Perch	15	3.7
31	ZENNE II	341560	26	Eel	14.3	3.5
31	ZENNE II	341560	27	Eel	14.1	3.5
32	GROTE NETE III	253000	28	Perch	14.6	3.6
32	GROTE NETE III	253000	29	Perch	13	3.1
32	GROTE NETE III	253000	30	Eel	13	3.2
32	GROTE NETE III	253000	31	Eel	13.4	3.3
33	MARK (Maas)	72000	32	Perch	14.5	3.6
33	MARK (Maas)	72000	33	Perch	14.9	3.7
33	MARK (Maas)	72000	34	Eel	15.9	4
33	MARK (Maas)	72000	35	Eel	16	4
34	HAVENGEUL IJZER	122	1	Eel	15.2	3.8
34	HAVENGEUL IJZER	122	2	Eel	16.1	4.1
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	765007	3	Perch	20.5	5.4
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	765007	4	Perch	20.3	5.3
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	765007	5	Eel	19.7	5.1
36	TOERISTISCHE LEIE	571900	6	Perch	16.1	4.1
36	TOERISTISCHE LEIE	571900	7	Perch	17.4	4.5
36	TOERISTISCHE LEIE	571900	8	Eel	16.7	4.2
37	DENDER IV	503500	9	Perch	18.5	4.8
37	DENDER IV	503500	10	Perch	19.7	5.1
37	DENDER IV	503500	11	Eel	18	4.6
38	DENDER V	499500	12	Perch	19	4.9
38	DENDER V	499500	13	Perch	18.9	4.9
38	DENDER V	499500	14	Eel	18.1	4.7
38	DENDER V	499500	15	Eel	19.1	4.9
39	ZENNE I	347000	16	Perch	17.3	4.4
39	ZENNE I	347000	17	Perch	16.6	4.2
39	ZENNE I	347000	18	Perch	16.4	4.2
40	DIJLE IV	216000	19	Eel	15.8	4
40	DIJLE IV	216000	20	Eel	16.2	4.1
40	DIJLE IV	216000	21	Eel	15.8	4
41	KLEINE NETE II	274000	22	Eel	15.2	3.8
41	KLEINE NETE II	274000	23	Eel	14.6	3.6
41	KLEINE NETE II	274000	24	Eel	15	3.7
42	KANAAL BOCHOLT-HERENTALS	848200	25	Perch	14.6	3.6
42	KANAAL BOCHOLT-HERENTALS	848200	26	Perch	14.7	3.6
42	KANAAL BOCHOLT-HERENTALS	848200	27	Perch	14.1	3.5

Table A6.1 (continued)

43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	851700	28	Perch	15.3	3.8
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	851700	29	Perch	15.9	4
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	851700	30	Eel	14.9	3.7
44	ALBERTKANAAL	824000	31	Perch	12.7	3.1
44	ALBERTKANAAL	824000	32	Perch	12.6	3
44	ALBERTKANAAL	824000	33	Eel	13.7	3.4

Table A6.2. Stable N isotopes ($\delta^{15}N$) in mussels.

No.	Waterbody	VMM code	Species	$\delta^{15}N$ (‰)
12	IJZER I	916000	*Dreissena polymorpha*	9.3
13	BLANKENBERGSE VAART + NOORDEDE	877000	*Corbicula fluminea*	5.5
14	LEOPOLDKANAAL I	12000	*Corbicula fluminea*	5.2
15	BOVEN-SCHELDE IV	172100	*Dreissena polymorpha*	8.9
15	BOVEN-SCHELDE IV	172100	*Dreissena bugensis*	6.4
16	ZEESCHELDE II	164000	*Dreissena polymorpha*	9.9
16	ZEESCHELDE II	164000	*Dreissena bugensis*	6.8
17	ZEESCHELDE III + RUPEL	162000	*Dreissena polymorpha*	9.7
17	ZEESCHELDE III + RUPEL	162000	*Dreissena bugensis*	6.8
18	GETIJDEDIJLE-GETIJDEZENNE	212000	*Dreissena polymorpha*	8.9
19	HERK + KLEINE HERK	446000	*Dreissena polymorpha*	9
20	MELSTERBEEK I+II	433900	*Dreissena polymorpha*	9.2
21	DOMMEL [a]	91000	*Dreissena polymorpha*	8.8
21	DOMMEL [a]	91000	*Dreissena bugensis*	6.3
22	DEMER I	401000	*Dreissena polymorpha*	8.3
23	KANAAL DUINKERKE-NIEUWPOORT	680000	*Corbicula fluminea*	8
24	KANAAL IEPER-IJZER	946000	*Dreissena bugensis*	5.4
25	LEOPOLDKANAAL II	6000	*Corbicula fluminea*	5.4
26	LEIE III	573300	*Dreissena bugensis*	6.7
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONKKANAAL I	768000	*Dreissena bugensis*	6.4
28	BOVEN-SCHELDE II+III	174000	*Dreissena bugensis*	6.5
29	BELLEBEEK	523000	*Dreissena bugensis*	5.7
30	ZEEKANAAL BRUSSEL-SCHELDE	351000	*Dreissena bugensis*	6.2
31	ZENNE II	341560	*Dreissena bugensis*	6.4
32	GROTE NETE III	253000	*Dreissena bugensis*	6.3
33	MARK (Maas)	72000	*Dreissena bugensis*	6.9
34	HAVENGEUL IJZER[b]	122	*Mytilus edulis*	10.4
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	765007	*Dreissena bugensis*	12.2
36	TOERISTISCHE LEIE	571900	*Dreissena bugensis*	6.7
37	DENDER IV	503500	*Dreissena bugensis*	12.5
38	DENDER V	499500	*Dreissena bugensis*	12.8
39	ZENNE I	347000	*Dreissena bugensis*	6.7
40	DIJLE IV	216000	*Dreissena bugensis*	8.8
41	KLEINE NETE II	274000	*Dreissena bugensis*	6.8
42	KANAAL BOCHOLT-HERENTALS	848200	*Dreissena bugensis*	5.9
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (deels) + KANAAL BRIEGDEN-NEERHAREN	851700	*Dreissena bugensis*	7.4
44	ALBERTKANAAL	824000	*Dreissena bugensis*	6.2

Appendix B: Chapter 3

Table B.1. Results Spearman correlation tests on length measurements in perch (correlation coefficient; p-value).

	Total length	***Fork length***	***Standard length***
Total length	*	0.99 (<0.001)	1.00 (<0.001)
Fork length		*	0.99 (<0.001)
Standard length			*

Table B.2. Results Spearman correlation tests on concentrations in wet weight (ww), dry weight (dw) and lipid weight (lw) in both muscle and liver tissue of perch (correlation coefficient; p-value).

	Hg muscle (ww)	***Hg liver (ww)***	***Hg muscle (dw)***	***Hg liver (dw)***	***Hg muscle (lw)***	***Hg liver (lw)***
Hg muscle (ww)	*	0.72 (<0.001)	0.97 (<0.001)	0.69 (<0.001)	0.94 (<0.001)	0.57 (<0.001)
Hg liver (ww)		*	0.75 (<0.001)	0.99 (<0.001)	0.65 (<0.001)	0.92 (<0.001)
Hg muscle (dw)			*	0.73 (<0.001)	0.95 (<0.001)	0.59 (<0.001)
Hg liver (dw)				*	0.63 (<0.001)	0.92 (<0.001)
Hg muscle (lw)					*	0.56 (<0.001)
Hg liver (lw)						*

Table B.3. Results Spearman correlation tests on concentrations in wet weight (ww), dry weight (dw) and lipid weight (lw) in both muscle and liver tissue of eel (correlation coefficient; p-value).

	Hg muscle (ww)	***Hg liver (ww)***	***Hg muscle (dw)***	***Hg liver (dw)***	***Hg muscle (lw)***	***Hg liver (lw)***
Hg muscle (ww)	*	0.56 (<0.001)	0.92 (<0.001)	0.57 (<0.001)	0.41 (<0.001)	0.52 (<0.001)
Hg liver (ww)		*	0.67 (<0.001)	0.97 (<0.001)	0.45 (<0.001)	0.92 (<0.001)
Hg muscle (dw)			*	0.68 (<0.001)	0.64 (<0.001)	0.63 (<0.001)
Hg liver (dw)				*	0.40 (<0.01)	0.95 (<0.001)
Hg muscle (lw)					*	0.40 (<0.01)
Hg liver (lw)						*

Table B.4: Individual length and weight, dry/wet weight ratios and lipid contents (%) for perch.

No.	Water body	Indi-vidual	Total length (mm)	Weight (g)	Dry/wet weight ratio		Total lipid content (%)	
					Muscle tissue	Liver tissue	Muscle tissue	Liver tissue
1	Boven-Schelde I	B1	207	120.8	0.22	0.25	NA	2.7
1	Boven-Schelde I	B2	173	76.3	0.22	0.24	0.76	2.3
1	Boven-Schelde I	B3	92	9.0	0.21	0.27	1.2	NA
1	Boven-Schelde I	B4	96	10.9	0.19	0.24	2.0	NA
1	Boven-Schelde I	B5	95	9.5	0.21	0.23	0.92	NA
1	Boven-Schelde I	B6	94	9.1	0.22	0.23	1.3	NA
1	Boven-Schelde I	B7	87	8.2	0.17	0.22	1.1	NA
1	Boven-Schelde I	B8	95	10.3	0.21	0.25	1.0	NA
1	Boven-Schelde I	B9	90	12.3	0.21	0.23	1.1	NA
1	Boven-Schelde I	B10	105	13.9	0.22	0.25	0.96	NA
1	Boven-Schelde I	B11	78	7.7	0.21	0.24	1.0	NA
1	Boven-Schelde I	B12	103	12.6	0.21	0.24	1.2	NA
1	Boven-Schelde I	B13	91	8.3	0.10	0.24	0.92	NA
1	Boven-Schelde I	B14	88	7.6	0.22	0.26	0.86	NA
1	Boven-Schelde I	B15	98	8.2	0.10	0.24	1.4	NA
1	Boven-Schelde I	B16	85	10.4	0.22	0.25	0.93	NA
1	Boven-Schelde I	B17	73	6.7	0.22	0.21	1.0	NA
1	Boven-Schelde I	B18	80	8.4	0.25	0.21	1.3	NA
1	Boven-Schelde I	B19	107	15.3	0.22	0.30	1.5	NA
1	Boven-Schelde I	B20	98	9.1	0.10	0.28	0.94	NA
2	Dender I	B1	151	38.5	0.19	0.25	0.68	2.3
2	Dender I	B2	164	80.4	0.19	0.22	0.52	3.2
2	Dender I	B3	156	44.1	0.19	0.23	0.53	2.5
2	Dender I	B4	156	48.9	0.21	0.24	0.67	2.2
2	Dender I	B5	164	49.5	0.19	0.23	0.64	2.7
2	Dender I	B6	165	51.4	0.19	0.26	0.60	2.7
2	Dender I	B7	213	124.2	0.18	0.25	0.64	2.5
2	Dender I	B8	156	47.7	0.20	0.24	0.74	2.4
2	Dender I	B9	179	78.7	0.21	NA	0.69	2.8
2	Dender I	B10	155	43.7	0.20	0.22	0.71	2.1
2	Dender I	B11	75	4.9	0.15	0.15	NA	NA
2	Dender I	B12	140	30.5	0.19	0.24	0.51	2.3
2	Dender I	B13	84	6.7	0.17	0.24	0.79	NA
2	Dender I	B14	92	8.3	0.21	0.26	1.1	NA
2	Dender I	B15	92	8.9	0.20	0.23	NA	NA
2	Dender I	B16	76	5.5	0.21	0.24	1.1	NA
2	Dender I	B17	90	7.8	0.20	0.25	0.94	NA
2	Dender I	B18	82	5.8	0.11	0.24	NA	NA
2	Dender I	B19	84	6.1	0.21	0.24	1.1	NA
2	Dender I	B20	76	4.5	0.22	0.30	NA	NA
3	Demer VII	B1	172	68.7	0.18	0.23	0.76	3.0
3	Demer VII	B2	125	22.6	0.21	0.26	0.88	NA
3	Demer VII	B3	174	66.3	0.22	0.26	0.85	4.0
3	Demer VII	B4	169	55.8	0.22	0.23	0.68	NA
3	Demer VII	B5	170	54.2	0.21	0.25	0.76	NA
3	Demer VII	B6	156	50.5	0.21	0.22	0.74	NA
3	Demer VII	B7	144	37.1	0.21	0.24	0.79	NA
3	Demer VII	B8	190	79.3	0.30	0.22	0.66	3.0
3	Demer VII	B9	98	8.9	0.42	0.25	0.83	NA
4	Maas I+II+III	B1	195	96.7	0.21	0.25	0.82	3.6
4	Maas I+II+III	B2	102	10.4	0.20	0.22	0.98	NA
4	Maas I+II+III	B3	108	11.5	0.19	0.22	0.77	NA
4	Maas I+II+III	B4	99	9.2	0.14	0.21	1.0	NA
4	Maas I+II+III	B5	228	160.1	0.21	0.25	0.91	2.9

Table B.4 (continued)

4	Maas I+II+III	B6	207	147.3	0.47	0.24	1.2	2.4
4	Maas I+II+III	B7	209	122.9	0.21	0.25	0.72	2.1
4	Maas I+II+III	B8	101	9.9	0.21	0.26	2.5	NA
4	Maas I+II+III	B9	214	138.2	0.21	0.27	0.84	4.2
4	Maas I+II+III	B10	122	15.8	0.19	0.22	0.86	NA
4	Maas I+II+III	B11	101	9.5	0.19	0.22	0.83	NA
4	Maas I+II+III	B12	109	12.8	0.20	0.21	0.88	NA
4	Maas I+II+III	B13	171	56.1	0.21	0.23	0.68	2.8
4	Maas I+II+III	B14	149	44.7	0.21	0.24	0.90	2.4
4	Maas I+II+III	B15	102	9.4	0.20	0.24	1.1	NA
4	Maas I+II+III	B16	214	124.6	0.20	0.23	0.74	2.4
4	Maas I+II+III	B17	114	13.6	0.20	0.22	0.87	NA
4	Maas I+II+III	B18	121	26.1	0.20	0.23	0.84	NA
4	Maas I+II+III	B19	105	10.3	0.19	0.21	1.1	NA
4	Maas I+II+III	B20	108	12.8	0.20	0.21	0.82	NA
4	Maas I+II+III	B21	95	10.1	0.21	0.23	1.1	NA
5	IJzer III	B1	132	44.5	0.21	0.25	0.90	NA
5	IJzer III	B2	182	57.5	0.18	0.24	0.67	2.4
5	IJzer III	B3	127	23.3	0.18	0.21	0.76	NA
5	IJzer III	B4	220	149.6	0.20	0.24	0.79	2.6
5	IJzer III	B5	178	76.2	0.21	0.24	0.71	2.4
5	IJzer III	B6	106	14.2	0.21	0.24	0.94	NA
5	IJzer III	B7	82	9.7	0.21	0.23	1.5	NA
5	IJzer III	B8	91	8.9	0.20	0.23	0.94	NA
5	IJzer III	B9	112	17.9	0.21	0.23	0.80	NA
5	IJzer III	B10	101	11.3	0.18	0.22	1.2	NA
5	IJzer III	B11	92	11.3	0.21	0.25	0.92	NA
5	IJzer III	B12	112	16.1	0.21	0.25	1.1	NA
5	IJzer III	B13	88	8.3	0.19	0.25	1.0	NA
5	IJzer III	B14	89	8.7	0.21	0.23	1.4	NA
5	IJzer III	B15	96	9.6	0.21	0.15	1.6	NA
5	IJzer III	B16	97	10.5	0.23	0.24	1.9	NA
5	IJzer III	B17	115	13.9	0.22	0.23	1.2	NA
5	IJzer III	B18	95	9.8	0.20	0.22	1.1	NA
5	IJzer III	B20	99	10.6	0.22	0.23	1.2	NA
6	Leie I	B1	106	16.6	0.20	0.26	1.1	NA
6	Leie I	B2	103	12.6	0.17	0.21	0.94	NA
6	Leie I	B3	219	138.3	0.21	0.25	0.92	2.3
6	Leie I	B4	181	114.1	0.10	0.30	0.78	NA
6	Leie I	B5	101	13.4	0.19	0.16	1.1	NA
6	Leie I	B6	112	17.8	0.21	0.24	0.85	NA
6	Leie I	B8	93	13.8	0.21	0.25	1.2	NA
6	Leie I	B10	114	18.5	0.10	0.18	1.3	NA
6	Leie I	B11	86	9.0	0.21	0.24	1.2	NA
6	Leie I	B12	222	145.3	0.19	0.26	0.78	2.6
6	Leie I	B13	99	10.8	0.22	0.24	1.1	NA
6	Leie I	B14	100	9.1	0.22	0.26	1.1	NA
6	Leie I	B15	91	8.9	0.17	0.23	0.90	NA
6	Leie I	B16	168	59.4	0.10	0.23	1.0	2.0
7	Kanaal Gent-Terneuzen	B1	103	19.9	0.21	0.24	0.99	NA
7	Kanaal Gent-Terneuzen	B2	124	20.4	0.22	0.25	1.0	NA
7	Kanaal Gent-Terneuzen	B3	117	19.7	0.21	0.24	1.0	NA
7	Kanaal Gent-Terneuzen	B4	126	26.4	0.21	0.24	1.1	NA
7	Kanaal Gent-Terneuzen	B5	123	20.3	0.22	0.24	1.2	NA

Table B.4 (continued)

7	Kanaal Gent-Terneuzen	B6	176	96.9	0.22	0.24	1.1	2.4
7	Kanaal Gent-Terneuzen	B7	214	127.8	0.21	0.24	0.95	2.7
7	Kanaal Gent-Terneuzen	B8	132	26.0	0.21	0.23	0.95	NA
7	Kanaal Gent-Terneuzen	B9	111	15.4	0.19	0.19	0.92	NA
7	Kanaal Gent-Terneuzen	B10	137	31.3	0.22	0.30	0.88	2.0
7	Kanaal Gent-Terneuzen	B11	125	24.9	0.21	0.24	0.92	NA
7	Kanaal Gent-Terneuzen	B12	116	25.0	0.21	0.23	0.84	NA
7	Kanaal Gent-Terneuzen	B13	114	18.0	0.21	0.24	1.1	NA
7	Kanaal Gent-Terneuzen	B14	206	102.3	0.22	0.28	0.91	4.0
7	Kanaal Gent-Terneuzen	B15	121	21.0	0.21	0.21	0.97	NA
7	Kanaal Gent-Terneuzen	B16	111	14.6	0.19	0.22	1.1	NA
7	Kanaal Gent-Terneuzen	B17	111	16.9	0.20	0.23	0.95	NA
7	Kanaal Gent-Terneuzen	B18	129	26.3	0.21	0.23	0.92	NA
7	Kanaal Gent-Terneuzen	B19	124	23.7	0.21	0.24	0.93	NA
7	Kanaal Gent-Terneuzen	B20	120	20.4	0.21	0.24	0.91	NA
8	Kanaal Gent-Oostende III	B1	148	41.8	0.21	0.24	0.77	2.7
8	Kanaal Gent-Oostende III	B2	154	46.3	0.21	0.26	0.83	2.7
8	Kanaal Gent-Oostende III	B3	153	45.6	0.19	0.25	0.74	2.5
8	Kanaal Gent-Oostende III	B4	175	71.9	0.20	0.24	0.80	3.1
8	Kanaal Gent-Oostende III	B5	115	17.0	0.22	0.21	0.89	NA
8	Kanaal Gent-Oostende III	B6	97	15.5	0.22	0.23	1.0	NA
8	Kanaal Gent-Oostende III	B7	108	14.3	0.21	0.22	0.92	NA
8	Kanaal Gent-Oostende III	B8	116	18.3	0.21	0.23	0.75	NA
8	Kanaal Gent-Oostende III	B9	114	18.6	0.22	0.22	0.87	NA
8	Kanaal Gent-Oostende III	B10	103	12.2	0.21	0.24	1.5	NA
8	Kanaal Gent-Oostende III	B11	103	12.8	0.21	0.22	1.2	NA
8	Kanaal Gent-Oostende III	B12	194	88.6	0.20	0.23	0.74	2.8
8	Kanaal Gent-Oostende III	B13	103	13.0	0.21	0.23	1.1	NA
8	Kanaal Gent-Oostende III	B14	113	17.2	0.22	0.23	1.1	NA

Table B.4 (continued)

8	Kanaal Gent-Oostende III	B15	108	14.8	0.21	0.23	0.84	NA
8	Kanaal Gent-Oostende III	B16	103	10.9	0.22	0.22	0.96	NA
8	Kanaal Gent-Oostende III	B17	103	11.8	0.22	0.24	1.2	NA
8	Kanaal Gent-Oostende III	B18	94	10.3	0.22	0.22	NA	NA
8	Kanaal Gent-Oostende III	B19	96	10.7	0.22	0.23	1.3	NA
8	Kanaal Gent-Oostende III	B20	90	8.0	0.21	0.23	1.0	NA
9	Kleine Nete I	B1	159	65.3	0.20	0.21	0.82	2.0
9	Kleine Nete I	B2	176	64.5	0.20	0.17	0.71	2.2
9	Kleine Nete I	B3	153	47.7	0.20	0.22	0.76	2.3
9	Kleine Nete I	B4	170	66.3	0.20	0.21	0.76	2.5
9	Kleine Nete I	B5	161	47.5	0.21	0.22	0.82	2.2
9	Kleine Nete I	B6	167	51.1	0.19	0.21	0.92	2.2
9	Kleine Nete I	B7	167	90.5	0.21	0.24	0.85	2.3
9	Kleine Nete I	B8	141	33.7	0.20	0.22	0.81	NA
9	Kleine Nete I	B9	175	62.6	0.20	0.23	0.97	2.6
9	Kleine Nete I	B10	140	33.0	0.18	0.21	0.92	NA
9	Kleine Nete I	B11	161	53.5	0.20	0.21	0.89	2.5
9	Kleine Nete I	B12	153	54.0	0.21	0.22	1.0	2.1
9	Kleine Nete I	B13	157	48.1	0.20	0.22	0.94	2.1
9	Kleine Nete I	B14	152	43.3	0.21	0.20	0.88	2.4
9	Kleine Nete I	B15	150	40.0	0.20	0.22	0.93	2.2
9	Kleine Nete I	B17	164	54.3	0.20	0.20	0.96	2.1
9	Kleine Nete I	B19	145	38.4	0.20	0.20	0.88	NA
12	IJzer I	B1	97	10.5	0.21	0.21	1.2	NA
12	IJzer I	B2	103	11.8	0.21	0.23	1.2	NA
12	IJzer I	B3	95	9.7	0.21	0.24	NA	NA
12	IJzer I	B4	92	7.5	0.21	0.22	NA	NA
12	IJzer I	B5	95	9.1	0.21	0.21	0.95	NA
12	IJzer I	B6	84	6.7	0.22	0.22	NA	NA
12	IJzer I	B7	85	6.2	0.22	0.22	NA	NA
12	IJzer I	B8	86	6.1	0.20	0.22	NA	NA
12	IJzer I	B9	99	9.6	0.20	0.25	1.4	NA
12	IJzer I	B10	94	8.1	0.20	0.25	0.88	NA
12	IJzer I	B11	100	10.4	0.21	0.23	1.0	NA
12	IJzer I	B12	100	10.2	0.20	0.23	0.98	NA
12	IJzer I	B13	80	6.5	0.21	0.23	1.1	NA
12	IJzer I	B14	84	5.4	0.20	0.23	1.1	NA
12	IJzer I	B15	85	5.8	0.20	0.23	1.8	NA
12	IJzer I	B16	75	4.9	0.20	0.24	1.4	NA
12	IJzer I	B17	92	7.8	0.20	0.25	0.98	NA
12	IJzer I	B18	85	6.8	0.20	0.21	1.2	NA
12	IJzer I	B19	89	6.9	0.21	0.19	1.3	NA
12	IJzer I	B20	89	5.8	0.23	0.23	1.5	NA
13	Blankenbergse vaart	B1	116	15.7	0.20	0.26	1.1	NA
13	Blankenbergse vaart	B2	239	201.3	0.19	0.23	0.72	2.5
13	Blankenbergse vaart	B3	112	23.0	0.20	0.23	0.69	2.2
13	Blankenbergse vaart	B4	122	19.4	0.21	0.22	NA	NA
13	Blankenbergse vaart	B5	102	10.5	0.18	0.20	1.1	NA
13	Blankenbergse vaart	B1B	87	6.7	0.21	0.23	1.1	2.1
14	Leopoldkanaal I	B1	72	3.5	0.22	0.22	NA	NA
14	Leopoldkanaal I	B2	92	8.1	0.20	0.25	0.78	NA
14	Leopoldkanaal I	B3	92	6.9	0.19	NA	NA	NA
14	Leopoldkanaal I	B4	73	3.8	0.21	0.25	0.96	NA

Table B.4 (continued)

14	Leopoldkanaal I	B5	79	5.1	0.20	NA	1.1	NA
14	Leopoldkanaal I	B6	78	4.4	0.21	NA	NA	NA
14	Leopoldkanaal I	B7	76	4.0	0.21	0.18	0.92	NA
14	Leopoldkanaal I	B8	70	3.7	0.22	0.22	1.1	NA
14	Leopoldkanaal I	B9	86	6.1	0.20	0.25	1.3	NA
14	Leopoldkanaal I	B10	83	5.0	0.19	NA	NA	NA
14	Leopoldkanaal I	B11	83	7.1	0.20	0.24	0.93	NA
14	Leopoldkanaal I	B12	75	4.6	0.20	0.21	1.0	NA
14	Leopoldkanaal I	B13	80	5.3	0.21	0.23	1.1	NA
14	Leopoldkanaal I	B14	80	6.0	0.20	0.24	0.80	NA
14	Leopoldkanaal I	B15	79	5.6	0.21	0.22	0.93	NA
14	Leopoldkanaal I	B16	81	3.3	0.23	0.21	1.2	NA
14	Leopoldkanaal I	B17	76	4.8	0.21	0.24	1.2	NA
14	Leopoldkanaal I	B18	78	4.3	0.20	NA	1.0	NA
14	Leopoldkanaal I	B19	84	6.3	0.20	0.24	NA	NA
14	Leopoldkanaal I	B20	78	5.0	0.21	0.24	1.0	NA
15	Boven-Schelde IV	B1	162	77.9	0.20	0.23	0.77	3.3
15	Boven-Schelde IV	B2	203	106.4	0.19	0.20	0.66	2.0
15	Boven-Schelde IV	B3	137	30.9	0.19	0.20	0.55	2.2
15	Boven-Schelde IV	B4	109	13.4	0.18	0.18	0.66	2.2
15	Boven-Schelde IV	B5	125	22.5	0.20	0.22	0.70	2.0
15	Boven-Schelde IV	B6	105	15.2	0.21	0.21	0.79	2.6
15	Boven-Schelde IV	B7	110	16.6	0.19	0.23	1.00	3.4
15	Boven-Schelde IV	B9	126	21.6	0.20	0.21	0.81	2.2
15	Boven-Schelde IV	B10	121	19.0	0.20	0.20	0.90	2.4
15	Boven-Schelde IV	B11	120	18.4	0.19	0.26	0.79	4.0
15	Boven-Schelde IV	B13	128	25.5	0.21	0.22	0.87	2.5
15	Boven-Schelde IV	B14	128	25.2	0.19	0.24	0.76	3.0
15	Boven-Schelde IV	B15	155	40.7	0.20	0.23	0.74	3.7
15	Boven-Schelde IV	B16	133	40.8	0.19	0.22	0.68	2.7
15	Boven-Schelde IV	B17	84	10.7	0.17	0.19	0.50	NA
15	Boven-Schelde IV	B18	114	17.6	0.19	0.24	0.72	3.1
15	Boven-Schelde IV	B19	107	11.1	0.18	0.19	0.57	NA
15	Boven-Schelde IV	B20	107	14.0	0.18	0.22	0.56	NA
16	Zeeschelde II	B1	112	16.7	0.20	0.23	0.83	3.0
16	Zeeschelde II	B2	90	13.5	0.18	0.20	0.64	2.9
16	Zeeschelde II	B3	120	21.2	0.21	0.10	0.62	2.5
18	Getijdedijle-Getijdezenne	B1	115	19.3	0.17	0.19	0.64	2.6
18	Getijdedijle-Getijdezenne	B2	135	31.3	0.17	0.20	0.73	2.3
18	Getijdedijle-Getijdezenne	B3	93	11.0	0.17	0.21	1.2	NA
18	Getijdedijle-Getijdezenne	B4	98	13.9	0.14	0.21	0.50	NA
21	Dommel	B1	143	42.4	0.19	0.21	0.73	2.7
21	Dommel	B2	165	64.9	0.20	0.22	0.93	1.9
21	Dommel	B3	154	47.2	0.20	0.21	0.84	2.3
21	Dommel	B4	147	43.5	0.20	0.17	0.86	1.7
21	Dommel	B5	135	42.2	0.21	0.21	0.96	1.6
21	Dommel	B6	145	43.1	0.21	0.21	0.95	2.1
21	Dommel	B7	151	50.1	0.20	0.22	0.98	1.7
21	Dommel	B8	157	52.5	0.19	0.18	0.82	1.9
21	Dommel	B9	157	54.4	0.20	0.22	0.92	1.7
21	Dommel	B10	147	31.5	0.20	0.29	0.75	1.9
21	Dommel	B11	160	63.7	0.21	0.23	0.96	2.4
21	Dommel	B12	160	63.1	0.19	0.22	0.85	2.1
21	Dommel	B13	165	61.4	0.20	0.22	0.81	1.8
21	Dommel	B14	171	74.8	0.20	0.21	0.83	2.0

Table B.4 (continued)

21	Dommel	B15	157	60.2	0.20	0.23	0.94	2.7
22	Demer I	B1	178	77.7	0.22	0.25	1.3	3.5
22	Demer I	B2	112	17.5	0.22	0.22	0.7	3.5
22	Demer I	B3	86	9.0	0.21	0.24	1.4	NA
22	Demer I	B4	99	10.2	NA	0.22	NA	NA
23	Polder van Lier	26	142	35.1	0.19	NA	NA	NA
23	Polder van Lier	27	118	17.3	0.16	NA	NA	NA
23	Polder van Lier	28	242	204.6	0.19	NA	NA	NA
23	Polder van Lier	29	126	20.4	0.19	NA	NA	NA
23	Polder van Lier	30	83	5.0	0.18	NA	NA	NA
23	Polder van Lier	31	85	6.5	0.20	NA	NA	NA
23	Polder van Lier	32	121	19.1	0.19	NA	NA	NA
23	Polder van Lier	33	86	6.2	0.19	NA	NA	NA
23	Polder van Lier	34	123	20.8	0.20	NA	NA	NA
23	Polder van Lier	35	86	5.9	0.19	NA	NA	NA
23	Polder van Lier	36	84	5.5	0.19	NA	NA	NA
23	Polder van Lier	37	128	21.1	0.19	NA	NA	NA
23	Polder van Lier	38	191	85.4	0.18	NA	NA	NA
23	Polder van Lier	39	130	26.5	0.18	NA	NA	NA
23	Polder van Lier	40	126	22.8	0.20	NA	NA	NA
23	Polder van Lier	41	73	3.5	0.22	NA	NA	NA
23	Polder van Lier	42	78	4.4	0.23	NA	NA	NA
23	Polder van Lier	43	79	5.0	0.21	NA	NA	NA
23	Polder van Lier	44	82	4.6	0.24	NA	NA	NA
23	Polder van Lier	45	87	6.3	0.20	NA	NA	NA
23	Polder van Lier	46	90	7.0	0.19	NA	NA	NA
23	Polder van Lier	47	89	6.5	0.19	NA	NA	NA
23	Polder van Lier	48	92	7.4	0.20	NA	NA	NA
23	Polder van Lier	49	82	5.6	0.20	NA	NA	NA
23	Polder van Lier	50	118	17.2	0.20	NA	NA	NA
24	Laakdal	1	188	82.1	0.17	NA	NA	NA
24	Laakdal	2	165	59.4	0.17	NA	NA	NA
24	Laakdal	3	158	49.2	0.19	NA	NA	NA
24	Laakdal	4	135	29.1	0.20	NA	NA	NA
24	Laakdal	5	145	32.8	0.17	NA	NA	NA
24	Laakdal	6	147	31.6	0.17	NA	NA	NA
24	Laakdal	7	128	23.2	0.17	NA	NA	NA
24	Laakdal	8	131	27.3	0.18	NA	NA	NA
24	Laakdal	9	119	19.9	0.20	NA	NA	NA
24	Laakdal	10	115	16.2	0.18	NA	NA	NA
24	Laakdal	11	112	13.8	0.18	NA	NA	NA
24	Laakdal	12	110	13.4	0.19	NA	NA	NA
24	Laakdal	13	111	13.9	0.19	NA	NA	NA
24	Laakdal	14	105	10.6	0.17	NA	NA	NA
24	Laakdal	15	101	11.7	0.18	NA	NA	NA
24	Laakdal	16	89	7.3	0.19	NA	NA	NA
24	Laakdal	17	84	6.8	0.19	NA	NA	NA
24	Laakdal	18	87	5.9	0.18	NA	NA	NA
24	Laakdal	19	82	5.0	0.18	NA	NA	NA
24	Laakdal	20	85	6.0	NA	NA	NA	NA
24	Laakdal	21	78	4.9	0.17	NA	NA	NA
24	Laakdal	22	76	4.0	0.17	NA	NA	NA
24	Laakdal	23	74	4.6	0.18	NA	NA	NA
24	Laakdal	24	78	4.6	0.18	NA	NA	NA
24	Laakdal	25	76	4.8	0.17	NA	NA	NA

Table B.5: Individual length and weight, dry/wet weight ratios and lipid contents (%) for eel.

No.	Water body	code	Total length (mm)	Weight (g)	Dry/wet weight ratio		Total lipid content (%)	
					Muscle tissue	*Liver tissue*	*Muscle tissue*	*Liver tissue*
1	Boven-Schelde I	P1	410	144	0.29	0.29	4.9	6.3
1	Boven-Schelde I	P2	318	61	0.29	0.32	7.8	4.8
1	Boven-Schelde I	P3	634	539	0.36	0.30	8.7	2.0
3	Demer VII	P1	489	228	0.31	0.22	16	2.9
3	Dender I	P1	720	708	0.29	0.22	6.6	2.4
3	Demer VII	P2	630	415	0.25	0.26	5.3	4.2
3	Dender I	P2	502	217	0.45	0.24	16	2.9
3	Demer VII	P3	650	763	0.31	0.23	7.5	1.8
3	Dender I	P3	562	348	0.44	0.25	18	2.3
4	Maas I+II+III	P1	402	102	0.21	0.23	1.6	2.5
4	Maas I+II+III	P2	365	85	0.21	0.24	1.6	3.1
4	Maas I+II+III	P3	534	234	0.23	0.22	4.0	2.9
4	Maas I+II+III	P4	452	163	0.23	NA	3.6	2.3
5	IJzer III	P1	494	235	0.25	0.28	5.1	3.1
5	IJzer III	P2	425	192	0.25	0.28	4.8	2.6
5	IJzer III	P3	385	134	0.25	0.26	3.8	2.4
6	Leie I	P1	705	573	0.46	0.23	18	2.6
6	Leie I	P2	673	630	0.44	0.29	25	5.6
6	Leie I	P3	840	978	NA	NA	25	7.9
8	Kanaal Gent-Oostende III	P1	700	790	0.30	0.20	7.1	3.1
8	Kanaal Gent-Oostende III	P2	447	183	0.35	0.20	10	2.7
8	Kanaal Gent-Oostende III	P3	485	192	0.36	0.22	14	3.2
9	Kleine Nete I	P1	468	209	0.35	0.23	7.5	2.5
9	Kleine Nete I	P2	496	245	0.30	0.22	13	2.5
9	Kleine Nete I	P3	527	344	0.35	0.20	7.7	2.5
10	Zeeschelde IV	P1	306	57	0.39	0.25	15	2.8
10	Zeeschelde IV	P2	314	70	0.27	0.21	7.4	3.1
10	Zeeschelde IV	P3	341	87	0.28	0.22	6.5	2.6
10	Zeeschelde IV	P4	281	46	0.22	0.21	5.6	2.9
10	Zeeschelde IV	P5	625	444	0.30	0.23	18	2.7
10	Zeeschelde IV	P6	411	125	0.30	0.23	10	3.6
10	Zeeschelde IV	P7	645	633	0.41	0.23	10	4.2
10	Zeeschelde IV	P8	456	165	0.28	0.22	6.2	2.5
10	Zeeschelde IV	P9	383	108	0.33	0.25	17	4.1
10	Zeeschelde IV	P10	479	257	0.46	0.23	23	2.9
10	Zeeschelde IV	P11	425	161	0.37	0.22	19	3.1
11	Dijle I	P1	453	196	0.27	0.21	6.9	2.5
11	Dijle I	P2	450	208	0.26	0.22	14	3.2
11	Dijle I	P3	485	243	0.26	0.21	16	2.1
12	IJzer I	P1	622	350	0.22	0.25	1.9	3.4
13	Blankenbergse vaart	P1	471	165	0.23	0.24	1.7	2.4
13	Blankenbergse vaart	P2	542	275	0.44	0.25	21	3.1
13	Blankenbergse vaart	P3	449	198	0.32	0.26	12	2.9
14	Leopoldkanaal I	P1	482	215	0.27	0.26	9.8	2.9
14	Leopoldkanaal I	P2	465	175	0.24	0.26	2.9	3.1
14	Leopoldkanaal I	P3	662	517	0.23	0.28	2.4	2.9
15	Boven-Schelde IV	P1	495	239	0.30	0.20	7.6	2.7
15	Boven-Schelde IV	P2	462	177	0.35	0.19	18	2.1
15	Boven-Schelde IV	P3	481	227	0.36	0.21	17	2.2
16	Zeeschelde II	P1	396	114	0.17	0.18	3.1	2.2
16	Zeeschelde II	P2	362	78	0.30	0.19	2.5	2.3
16	Zeeschelde II	P3	431	137	0.25	0.20	5.9	2.3
16	Zeeschelde II	P4	414	117	0.22	0.20	4.8	2.4
17	Zeeschelde III+Rupel	P1	411	98	0.23	0.21	3.6	2.2

Table B.5 (continued)

17	*Zeeschelde III+Rupel*	*P2*	*444*	*154*	*0.30*	*0.21*	*15*	2.5
17	Zeeschelde III+Rupel	P3	429	100	0.21	0.20	1.9	2.2
18	Getijdedijle-Getijdezenne	P1	395	107	0.20	0.19	2.3	2.3
18	Getijdedijle-Getijdezenne	P2	425	163	0.40	0.18	22	2.8
18	Getijdedijle-Getijdezenne	P3	432	151	0.33	0.25	7.1	3.2
19	Herk + Kleine Herk	P1	585	295	0.30	0.26	7.4	4.0
19	Herk + Kleine Herk	P2	610	440	0.34	0.20	14	2.7
20	Melsterbeek I+II	P1	595	430	0.27	0.21	11	2.7
20	Melsterbeek I+II	P2	447	156	0.30	0.21	10	2.7
21	Dommel	P1	732	822	0.50	0.30	NA	10
21	Dommel	P2	820	1082	0.58	0.34	28	NA
22	Demer I	P1	352	83	0.31	0.21	6.9	2.1
23	Polder van Lier	26	815	1171	0.35	NA	NA	NA
23	Polder van Lier	27	650	613	0.34	NA	NA	NA
23	Polder van Lier	28	680	668	0.37	NA	NA	NA
23	Polder van Lier	29	684	586	0.31	NA	NA	NA
23	Polder van Lier	30	750	850	0.32	NA	NA	NA
24	Laakdal	31	789	1021	0.27	NA	NA	NA
24	Laakdal	32	768	987	0.33	NA	NA	NA
24	Laakdal	33	827	1324	0.40	NA	NA	NA
24	Laakdal	34	818	1393	0.37	NA	NA	NA
25	Camerlinckxgeleed	1	135	5.6	0.28	NA	NA	NA
25	Camerlinckxgeleed	2	375	107	0.29	NA	NA	NA
25	Camerlinckxgeleed	3	300	53	0.28	NA	NA	NA
25	Camerlinckxgeleed	4	331	60	0.23	NA	NA	NA
25	Camerlinckxgeleed	5	292	55	0.26	NA	NA	NA
26	Bergenmeersen	6	405	NA	0.29	NA	NA	NA
26	Bergenmeersen	7	547	332	0.32	NA	NA	NA
26	Bergenmeersen	8	472	191	0.39	NA	NA	NA
26	Bergenmeersen	9	500	211	0.24	NA	NA	NA
26	Bergenmeersen	10	327	52	0.22	NA	NA	NA
26	Bergenmeersen	11	391	102	0.30	NA	NA	NA
26	Bergenmeersen	12	418	115	0.30	NA	NA	NA
26	Bergenmeersen	13	394	102	0.32	NA	NA	NA
26	Bergenmeersen	14	403	99	0.28	NA	NA	NA
26	Bergenmeersen	15	423	103	0.25	NA	NA	NA
26	Bergenmeersen	16	464	176	0.36	NA	NA	NA
26	Bergenmeersen	17	502	232	0.27	NA	NA	NA
26	Bergenmeersen	18	435	123	0.31	NA	NA	NA
26	Bergenmeersen	19	442	132	0.36	NA	NA	NA
26	Bergenmeersen	20	502	192	0.31	NA	NA	NA
26	Bergenmeersen	21	505	245	0.43	NA	NA	NA
26	Bergenmeersen	22	472	178	0.32	NA	NA	NA
26	Bergenmeersen	23	579	319	0.35	NA	NA	NA
26	Bergenmeersen	24	597	411	0.39	NA	NA	NA
26	Bergenmeersen	25	504	277	0.37	NA	NA	NA

Table B.6: Total length and weight (range; median), and accumulated mercury concentrations (range; median).

No.	Sampling Site	Water body	Total length (mm)		Weight (g)		Hg muscle (µg g^{-1} dw)		Hg liver (µg g^{-1} dw)	
			Eel	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*
1	Pecq	Boven-Schelde I	318-634 (410)	73-207 (95)	60.7-539 (145)	6.7-120 (89.3)	0.14-0.38 (0.18)	<LOQ-0.53 (0.12)	0.11-0.20 (0.12)	0.03-0.26 (0.05)
2	Geraardsbergen	Dender I	489-720 (630)	75-213 (146)	228-708 (415)	4.5-124 (34.5)	0.53-0.97 (0.58)	0.01-1.73 (0.66)	0.14-0.83 (0.61)	0.01-0.77 (0.10)
3	Werchter	Demer VII	502-650 (562)	98-190 (169)	217-763 (348)	8.9-79.3 (54.2)	0.98-1.15 (1.06)	0.23-0.81 (0.35)	1.01-1.26 (1.07)	0.08-0.24 (0.13)
4	Kinrooi	Maas I+II+III	365-534 (427)	95-228 (114)	85-234 (133)	9.2-160 (13.6)	0.76-1.31 (1.09)	0.02-0.89 (0.41)	0.60-1.32 (0.68)	0.03-0.30 (0.18)
5	Nieuwpoort	IJzer III	385-494 (425)	82-220 (101)	134-235 (192)	8.3-150 (11.3)	0.28-0.79 (0.64)	0.06-1.12 (0.30)	0.08-0.97 (0.84)	0.03-0.34 (0.11)
6	Wevelgem	Leie I	673-840 (705)	86-222 (105)	573-978 (630)	8.9-145 (15.2)	0.13-0.23 (0.18)	<LOQ-0.44 (0.12)	0.41-0.48 (0.45)	0.03-0.14 (0.07)
7	Zelzate	Kanaal Gent-Terneuzen	NA	103-214 (124)	NA	14.6-128 (22.4)	NA	0.26-0.78 (0.43)	NA	0.13-0.30 (0.20)
8	Oostende	Kanaal Gent-Oostende III	447-700 (485)	90-194 (108)	183-790 (192)	8.0-88.6 (15.1)	0.52-1.21 (0.53)	0.22-0.78 (0.45)	0.51-0.67 (0.60)	0.07-0.27 (0.16)
9	Retie	Kleine Nete I	468-527 (496)	140-176 (159)	209-344 (245)	33.0-90.5 (51.1)	0.37-0.54 (0.44)	0.07-0.27 (0.21)	0.40-0.72 (0.42)	0.02-0.24 (0.06)
10	Antwerpen	Zeeschelde IV	281-645 (411)	NA	45.8-633 (125)	NA	0.24-0.71 (0.33)	NA	0.37-0.62 (0.49)	NA
11	Sint-Joris-Weert	Dijle I	450-485 (453)	NA	196-243 (208)	NA	0.88-1.30 (1.22)	NA	0.77-1.24 (1.22)	NA
12	Poperinge	IJzer I	622	75-103 (91)	351	4.9-11.8 (7.2)	1.06	0.03-0.38 (0.14)	0.87	0.03-0.18 (0.09)
13	Blankenberge	Blankenbergse vaart	449-542 (471)	87-239 (114)	165-275 (198)	6.7-201 (17.6)	0.15-0.73 (0.26)	0.44-1.05 (0.58)	0.31-0.74 (0.63)	0.28-0.67 (0.37)
14	Oostburg	Leopoldkanaal I	465-662 (482)	70-92 (79)	175-517 (215)	3.3-8.1 (5.0)	0.31-0.65 (0.61)	0.13-0.39 (0.22)	0.57-1.04 (0.68)	0.06-0.29 (0.09)
15	Gent	Boven-Schelde IV	462-495 (481)	84-203 (123)	177-239 (227)	10.7-106 (20.3)	0.26-0.50 (0.48)	0.23-0.68 (0.30)	0.45-1.38 (0.63)	0.08-0.54 (0.19)
16	Dendermonde	Zeeschelde II	362-431 (405)	90-120 (93)	78.3-137 (115)	13.5-21.2 (16.7)	0.37-0.47 (0.44)	0.16-0.20 (0.19)	0.39-0.70 (0.53)	0.10-0.26 (0.22)
17	Hemiksem	Zeeschelde III + Rupel	411-444 (429)	NA	97.6-154 (100)	NA	0.24-0.47 (0.28)	NA	0.33-0.57 (0.55)	NA
18	Mechelen	Getijdedijle-Getijdezenne	395-432 (425)	93-135 (107)	107-163 (151)	11.0-31.3 (16.6)	0.07-0.29 (0.13)	0.22-0.34 (0.29)	0.18-0.26 (0.24)	0.14-0.35 (0.23)

Table B.6 (continued)

19	Herk-de-Stad	Herk + Kleine Herk	585-610 (498)	NA	295-440 (368)	NA	0.29-0.41 (0.35)	NA	0.32-0.91 (0.61)	NA
20	Herk-de-Stad	Melsterbeek I+II	447-595 (521)	NA	156-430 (293)	NA	0.51-0.58 (0.55)	NA	0.74-1.18 (0.96)	NA
21	Neerpelt	Dommel	732-820 (776)	135-171 (157)	822-1082 (952)	31.5-74.8 (52.5)	0.12-0.20 (0.16)	0.16-0.31 (0.20)	0.14-0.39 (0.27)	0.06-0.17 (0.10)
22	Bilzen	Demer I	352	86-178 (106)	82.7	9.0-77.7 (13.9)	0.17	0.09-0.30 (0.10)	0.50	0.07-0.11 (0.09)
23	Lier	Polder van Lier	650-815 (684)	73-242 (90)	586-1171 (668)	3.5-205 (7.0)	0.42-0.81 (0.68)	0.22-1.06 (0.36)	NA	NA
24	Westerlo	Laakdal	768-827 (804)	74-188 (110)	988-1393 (1172)	4.0-82.1 (13.4)	0.65-1.03 (0.85)	0.51-1.92 (1.09)	NA	NA
25	Camerlinckxgeleed	Camerlinckxgeleed	135-375 (300)	NA	5.6-107 (55.5)	NA	0.13-0.53 (0.39)	NA	NA	NA
26	Bergenmeersen	Boven-Schelde	327-597 (468)	NA	51.9-411 (178)	NA	0.18-0.41 (0.21)	NA	NA	NA

NA: no data available.

Table B.7: Accumulated mercury concentrations (range; median) after correction for lipid content in muscle and liver tissue of eel and perch.

No.	Sampling Site	Water body	Hg muscle (µg g^{-1} dw)		Hg liver (µg g^{-1} dw)	
			Eel	***Perch***	***Eel***	***Perch***
1	Pecq	Boven-Schelde I	0.15-0.40 (0.19)	<LOQ-0.49 (0.12)	0.12-0.21 (0.12)	0.21-0.27 (0.24)
2	Geraardsbergen	Dender I	0.57-1.17 (0.69)	0.08-1.27 (0.68)	0.14-0.85 (0.62)	0.10-0.79 (0.17)
3	Werchter	Demer VII	1.12-1.24 (1.17)	0.23-0.81 (0.36)	1.04-1.28 (1.12)	0.13-0.25 (0.22)
4	Kinrooi	Maas I+II+III	0.77-1.34 (1.13)	0.01-0.90 (0.42)	0.62-1.36 (0.70)	0.20-0.30 (0.26)
5	Nieuwpoort	IJzer III	0.29-0.84 (0.67)	0.07-1.13 (0.28)	0.08-1.00 (0.86)	0.22-0.33 (0.26)
6	Wevelgem	Leie I	0.16-0.30 (0.23)	<LOQ-0.45 (0.14)	0.43-0.51 (0.47)	0.10-0.12 (0.10)
7	Zelzate	Kanaal Gent-Terneuzen	NA	0.27-0.87 (0.43)	NA	0.20-0.31 (0.26)
8	Oostende	Kanaal Gent- Oostende III	0.57-1.35 (0.61)	0.23-0.79 (0.46)	0.53-0.69 (0.61)	0.15-0.28 (0.21)
9	Retie	Kleine Nete I	0.40-0.62 (0.48)	0.07-0.27 (0.21)	0.41-0.74 (0.43)	0.02-0.25 (0.07)
10	Antwerpen	Zeeschelde IV	0.28-0.87 (0.37)	NA	0.38-0.64 (0.50)	NA
11	Sint-Joris-Weert	Dijle I	1.02-1.31 (1.17)	NA	0.18-0.27 (0.25)	NA
12	Poperinge	IJzer I	1.08	0.04-0.30 (0.14)	0.90	NA
13	Blankenberge	Blankenbergse vaart	0.18-0.74 (0.33)	0.45-1.04 (0.59)	0.32-0.75 (0.65)	0.28-0.39 (0.37)
14	Oostburg	Leopoldkanaal I	0.35-0.67 (0.63)	0. 13-0.36 (0.22)	0.59-1.07 (0.70)	NA
15	Gent	Boven-Schelde IV	0.31-0.60 (0.52)	0.23-0.68 (0.30)	0.46-1.42 (0.64)	0.08-0.56 (0.18)
16	Dendermonde	Zeeschelde II	0.38-0.50 (0.46)	0.17-0.20 (0.19)	0.40-0.72 (0.54)	0.11-0.27 (0.23)
17	Hemiksem	Zeeschelde III + Rupel	0.28-0.48 (0.29)	NA	0.34-0.58 (0.56)	NA
18	Mechelen	Getijdedijle-Getijdezenne	0.10-0.30 (0.14)	0.23-0.34 (0.29)	0.18-0.27 (0.25)	0.15-0.31 (0.23)
19	Herk-de-Stad	Herk + Kleine Herk	0.31-0.47 (0.39)	NA	0.33-0.93 (0.63)	NA
20	Herk-de-Stad	Melsterbeek I+II	0.58-0.65 (0.61)	NA	0.76-1.21 (0.99)	NA
21	Neerpelt	Dommel	0.17	0.16-0.31 (0.21)	0.44	0.06-0.18 (0.11)
22	Bilzen	Demer I	0.18	0.09-0.30 (0.10)	0.52	0.10-0.12 (0.11)
23	Lier	Polder van Lier	NA	NA	NA	NA
24	Westerlo	Laakdal	NA	NA	NA	NA
25	Camerlinckxgeleed	Camerlinckxgeleed	NA	NA	NA	NA
26	Bergenmeersen	Boven-Schelde	NA	NA	NA	NA

NA: no data available.

Table B.8: Determination of human health risk through consumption of contaminated fish. Range and median of mercury concentrations in muscle tissue of perch and eel are given. Maximum amount (g) of contaminated fish muscle a 70 kg person can consume per day without posing health risks (MADC) were calculated for the median percentile of the observed mercury concentrations in fish muscle tissue in each sampling location, based on MRL (ATSDR, 2018), RfD (UNEP, 2008) and PTWI (FAO/WHO, 2010). HQ was determined by dividing the estimated daily intake (EDI) for perch (2.7 g day^{-1}) and eel (18 g day^{-1}) with the MADC.

		***Perca fluviatilis* (European perch)**				***Anguilla anguilla* (European eel)**			
			MADC (g/day/70 kg adult) and HQ				***MADC (g/day/70 kg adult) and HQ***		
No.	**Water body**	**Hg in muscle tissue (µg g^{-1} ww)**	**MRL**	**RfD**	**PTWI**	**Hg in muscle tissue (µg g^{-1} ww)**	**MRL**	**RfD**	**PTWI**
1	Boven-Schelde I	<LOQ-0.12 (0.03)	782 (<0.01)	261 (0.01)	599 (<0.01)	0.05-0.11 (0.05)	410 (0.04)	137 (0.13)	315 (0.06)
2	Dender I	0.001-0.31 (0.12)	169 (0.02)	56 (0.05)	129 (0.02)	0.15-0.42 (0.26)	80 (0.23)	27 (0.69)	61 (0.30)
3	Demer VII	0.05-0.17 (0.09)	225 (0.01)	75 (0.04)	173 (0.02)	0.26-0.36 (0.31)	69 (0.27)	23 (0.80)	53 (0.35)
4	Maas I+II+III	0.004-0.34 (0.08)	251 (0.01)	84 (0.03)	192 (0.01)	0.16-0.27 (0.25)	83 (0.22)	28 (0.66)	64 (0.29)
5	IJzer III	0.02-0.22 (0.06)	327 (0.01)	109 (0.02)	251 (0.01)	0.07-0.20 (0.16)	133 (0.14)	44 (0.41)	102 (0.18)
6	Leie I	<LOQ-0.09 (0.02)	921 (<0.01)	307 (0.01)	706 (<0.01)	0.06-0.10 (0.08)	262 (0.07)	87 (0.21)	201 (0.09)
7	Kanaal Gent-Terneuzen	0.06-0.16 (0.09)	234 (0.01)	78 (0.03)	179 (0.02)	NA	NA	NA	NA
8	Kanaal Gent- Oostende III	0.05-0.16 (0.10)	217 (0.01)	72 (0.04)	166 (0.02)	0.16-0.43 (0.19)	112 (0.16)	37 (0.49)	86 (0.21)
9	Kleine Nete I	0.01-0.05 (0.04)	512 (0.01)	171 (0.02)	393 (0.01)	0.13-0.16 (0.16)	135 (0.14)	45 (0.41)	103 (0.18)
10	Zeeschelde IV	NA	NA	NA	NA	0.07-0.22 (0.11)	186 (0.10)	62 (0.30)	142 (0.13)
11	Dijle I	NA	NA	NA	NA	0.23-0.34 (0.33)	64 (0.29)	21 (0.86)	49 (0.37)
12	IJzer I	0.005-0.08 (0.03)	734 (<0.01)	245 (0.01)	562 (<0.01)	0.23	90 (0.20)	30 (0.61)	69 (0.26)
13	Blankenbergse vaart	0.09-0.22 (0.12)	175 (0.02)	58 (0.05)	134 (0.02)	0.05-0.17 (0.11)	185 (0.10)	62 (0.30)	142 (0.13)
14	Leopoldkanaal I	0.03-0.08 (0.05)	448 (0.01)	149 (0.02)	343 (0.01)	0.09-0.15 (0.15)	143 (0.13)	48 (0.38)	109 (0.17)
15	Boven-Schelde IV	0.04-0.13 (0.06)	357 (0.01)	119 (0.02)	273 (0.01)	0.09-0.17 (0.14)	148 (0.12)	49 (0.37)	113 (0.16)
16	Zeeschelde II	0.03-0.04 (0.04)	592 (<0.01)	197 (0.01)	454 (0.01)	0.08-0.11 (0.10)	207 (0.09)	69 (0.27)	159 (0.12)
17	Zeeschelde III + Rupel	NA	NA	NA	NA	0.06-0.10 (0.07)	291 (0.06)	97 (0.19)	223 (0.08)
18	Getijdedijle-Getijdezenne	0.04-0.06 (0.04)	473 (0.01)	158 (0.02)	363 (0.01)	0.03-0.06 (0.04)	476 (0.04)	159 (0.12)	365 (0.05)
19	Herk + Kleine Herk	NA	NA	NA	NA	0.09-0.14 (0.11)	185 (0.10)	62 (0.30)	142 (0.13)
20	Melsterbeek I+II	NA	NA	NA	NA	0.14-0.18 (0.16)	133 (0.14)	44 (0.41)	102 (0.18)
21	Dommel	0.04-0.06 (0.04)	531 (0.01)	177 (0.02)	407 (0.01)	0.07-0.10 (0.09)	246 (0.07)	82 (0.22)	189 (0.10)
22	Demer I	0.02-0.06 (0.02)	930 (<0.01)	310 (0.01)	713 (<0.01)	0.05	405 (0.05)	135 (0.14)	310 (0.06)
23	Polder van Lier	0.04-0.20 (0.07)	300 (0.01)	100 (0.03)	230 (0.01)	0.16-0.29 (0.22)	95 (0.19)	32 (0.58)	73 (0.25)
24	Laakdal	0.09-0.35 (0.20)	106 (0.03)	35 (0.08)	81 (0.03)	0.26-0.30 (0.29)	73 (0.25)	24 (0.75)	56 (0.33)
25	Camerlinckxgeleed	NA	NA	NA	NA	0.04-0.15 (0.09)	240 (0.08)	80 (0.23)	184 (0.10)
26	Boven-Schelde	NA	NA	NA	NA	0.04-0.16 (0.07)	314 (0.06)	105 (0.17)	241 (0.08)

HQ: hazard quotient. Risk group: fishermen that take home eel on a regular base. Calculations were performed on the median value per location.

Table B.9: Determination of human health risk through consumption of contaminated eel for 'worst case consumption scenario'. Range and median of mercury concentrations in muscle tissue of eel are given. Maximum amount (g) of contaminated fish muscle a 70 kg person can consume per day without posing health risks (MADC) were calculated for the median percentile of the observed mercury concentrations in fish muscle tissue in each sampling location, based on MRL (ATSDR, 2018), RfD (UNEP, 2008) and PTWI (FAO/WHO, 2010). HQ was determined by dividing the estimated daily intake (EDI) 'worst scenario' (Bilau et al., 2007) for eel (71.14 g day^{-1}) with the MADC.

		***Anguilla anguilla* (European eel)**			
			MADC (g/day/70 kg adult) and HQ		
No.	**Water body**	**Hg in muscle tissue (µg g^{-1} ww)**	**MRL**	**RfD**	**PTWI**
1	Boven-Schelde I	0.05-0.11 (0.05)	410 (0.17)	137 (0.52)	315 (0.23)
2	Dender I	0.15-0.42 (0.26)	80 (0.89)	27 (**2.63**)	61 (**1.17**)
3	Demer VII	0.26-0.36 (0.31)	69 (**1.03**)	23 (**3.09**)	53 (**1.34**)
4	Maas I+II+III	0.16-0.27 (0.25)	83 (0.86)	28 (**2.54**)	64 (**1.11**)
5	IJzer III	0.07-0.20 (0.16)	133 (0.53)	44 (**1.62**)	102 (0.70)
6	Leie I	0.06-0.10 (0.08)	262 (0.27)	87 (0.82)	201 (0.35)
7	Kanaal Gent-Terneuzen	NA	NA	NA	NA
8	Kanaal Gent- Oostende III	0.16-0.43 (0.19)	112 (0.64)	37 (**1.92**)	86 (0.83)
9	Kleine Nete I	0.13-0.16 (0.16)	135 (0.53)	45 (**1.58**)	103 (0.69)
10	Zeeschelde IV	0.07-0.22 (0.11)	186 (0.38)	62 (**1.15**)	142 (0.50)
11	Dijle I	0.23-0.34 (0.33)	64 (**1.11**)	21 (**3.39**)	49 (**1.45**)
12	IJzer I	0.23	90 (0.79)	30 (**2.37**)	69 (**1.03**)
13	Blankenbergse vaart	0.05-0.17 (0.11)	185 (0.38)	62 (**1.15**)	142 (0.50)
14	Leopoldkanaal I	0.09-0.15 (0.15)	143 (0.50)	48 (**1.48**)	109 (0.65)
15	Boven-Schelde IV	0.09-0.17 (0.14)	148 (0.48)	49 (**1.45**)	113 (0.63)
16	Zeeschelde II	0.08-0.11 (0.10)	207 (0.34)	69 (**1.03**)	159 (0.45)
17	Zeeschelde III + Rupel	0.06-0.10 (0.07)	291 (0.24)	97 (0.73)	223 (0.32)
18	Getijdedijle-Getijdezenne	0.03-0.06 (0.04)	476 (0.15)	159 (0.45)	365 (0.19)
19	Herk + Kleine Herk	0.09-0.14 (0.11)	185 (0.38)	62 (**1.15**)	142 (0.50)
20	Melsterbeek I+II	0.14-0.18 (0.16)	133 (0.53)	44 (**1.62**)	102 (0.70)
21	Dommel	0.07-0.10 (0.09)	246 (0.29)	82 (0.87)	189 (0.38)
22	Demer I	0.05	405 (0.18)	135 (0.53)	310 (0.23)
23	Polder van Lier	0.16-0.29 (0.22)	95 (0.75)	32 (**2.22**)	73 (0.97)
24	Laakdal	0.26-0.30 (0.29)	73 (0.97)	24 (**2.96**)	56 (**1.27**)
25	Camerlinckxgeleed	0.04-0.15 (0.09)	240 (0.30)	80 (0.89)	184 (0.39)
26	Boven-Schelde	0.04-0.16 (0.07)	314 (0.23)	105 (0.68)	241 (0.30)

HQ: hazard quotient. Risk group: fishermen that take home eel on a regular base. Calculations were performed on the median value per location.

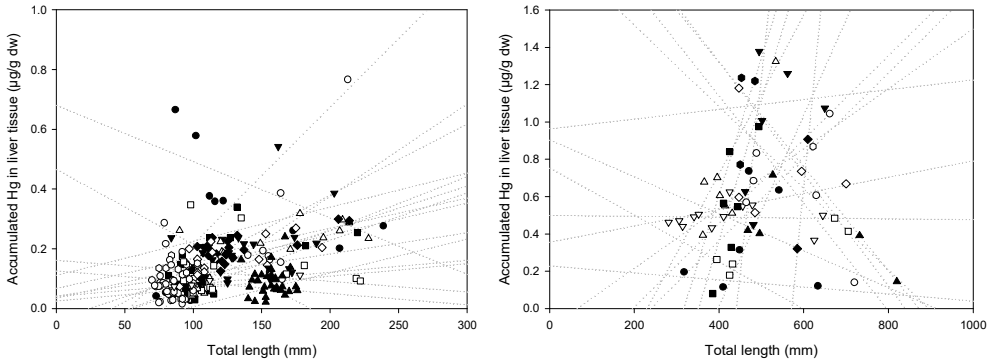

Figure B.1: Regression between total length of the individual and the accumulated mercury concentration in liver tissue for perch (LEFT; F=72.44, p<0.001) and for eel (RIGHT; F=0.30, p=0.59). Every symbol refers to a different location. The dotted lines give regression lines for each location, not necessarily significant.

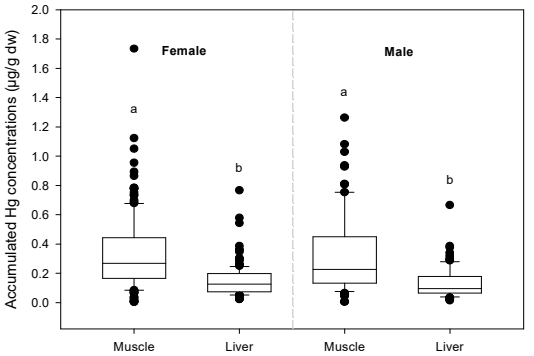

Figure B.2: Boxplots accumulated mercury concentrations in perch, depending on sex. Different letters stand for a significant difference (p <0.001).

Appendix C: Chapter 4

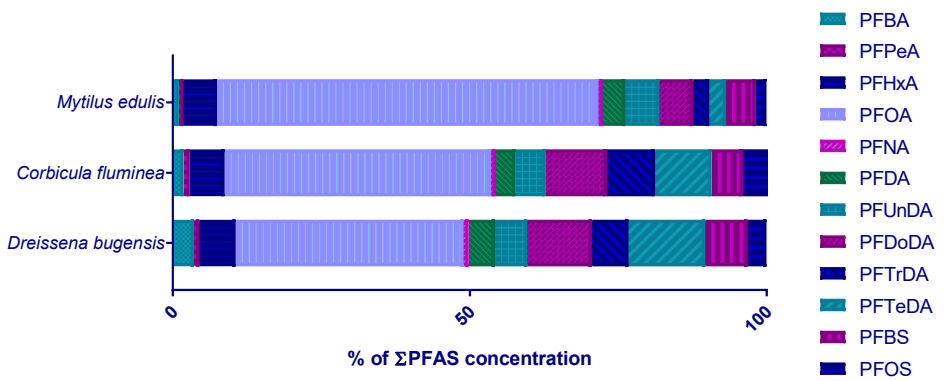

*Figure C.1: PFAS profiles in quagga mussel (*Dreissena bugensis*; N = 143), Asiatic clam (*Corbicula fluminea*; N = 30) and blue mussel (*Mytilus edulis*; N = 5). PFHpA, PFHxS and PFDS were excluded as their concentrations were <LOQ in all the samples.*

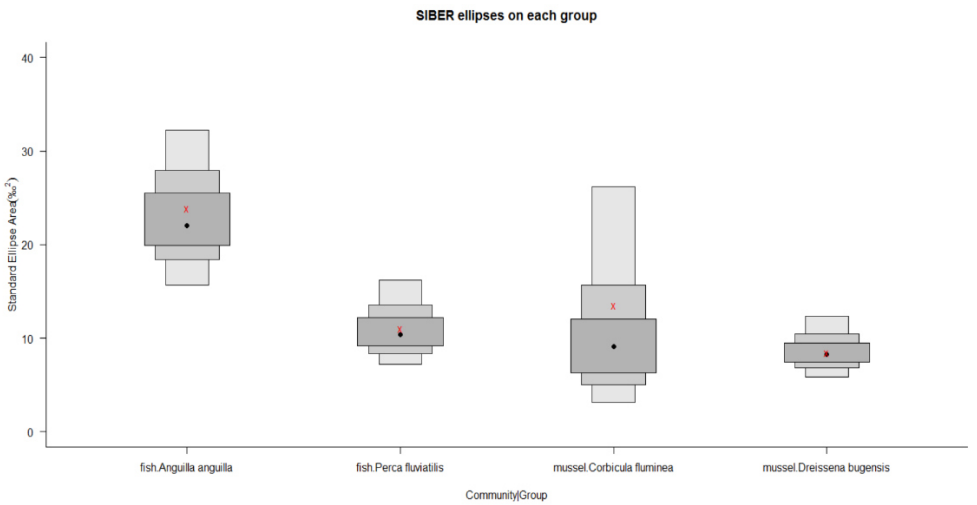

Figure C.2: Standard Ellipse Areas (SEA, ‰^{2}) of the Bayesian ellipses for each species; perch (Perca fluviatilis, N = 24), eel (Anguilla anguilla, N = 31), quagga mussel (Dreissena bugensis, N = 30) and Asian clam (Corbicula fluminea, N = 5). The red crosses represent the corrected median SEA (SEAc).

Table C.1: Condition Index, stable isotope data ($\delta^{13}C$ and $\delta^{15}N$), and PFAS concentrations (mean and range (between brackets); ng g^{-1} ww) of the mussels (quagga mussel (Dreissena bugensis; Db), Asian clam (Corbicula fluminea; Cf) and blue mussel (Mytilus edulis; Me) at each location. PFHpA, PFHxS and PFDS were not detected in any of the samples and therefore excluded from the Table. No range is given when none of the samples had concentrations above the LOQ. Location numbers are used according to Figure 4.1 and Table 4.1.

No.	sp.	Condition Index	PFBA	PFPeA	PFHxA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS	$\delta^{13}C$	$\delta^{15}N$
1	*Db*	0.71 (0.55 – 1.15)	<LOQ	<LOQ	<LOQ	0.389 (<LOQ – 0.629)	<LOQ	0.402 (<LOQ – 0.688)	0.644 (<LOQ – 1.170)	1.581 (<LOQ – 2.218)	<LOQ (<LOQ – 0.769)	1.607 (<LOQ – 2.736)	<LOQ	0.602 (<LOQ – 1.531)	-27.5	5.9
2	*Db*	0.78 (0.69 – 0.90)	0.508 (<LOQ – 0.839)	<LOQ	<LOQ	1.353 (<LOQ – 2.382)	<LOQ	0.548 (<LOQ – 1.764)	0.352 (<LOQ – 0.912)	0.739 (<LOQ – 1.371)	<LOQ	<LOQ (<LOQ – 1.279)	<LOQ	<LOQ (<LOQ – 0.327)	-30.2	5.5
3	*Db*	0.43 (0.33 – 0.51)	<LOQ (<LOQ – 0.577)	<LOQ	<LOQ	0.324 (<LOQ – 0.506)	<LOQ	0.378 (<LOQ – 0.635)	0.637 (<LOQ – 1.119)	1.084 (<LOQ – 1.861)	<LOQ	1.083 (<LOQ – 2.282)	<LOQ	<LOQ	-30.7	10.3
4	*Db*	0.72 (0.39 – 1.17)	2.017 (1.004 – 5.283)	<LOQ	<LOQ	0.853 (<LOQ – 2.124)	<LOQ	<LOQ	0.455 (<LOQ – 1.893)	<LOQ	0.912 (<LOQ – 1.993)	<LOQ	<LOQ	<LOQ	-26.5	7.7
5	*Cf*	0.17 (0.13 – 0.20)	<LOQ	0.206 (<LOQ – 0.658)	<LOQ	0.601 (<LOQ – 1.052)	<LOQ (<LOQ – 0.178)	0.732 (<LOQ – 1.343)	1.103 (0.294 – 1.482)	1.264(0.742 – 1.992)	<LOQ (<LOQ – 0.687)	1.114 (<LOQ – 2.409)	<LOQ	0.617 (<LOQ – 0.921)	-27.6	7.1
6	*Db*	0.66 (0.40 – 1.15)	1.899 (1.161 – 3.370)	<LOQ	<LOQ	0.716 (<LOQ – 1.421)	<LOQ	0.281 (<LOQ – 0.713)	1.222 (<LOQ – 5.136)	0.998 (<LOQ – 3.637)	<LOQ (<LOQ – 1.548)	6.232 (<LOQ – 15.3)	<LOQ	<LOQ	-27.6	7.6
7	*Cf*	0.19 (0.17 – 0.21)	<LOQ	<LOQ	<LOQ	0.439 (0.378 – 0.538)	<LOQ	0.631 (0.460 – 0.822)	0.906 (0.687 – 1.185)	3.514 (2.765 – 4.292)	4.532 (3.816 – 5.186)	2.944 (2.541 – 3.368)	<LOQ	2.290 (0.525 – 5.608)	-28.2	10.3
9	*Db*	0.42 (0.32 – 0.55)	<LOQ	<LOQ	<LOQ	0.399 (<LOQ – 0.654)	<LOQ	0.551 (0.188 – 0.822)	0.763 (<LOQ – 1.612)	<LOQ (<LOQ – 0.813)	<LOQ	<LOQ (<LOQ – 1.126)	<LOQ	0.400 (<LOQ – 0.836)	-27.7	6.5
11	*Db*	0.48 (0.31 – 0.65)	<LOQ	0.213 (<LOQ – 0.421)	<LOQ	0.339 (<LOQ – 0.598)	<LOQ	0.544 (0.330 – 0.847)	0.547 (0.386 – 0.693)	1.907 (1.549 – 2.200)	<LOQ (<LOQ – 0.817)	2.465 (1.872 – 3.332)	<LOQ	<LOQ (<LOQ – 0.480)	-27.9	7.7

Table C.1 (continued).

12	*Db*	0.46 (0.38 – 0.50)	<LOQ	<LOQ (<LOQ – 0.496)	<LOQ	0.439 (<LOQ – 0.632)	<LOQ	0.577 (0.266 – 1.183)	0.918 (0.470 – 1.194)	0.997 (<LOQ – 1.409)	<LOQ	1.457 (<LOQ – 2.368)	<LOQ	0.317 (<LOQ – 0.651)	-26.7	6.3
15	*Db*	0.78 (0.52 – 1.00)	0.799 (<LOQ – 1.667)	<LOQ	<LOQ	0.515 (<LOQ – 1.262)	0.198 (<LOQ – 0.639)	0.643 (<LOQ – 2.839)	0.488 (<LOQ – 2.055)	<LOQ	<LOQ	<LOQ	<LOQ	0.743 (<LOQ – 2.155)	-28.3	8.2
16	*Db*	0.55 (0.40 – 0.75)	<LOQ	<LOQ	<LOQ	0.395 (<LOQ – 0.593)	<LOQ	0.370 (<LOQ – 0.629)	0.547 (<LOQ – 0.904)	1.700 (<LOQ – 2.342)	1.027 (<LOQ – 1.357)	1.905 (<LOQ – 2.665)	<LOQ	1.529 (0.517 – 4.664)	-27.6	7.2
23	*Cf*	0.29 (0.20 – 0.39)	<LOQ	<LOQ	<LOQ	15.4 (7.213 – 22.5)	<LOQ	0.475 (<LOQ – 0.768)	<LOQ (<LOQ – 0.543)	1.416 (1.041 – 2.195)	<LOQ (<LOQ – 0.887)	1.022 (<LOQ – 1.780)	<LOQ	<LOQ (<LOQ – 0.294)	-31.5	8.0
25	*Cf*	0.22 (0.20 – 0.26)	<LOQ	<LOQ	<LOQ	15.9 (6.279 – 28.9)	<LOQ	0.466 (<LOQ – 0.870)	0.275 (<LOQ – 0.619)	0.960 (<LOQ – 2.007)	<LOQ	<LOQ (<LOQ – 1.221)	<LOQ	<LOQ (<LOQ – 0.388)	-29.4	5.4
26	*Db*	0.65 (0.56 – 0.80)	<LOQ	<LOQ	<LOQ	9.197 (0.582 – 18.5)	<LOQ	0.633 (0.236 – 1.050)	0.781 (0.512 – 1.253)	3.125 (1.829 – 4.143)	2.579 (2.154 – 3.144)	2.463 (1.903 – 3.168)	<LOQ	<LOQ (<LOQ – 0.270)	-27.8	6.7
27	*Db*	0.73 (0.45 – 1.17)	<LOQ	<LOQ	<LOQ	12.1 (0.673 – 26.8)	<LOQ	0.451 (0.217 – 0.706)	1.155 (0.735 – 1.791)	3.670 (2.490 – 4.770)	2.943 (1.755 – 3.752)	2.846 (1.612 – 4.034)	<LOQ	<LOQ (<LOQ – 0.375)	-27.8	6.4
28	*Db*	0.66 (0.34 – 0.95)	0.285 (<LOQ – 0.905)	<LOQ	<LOQ	22.7 (14.2 – 34.8)	<LOQ	0.475 (<LOQ – 0.885)	0.296 (<LOQ – 0.953)	1.730 (0.949 – 2.442)	2.504 (2.016 – 3.066)	7.086 (5.071 – 9.309)	<LOQ	<LOQ (<LOQ – 0.503)	-28.3	6.5
29	*Db*	0.60 (0.42 – 0.93)	<LOQ	<LOQ	<LOQ	21.6 (12.1 – 33.9)	<LOQ	0.443 (<LOQ – 1.410)	0.539 (<LOQ – 1.021)	2.027 (<LOQ – 6.119)	<LOQ (<LOQ – 1.843)	0.869 (<LOQ – 2.369)	<LOQ	<LOQ	-28.2	5.7

Table C.1 (continued).

30	*Db*	0.43 (0.34 – 0.55)	<LOQ	<LOQ	<LOQ	32.0 (18.5 – 49.2)	0.198 (<LOQ – 0.517)	0.649 (<LOQ – 1.209)	0.961 (<LOQ – 4.419)	3.031 (<LOQ – 4.964)	1.651 (<LOQ – 3.255)	14.5 (6.403 – 23.2)	<LOQ	1.179 (0.350 – 1.655)	-28.1	6.2
31	*Db*	0.39 (0.24 – 0.53)	<LOQ	<LOQ	<LOQ	21.3 (10.8 – 41.2)	<LOQ	0.436 (<LOQ – 0.655)	0.382 (<LOQ – 0.887)	0.819 (<LOQ – 2.000)	<LOQ (<LOQ – 0.969)	3.010 (1.571 – 4.881)	<LOQ	<LOQ (<LOQ – 0.348)	-27.1	6.4
	Cf	0.23 (0.11 – 0.36)	<LOQ	<LOQ	<LOQ	7.832 (2.480 – 16.0)	<LOQ	<LOQ (<LOQ – 0.303)	0.314 (<LOQ – 0.562)	<LOQ (<LOQ – 1.375)	<LOQ	0.626 (<LOQ – 1.449)	<LOQ	<LOQ	NA	NA
32	*Db*	0.70 (0.52 – 0.82)	<LOQ (<LOQ – 0.542)	<LOQ	<LOQ	27.3 (15.6 – 57.4)	<LOQ	1.274 (0.795 – 1.866)	0.402 (<LOQ – 1.625)	<LOQ (<LOQ – 0.922)	<LOQ	<LOQ	<LOQ	0.328 (<LOQ – 0.927)	-28.5	6.3
33	*Db*	0.70 (0.41 – 1.13)	<LOQ	<LOQ	<LOQ	20.6 (14.3 – 30.0)	<LOQ	0.602 (0.353 – 1.519)	0.319 (<LOQ – 1.209)	<LOQ (<LOQ – 1.555)	<LOQ	<LOQ	<LOQ	<LOQ (<LOQ – 0.484)	-27.4	6.9
34	*Me*	0.87 (0.43 – 1.44)	<LOQ	<LOQ	<LOQ	12.0 (0.715 – 16.2)	<LOQ	0.441 (0.313 – 0.537)	0.718 (0.469 – 0.818)	<LOQ (<LOQ – 1.045)	<LOQ	<LOQ (<LOQ – 0.709)	<LOQ	0.282 (<LOQ – 0.738)	-20.5	10.4
35	*Db*	0.73 (0.65 – 0.78)	<LOQ	<LOQ	<LOQ	0.493 (0.353 – 0.763)	<LOQ	0.814 (0.663 – 0.958)	1.896 (1.616 – 2.239)	6.944 (5.985 – 8.708)	3.088 (2.329 – 3.748)	3.773 (2.974 – 4.142)	<LOQ	0.819 (0.523 – 1.169)	-33.8	12.2
36	*Db*	0.72 (0.55 – 1.05)	0.718 (<LOQ – 2.932)	<LOQ	<LOQ	0.488 (<LOQ – 0.910)	<LOQ	0.754 (<LOQ – 1.193)	1.319 (<LOQ – 2.431)	5.716 (<LOQ – 11.4)	5.856 (4.124 – 7.933)	6.989 (<LOQ – 10.6)	12.8 (<LOQ – 51.6)	0.338 (<LOQ – 0.502)	-29.9	6.7
37	*Db*	0.84 (0.61 – 1.25)	0.980 (<LOQ – 2.711)	<LOQ	<LOQ	0.337 (<LOQ – 0.627)	<LOQ (<LOQ – 0.286)	0.354 (<LOQ – 0.746)	0.628 (<LOQ – 1.418)	1.498 (<LOQ – 2.315)	<LOQ (<LOQ – 0.776)	1.248 (<LOQ – 2.434)	31.3 (<LOQ – 147.5)	0.668 (0.382 – 1.210)	-34.4	12.5

Table C.1 (continued).

38	*Db*	0.75 (0.39 – 1.04)	<LOQ (<LOQ – 0.358)	<LOQ	<LOQ	24.4 (16.9 – 32.2)	<LOQ (<LOQ – 0.254)	0.799 (0.526 – 0.958)	1.075 (0.854 – 1.524)	2.700 (0.832 – 3.694)	1.643 (1.486 – 1.754)	4.558 (3.695 – 5.410)	<LOQ	0.491 (0.331 – 0.603)	-36.2	12.8
39	*Db*	0.72 (0.42 – 1.00)	<LOQ	<LOQ	<LOQ	19.5 (14.4 – 24.8)	<LOQ (<LOQ – 0.193)	0.717 (0.379 – 1.059)	0.790 (0.336 – 1.517)	1.287 (<LOQ – 3.168)	<LOQ (<LOQ – 0.902)	0.857 (<LOQ – 1.918)	<LOQ	<LOQ (<LOQ – 0.325)	-27.8	6.7
40	*Db*	0.88 (0.48 – 1.31)	<LOQ	<LOQ	<LOQ	11.7 (10.1 – 16.0)	<LOQ	0.446 (0.403 – 0.500)	0.579 (0.313 – 0.743)	1.454 (1.152 – 1.643)	<LOQ	1.657 (1.181 – 2.454)	<LOQ	<LOQ	-29.2	8.8
41	*Db*	0.50 (0.31 – 0.73)	<LOQ (<LOQ – 0.267)	<LOQ	<LOQ (<LOQ – 1.447)	0.777 (0.561 – 1.112)	<LOQ (<LOQ – 0.221)	1.687 (1.166 – 3.047)	1.400 (0.609 – 2.244)	1.243 (<LOQ – 2.578)	<LOQ	<LOQ	<LOQ	<LOQ (<LOQ – 0.268)	-28.0	6.8
42	*Db*	0.46 (0.29 – 0.87)	<LOQ (<LOQ – 0.345)	<LOQ	<LOQ (<LOQ – 1.601)	30.8 (16.8 – 58.6)	<LOQ	1.200 (0.278 – 2.405)	1.219 (0.315 – 2.586)	2.311 (<LOQ – 5.158)	0.879 (<LOQ – 1.970)	2.849 (0.769 – 5.576)	<LOQ	<LOQ (<LOQ – 0.329)	-28.6	5.9
43	*Db*	0.69 (0.46 – 0.82)	<LOQ	<LOQ	<LOQ	20.7 (16.7 – 24.7)	<LOQ (<LOQ – 0.204)	0.698 (0.266 – 1.275)	1.143 (0.823 – 1.366)	2.282 (1.523 – 3.965)	1.026 (<LOQ – 1.549)	4.049 (2.823 – 5.399)	<LOQ	<LOQ (<LOQ – 0.522)	-29.9	7.4
R1	*Db*	0.73 (0.51 – 0.94)	<LOQ	<LOQ	<LOQ	12.7 (7.896 – 16.8)	LOQ	0.321 (0.238 – 0.567)	0.511 (<LOQ – 1.333)	<LOQ	<LOQ (<LOQ – 0.802)	0.741 (<LOQ – 1.698)	<LOQ	<LOQ	-26.4	5.3
	Cf	0.23 (0.20 – 0.27)	<LOQ	<LOQ	<LOQ	12.2 (6.426 – 21.3)	<LOQ	0.191 (<LOQ – 0.317)	0.519 (<LOQ – 0.836)	<LOQ (<LOQ – 0.802)	<LOQ	<LOQ	<LOQ	<LOQ	-27.4	6.1
R2	*Dp*[a]	NA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.402 (<LOQ-0.774)	<LOQ	<LOQ	NA	NA

[a] Concentrations were measured outside the scope of the present study (Dp: *Dreissena polymorpha*). NA: not determined.

Table C.2: MRM transitions (precursor and product ions), internal standards (ISTDs), cone voltages (V) and collision energy (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
PFTrDA	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 C.3: Mean and range trophic levels (TLs) of the organisms. TLs were calculated as $\delta^{15}N$ divided by 3.4, the mean trophic fractionation of $\delta^{15}N$ (Borgå et al., 2011).

	Perch (*Perca fluviatilis*) (N = 24)	**Eel (*Anguilla anguilla*) (N = 31)**	**quagga mussel (*Dreissena bugensis*) (N = 28)**	**Asian clam (*Corbicula fluminea*) (N = 5)**	**Blue mussel (*Mytilus edulis*) (N = 1)**
Average (± st. error)	4.97 ± 0.15	4.86 ± 0.14	2.20 ± 0.11	2.17 ± 0.25	3.05
Range (min – max)	3.72 – 6.62	3.38 – 6.86	1.55 – 3.76	1.58 – 3.04	-

Table C.4: PFAS concentrations (ng g^{-1} ww) and stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) in fish muscle tissue (eel (Anguilla anguilla) and perch (Perca fluviatilis)) at each location. PFPeA, PFHpA, PFBS and PFHxS were not detected in any of the samples and therefore excluded from the Table. No range is given when none of the samples had concentrations above the LOQ. Location numbers are abbreviated according to Figure 4.1 and Table 4.1. NA = not assessed.

No.	Species	PFBA	PFHxA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFOS	PFDS	$\delta^{13}C$	$\delta^{15}N$
1	*Perch*	<LOQ	<LOQ	0.270	<LOQ	0.858	1.389	0.981	0.168	<LOQ	8.081	<LOQ	NA	NA
	Eel	<LOQ	<LOQ	0.461	<LOQ	0.853	2.777	2.509	1.780	0.369	9.675	<LOQ	NA	NA
2	*Perch*	<LOQ	<LOQ	0.248	<LOQ	1.047	<LOQ	0.735	<LOQ	<LOQ	5.349	<LOQ	NA	NA
	Eel	<LOQ	<LOQ	0.228	<LOQ	0.929	<LOQ	1.419	0.382	<LOQ	6.984	<LOQ	NA	NA
3	*Perch*	<LOQ	<LOQ	0.249	<LOQ	1.643	<LOQ	1.700	0.194	<LOQ	37.298	<LOQ	NA	NA
	Eel	<LOQ	<LOQ	0.298	<LOQ	1.136	<LOQ	3.544	0.855	0.535	7.939	<LOQ	NA	NA
4	*Perch*	<LOQ	<LOQ	0.245	<LOQ	3.372	<LOQ	0.991	0.284	<LOQ	12.411	<LOQ	NA	NA
	Eel	<LOQ	<LOQ	0.331	<LOQ	3.369	<LOQ	6.702	1.077	<LOQ	6.851	<LOQ	NA	NA
5	*Perch*	<LOQ	<LOQ	0.219	<LOQ	4.624	<LOQ	1.507	<LOQ	<LOQ	28.790	<LOQ	NA	NA
	Eel	<LOQ	0.757	0.354	<LOQ	3.154	<LOQ	1.661	<LOQ	<LOQ	14.524	<LOQ	NA	NA
6	*Perch*	<LOQ	<LOQ	0.238	<LOQ	1.645	<LOQ	1.824	0.835	0.257	17.091	<LOQ	NA	NA
	Eel	<LOQ	0.381	0.263	<LOQ	<LOQ	<LOQ	2.135	1.740	1.653	5.908	0.133	NA	NA
7	*Perch*	0.159	<LOQ	0.419	<LOQ	4.035	3.257	5.429	2.616	0.235	41.943	0.079	NA	NA
8	*Perch*	<LOQ	<LOQ	0.787	<LOQ	4.332	<LOQ	2.443	1.633	0.125	25.593	<LOQ	NA	NA
	Eel	<LOQ	0.488	0.390	<LOQ	2.961	4.632	3.056	2.947	<LOQ	22.912	<LOQ	NA	NA
9	*Perch*	<LOQ	<LOQ	0.476	<LOQ	0.908	<LOQ	0.839	<LOQ	<LOQ	8.247	<LOQ	NA	NA
	Eel	<LOQ	<LOQ	0.380	<LOQ	1.262	<LOQ	1.601	0.202	<LOQ	11.954	<LOQ	NA	NA
10	*Eel*	<LOQ	0.652	0.587	<LOQ	1.432	1.088	3.266	1.069	0.171	28.799	<LOQ	NA	NA
11	*Eel*	<LOQ	<LOQ	0.353	<LOQ	<LOQ	<LOQ	1.739	0.251	0.107	3.314	<LOQ	NA	NA
12	*Perch*	<LOQ	<LOQ	0.151	<LOQ	2.253	NA	1.499	0.282	0.501	10.352	<LOQ	-32.8	19.2
	Eel	<LOQ	<LOQ	0.259	<LOQ	<LOQ	NA	0.464	<LOQ	0.129	3.571	<LOQ	-32.6	19.5

Table C.4 (continued)

13	*Perch*	<LOQ	<LOQ	0.197	0.730	3.533	NA	0.556	<LOQ	0.076	11.258	<LOQ	-31.7	17.0
	Eel	<LOQ	<LOQ	0.546	0.608	1.718	NA	0.472	<LOQ	0.066	10.233	<LOQ	-33.8	17.4
14	*Perch*	<LOQ	<LOQ	0.203	<LOQ	<LOQ	NA	0.159	<LOQ	<LOQ	3.535	<LOQ	-33.4	20.5
	Eel	<LOQ	<LOQ	0.351	<LOQ	<LOQ	NA	0.475	0.135	0.155	6.895	<LOQ	-31.7	19.0
15	*Perch*	<LOQ	<LOQ	0.147	<LOQ	1.727	NA	3.168	1.950	2.021	15.452	0.138	-30.3	15.1
	Eel	<LOQ	<LOQ	0.292	<LOQ	1.385	NA	5.390	3.281	3.188	17.126	0.204	-32.7	13.9
16	*Perch*	<LOQ	<LOQ	0.297	<LOQ	1.273	NA	1.254	0.412	0.434	25.940	<LOQ	-29.4	16.7
	Eel	<LOQ	<LOQ	0.727	<LOQ	0.907	NA	3.295	1.736	4.326	19.737	<LOQ	-29.5	20.3
17	*Eel*	<LOQ	0.406	0.371	<LOQ	1.077	NA	4.066	2.080	2.732	27.879	<LOQ	-27.6	20.4
18	*Perch*	<LOQ	<LOQ	0.164	<LOQ	<LOQ	NA	1.871	0.678	<LOQ	10.679	<LOQ	-28.2	16.5
	Eel	<LOQ	<LOQ	0.308	<LOQ	<LOQ	NA	0.691	0.209	0.568	7.300	0.073	-27.7	15.6
19	*Eel*	<LOQ	<LOQ	<LOQ	<LOQ	1.077	NA	3.371	0.796	1.665	8.255	<LOQ	-30.0	16.3
20	*Eel*	<LOQ	0.417	0.150	<LOQ	<LOQ	NA	1.314	0.376	0.912	64.604	<LOQ	-30.0	14.6
21	*Perch*	<LOQ	<LOQ	0.194	<LOQ	<LOQ	NA	1.115	2.069	0.254	4.129	<LOQ	-27.5	14.5
	Eel	<LOQ	<LOQ	0.272	<LOQ	1.266	NA	2.404	0.514	0.834	6.722	<LOQ	-30.6	15.1
22	*Perch*	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	NA	<LOQ	<LOQ	<LOQ	5.933	<LOQ	-26.8	13.9
	Eel	<LOQ	<LOQ	0.260	<LOQ	<LOQ	NA	<LOQ	<LOQ	1.827	11.372	<LOQ	-28.5	11.5
23	*Perch*	<LOQ	<LOQ	0.207	<LOQ	1.208	<LOQ	0.385	<LOQ	0.129	2.672	<LOQ	-30.5	22.5
	Eel	<LOQ	<LOQ	0.482	<LOQ	<LOQ	0.542	0.239	<LOQ	<LOQ	2.373	<LOQ	-32.4	23.3
24	*Eel*	<LOQ	0.876	0.351	<LOQ	1.199	1.051	2.040	0.271	0.704	52.317	<LOQ	-29.8	16.2
25	*Perch*	<LOQ	<LOQ	<LOQ	<LOQ	3.138	1.293	0.621	<LOQ	<LOQ	9.727	<LOQ	-32.6	18.7
	Eel	<LOQ	<LOQ	0.300	<LOQ	0.868	<LOQ	<LOQ	<LOQ	<LOQ	5.349	<LOQ	-36.8	18.2
26	*Perch*	<LOQ	<LOQ	0.114	<LOQ	2.404	10.234	4.547	3.086	3.126	19.841	<LOQ	-29.8	16.0
	Eel	<LOQ	<LOQ	0.377	<LOQ	1.728	7.081	4.346	4.743	3.039	15.528	<LOQ	-31.2	14.2

Table C.4 (continued)

27	*Perch*	<LOQ	<LOQ	<LOQ	<LOQ	1.883	10.605	3.335	4.693	2.483	14.495	<LOQ	-29.8	18.0
	Eel	<LOQ	<LOQ	0.157	<LOQ	1.147	7.202	6.998	6.579	3.112	9.827	<LOQ	-33.4	18.6
28	*Eel*	<LOQ	<LOQ	0.214	<LOQ	1.574	2.820	5.133	3.066	4.530	5.601	<LOQ	-34.1	14.8
29	*Eel*	<LOQ	<LOQ	0.324	<LOQ	<LOQ	0.580	2.091	0.746	1.292	9.390	<LOQ	-29.7	12.1
30	*Perch*	<LOQ	<LOQ	<LOQ	<LOQ	2.172	1.920	4.981	1.730	4.259	45.100	<LOQ	-32.5	19.0
	Eel	<LOQ	<LOQ	0.223	<LOQ	1.789	3.989	8.289	2.653	8.278	35.149	<LOQ	-33.1	19.6
31	*Perch*	<LOQ	<LOQ	0.141	<LOQ	4.111	0.965	1.088	0.333	0.472	53.482	<LOQ	-29.3	15.0
	Eel	<LOQ	<LOQ	0.280	<LOQ	<LOQ	0.684	2.270	0.961	3.031	7.934	<LOQ	-28.1	14.2
32	*Perch*	<LOQ	<LOQ	<LOQ	<LOQ	1.204	0.984	0.746	0.239	0.303	9.516	<LOQ	-30.4	13.8
	Eel	<LOQ	<LOQ	0.207	<LOQ	<LOQ	1.108	1.373	1.181	1.483	5.600	<LOQ	-29.0	13.2
33	*Perch*	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.321	<LOQ	0.289	3.688	<LOQ	-27.9	14.7
	Eel	<LOQ	<LOQ	0.404	<LOQ	0.835	1.198	1.434	0.301	<LOQ	9.016	<LOQ	-28.9	16.0
34	*Eel*	<LOQ	<LOQ	0.119	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.080	1.521	<LOQ	-21.6	15.7
35	*Perch*	<LOQ	<LOQ	0.182	<LOQ	3.658	3.468	0.905	0.848	0.664	36.802	<LOQ	-30.6	20.4
	Eel	<LOQ	<LOQ	0.160	<LOQ	2.174	2.921	2.010	2.444	1.277	19.391	<LOQ	-30.0	19.7
36	*Perch*	<LOQ	<LOQ	0.169	<LOQ	3.368	6.903	4.105	3.871	3.325	14.429	<LOQ	-30.1	16.8
	Eel	<LOQ	0.631	0.281	<LOQ	1.345	5.282	5.958	6.468	5.845	12.576	<LOQ	-34.0	16.7
37	*Perch*	<LOQ	<LOQ	0.114	<LOQ	1.071	0.989	1.008	0.248	0.609	5.534	<LOQ	-31.1	19.2
	Eel	<LOQ	<LOQ	0.152	<LOQ	1.419	<LOQ	1.233	0.349	0.891	6.189	<LOQ	-33.7	18.0
38	*Perch*	<LOQ	<LOQ	<LOQ	<LOQ	1.194	0.918	1.701	0.361	1.505	7.822	<LOQ	-30.5	19.0
	Eel	<LOQ	0.411	0.431	<LOQ	1.736	1.460	3.281	1.243	3.140	8.120	<LOQ	-31.3	18.6
39	*Perch*	<LOQ	<LOQ	0.114	<LOQ	1.909	1.174	1.804	0.367	0.676	6.615	<LOQ	-27.8	17.0
40	*Eel*	<LOQ	<LOQ	0.179	<LOQ	<LOQ	0.800	1.473	0.483	1.853	1.836	<LOQ	-29.1	15.9
41	*Eel*	<LOQ	<LOQ	0.257	<LOQ	<LOQ	0.696	1.271	1.024	1.659	3.424	<LOQ	-32.0	14.9

Table C.4 (continued)

42	*Perch*	<LOQ	<LOQ	0.133	<LOQ	1.358	0.709	1.099	0.380	0.878	9.022	<LOQ	-28.3	14.5
43	*Perch*	<LOQ	<LOQ	0.214	<LOQ	1.718	0.583	1.145	0.408	1.025	5.606	<LOQ	-28.4	15.6
	Eel	<LOQ	<LOQ	0.157	<LOQ	1.005	0.902	1.940	1.148	1.832	3.135	<LOQ	-32.0	14.9
44	*Perch*	<LOQ	<LOQ	0.175	<LOQ	1.565	1.075	1.918	0.740	1.774	5.626	<LOQ	-26.5	12.7
	Eel	0.219	<LOQ	0.187	<LOQ	2.502	2.106	5.875	3.642	4.500	6.432	<LOQ	-31.2	13.7

Table C.5: Overlap in the corrected Standard Ellipse Area (SEAc; ‰²) of the ellipses of quagga mussel (Dreissena bugensis, N = 30), Asian clam (Corbicula fluminea, N = 5), perch (Perca fluviatilis, N = 43) and eel (Anguilla anguilla, N = 56).

	Dreissena bugensis	***Corbicula fluminea***	***Perca fluviatilis***	***Anguilla anguilla***
Dreissena bugensis	*	6.77	<0.001	<0.001
Corbicula fluminea		*	<0.001	<0.001
Perca fluvitilis			*	11.38

Table C.6: Slopes and trophic magnification factors (TMFs) for the target analytes that were significantly related with trophic level (TLs) of the organisms.

	PFOA	**PFDA**	**PFUnDA**	**PFTeDA**	**PFOS**
Slope	-0.381	0.101	0.097	-0.137	0.499
TMF	0.416	1.262	1.251	0.729	3.155

Table C.7. Summary of results of PFCs in the environmental study samples (fish, results in ng g^{-1} ww) of the interlaboratory study of the reference material (Van Leeuwen et al., 2011) and reference concentrations (average values) measured in the present study.

Fish	Assigned value	Average	Median	Min.	Max.	SD	%RSD	n°	Present Study
PFBA	NA	0.16	0.16	NA	NA	NA	NA	1	<LOQ
PFPeA	NA	0.24	0.24	NA	NA	NA	NA	1	<LOQ
PFHxA	NA	2.07	2.15	0.09	3.90	1.56	75	4	<LOQ
PFHpA	NA	1.28	1.00	0.80	2.05	0.67	52	3	<LOQ
PFOA	NA	3.80	0.39	0.09	33.0	10.3	270	10	0.41
PFNA	0.52	1.23	0.60	0.23	6.75	1.63	132	19	0.67
PFDA	2.62	4.04	2.69	0.66	27.0	5.11	127	24	2.62
PFUdA	1.43	1.73	1.40	0.38	4.70	1.12	65	19	1.60
PFDoA	0.27	1.05	0.30	0.20	5.30	1.57	149	13	0.32
PFTrDA	NA	0.73	0.39	0.10	2.16	0.83	114	5	<LOQ
PFTeDA	NA	0.08	0.08	NA	NA	NA	NA	1	<LOQ
PFHxDA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PFODA	NA	NA	NA	NA	NA	NA	NA	NA	NA
L-PFBS	NA	0.10	0.10	0.00	0.00	0.14	141	2	<LOQ
L-PFHxS	NA	0.89	0.11	0.00	5.10	1.87	210	7	<LOQ
L-PFOS	65.4	61.6	67.0	2.48	110	24.8	40	27	46.4
L-PFDS	NA	1.93	0.29	0.14	5.35	2.96	154	3	<LOQ
PFOSA	1.44	1.80	1.60	0.88	3.60	0.99	55	8	NA

n°: number of submitted datasets. NA: not assessed.

Appendix D: Chapter 5

D.1 Detailed methods, materials, reagents and QC/QA for analysis for HOCs, Hg and PFOS

All polypropylene tubes used for sample preparation and extraction were from Greiner (Bio-One, Vilvoorde, Belgium), unless stated otherwise.

Hexachlorobenzene (HCB) and Hexachlorobutadiene (HCBD)

These analyses were performed by the Flanders Environment Agency (see 2.4 Analysis of water and sediment samples). Freeze-dried samples (1 g; Virtis genesis 2.0, Virtis genesis company inc., USA) were spiked with ^{13}C-labeled internal standards (ISTDs; ^{13}C-HCB and ^{13}C-1,2,4,5 tetrachlorobenzene, LGC, UK) dissolved in hexane (>99%, Chem-Lab) and extracted with accelerated solvent extraction (ASE, Dionex, Thermo) with a cleaning step on 5 g activated aluminium oxide (Sigma-Aldrich) in 5 g acid silica (Davison 923, VWR) and 2 g florisil (Merck). The extraction was performed with hexane:dichloromethane (DCM; >99%, Chem-Lab) (2:1, *v/v*) in two extraction cycles. After evaporation under N_2-stream (Zymark Turbo VAP 500, Biotage, Sweden) of the extract to 1 mL, HCB and HCBD concentrations were measured using Gas Chromatography with a High Resolution Mass Spectrometer (GC-HRMS; Agilent 7890A GC, AutoSpec Premier HRMS, Waters, USA) using a HT 8 column (50 m x 0.22 mm, 0.25 µm, Waters, USA). The carrier gas existed of Helium (1mL minute^{-1}).

Quality assurance/quality control: For each analyte, the limit of detection (LOD) was calculated as three times the standard deviation of 6 repetitions of samples spiked with the assumed limit of quantification (LOQ). The LOQ was 2 times the LOD. Both LOQ and LOD were defined during method development and verified with every batch of twenty samples (2x10). The quality control was performed by regular analyses of procedural blanks, sample replicates, random injection of standards, spiked samples and solvent blanks. The calibration curve in hexane (>99%, Chem-Lab) ranged from 1-20 ng ml^{-1}. Procedural blanks were analysed simultaneously with every batch of twenty samples (2x10) to check for interferences. An external standard (^{13}C-PCB 178, LGC, UK) was added after extraction. Recoveries for individual compounds ranged between 70-130 %.

Polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDEs) and Hexabromocyclododecane (HBCD)

Freeze-dried samples (0.5 g of eel or 1 g of perch; Heto PowerDry LL3000, Thermo Scientific) were mixed with anhydrous Na_2SO_4 (VWR) and spiked with internal standards (CB143, BDE77, BDE128, and ^{13}C-HBCD alpha-, beta- and gamma-isomers, Accustandard, New Haven, CT, USA and Wellington Laboratories, Guelph, ON, Canada). Further, the samples were extracted twice using 6 mL of an hexane:acetone mixture (3:1, *v/v, Acros Organics and Merck*) by applying ultra-sonication (Bransonic 2800 Ultrasonic Cleaner, VWR) twice for 20 min, with vortexing for 2 min between each sonication period. After each sonication, the mixture was centrifuged at 3500 rpm (Eppendorf centrifuge 5804, VWR), and the supernatants were combined afterwards. The lipid content was determined gravimetrically (Sartorius Analytical Balance QUINTIX124-1S, accuracy 0.1 mg) on an aliquot of the extract (105 °C, 1 h; HERAEUS RTV200, F-no. 7603713). The rest of the extract was further evaporated to dryness under a N_2-stream (Thermo Reacti-Therm III TS-18824), reconstituted in 0.5 mL of hexane (>99%), purified by using a ~6 g acid silica (44%, Merck) cartridge, from which analytes were eluted with 20 mL hexane (>99%) and 15 mL DCM (>99%, Merck). The cleaned extract was evaporated to 1-2 mL using a rotary evaporator (Rotavapor R-300, BUCHI, Switzerland). Furthermore, the extract was evaporated to 0.5 mL and re-dissolved in 0.5 mL hexane and eluted from pre-packed silica cartridges (3 mL 500 mg^{-1}, Bond Elut), topped with 100 mg acid silica (44%). The first fraction (A) was eluted with 6 mL hexane and contained PCBs and PBDEs. This fraction was evaporated to dryness, redissolved in 100 µL iso-octane (Merck) and analysed by GC-ECNI/MS (Gas chromatography coupled to electron capture negative ion mass spectrometer; Agilent 6890 GC and 5973 MS) and by GC-EI/MS (Agilent GC 6890 coupled to an Agilent 5973 MS operated in electron ionization (EI) mode) using a DB-5ms column (30 m x 0.25 mm x 0.25 µm, J&W Scientific (Agilent Technologies)). The mobile phase of this analysis existed of water:methanol (1:1) containing 2mM ammonium acetate. The second fraction (B) was eluted with 10 mL DCM and contained HBCDs. This fraction was evaporated to dryness, re-dissolved in 100µL methanol (>99%, Merck) and analysed by LC-MS/MS (Agilent 1200 Infinity LC and Agilent 6410 MS/MS) using a Luna C18 column (150 mm x 2 mm, 3µm, Phenomenex). The mobile phase existed of methanol.

Quality assurance/quality control: Multi-level calibration curves in the linear response interval of the detector were created for the quantification to cover the whole range of concentrations measured in the samples, and a good correlation ($r^2 > 0.999$) was achieved. The identification of analytes was based on the relative retention times (RRTs) to the internal standards used for quantification, ion chromatograms and intensity ratios of the

monitored ions. The peaks were quantified as target compounds if: (1) the retention time matched that of the standard compound within ± 0.1 min and (2) the signal-to-noise ratio (S/N) was higher than 3:1. For each analyte, the limit of quantification (LOQ) was calculated as three times the standard deviation of the mean of the blank measurements. Procedural blanks were analysed simultaneously with every batch of ten samples to check for interferences. Procedural blanks were consistent (RSD < 20%), and therefore the mean value was calculated for each compound and subtracted from the values in the samples. The quality control was performed by regular analyses of procedural blanks, sample replicates, by random injection of standards, spiked samples and solvent blanks. Recoveries for individual PCB, PBDE and HBCD congeners ranged between 86 and 104% (RSD < 12%). A NIST standard reference material SRM 1945 (PCBs, OCPs, and PBDEs in whale blubber) was used to test the accuracy of the method. Measured values did not deviate more than 15% of the certified values. For a more detailed description of the analytical method of the quality assurance procedures, we refer to Malarvannan et al. (2014).

Mercury

Freeze-dried samples (0.1-0.5 g; Heto PowerDry LL3000, Thermo Scientific) were digested in a 1:3 mixture of HNO_3 (69%, Fisher Chemical) and HCl (30%, VWR) ("Aqua Regia") at room temperature. After 24h, H_2O_2 (30%, Fisher Chemical) was added to the samples for further digestion, which was conducted in a pressurized microwave digestion system, Discover SP-D (CEM Corporation, Matthews, NC 28106, USA). The analysis was performed using a High-Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS; Element XR, Thermo Scientific, Bremen, Germany). Settings for digestion, analysis and quality control/assurance were performed as described in Mataba et al., 2016.

Quality assurance/quality control: The reference material (0.05 g) used was freeze-dried mussel tissue (NIST-2976; National Institute of Standards and Technology, USA). Recoveries ranged from 70 to 136 %. Concentrations below 90% or above 110 % were corrected for recovery.

Perfluorooctane sulfonate (PFOS)

To 0.5 g of homogenized samples, 10 mL acetonitrile (≥99.9%, Fisher Chemical) was added. Samples were spiked with internal standards, sonicated (3x10 min, Branson 2510) and shaken overnight (135 rpm, GFL 3020, VWR International, Leuven, Belgium). The isotopically mass-labelled internal standards (ISTDs) contained $^{13}C_4$-PFBA, [1,2-$^{13}C_2$]PFHxA, [1,2,3,4-$^{13}C_4$]PFOA, [1,2,3,4,5-$^{13}C_5$]PFNA, [1,2-$^{13}C_2$]PFDA, [1,2-$^{13}C_2$]PFUnDA, [1,2-$^{13}C_2$]PFoDA, $^{18}O_2$-PFHxS and [1,2,3,4-[1,2-$^{13}C_4$]PFOS (Wellington

Laboratories, Guelph, Canada). After centrifugation (Centrifuge 5804 R, Eppendorf), the supernatant was evaporated to 0.5 mL (Eppendorf rotational-vacuum-concentrator; 30 °C, type 5301, Hamburg, Germany) and cleaned with graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Belgium) and glacial acetic acid (≥99.7%, Fisher Chemical). Furthermore, the tube was washed twice to obtain a total volume of 1 mL extract. The extract was evaporated to dryness, redissolved in 200µl 2% ammonium hydroxide (Acros Organics) and filtrated. The perfluoroalkyl compounds were analysed using an Ultra Performance Liquid Chromatograph connected to a tandem quadrupole mass spectrometer (UPLC-MS/MS; ACQUITY, TQD, Waters, Milford, MA, USA). An ACQUITY BEH C18 column (2.1 x50mm; 1.7 µm, Waters, USA) was used. The mobile phase existed of acetonitrile and water (both 0.1% formic acid).

Quality assurance/quality control: For a more detailed description of the method, UPLC settings and quality assurance, we refer to Groffen et al. (2019).

Dicofol

This analysis was performed by the private, accredited and officially recognised service laboratory of Primoris Belgium (http://www.primoris-lab.com/en, Technologiepark 2/3, B-9052 Zwijnaarde - Ghent) holding a BELAC accreditation to ISO/CEI 17025 for pesticide residues in foodstuffs. Samples were analysed using a standard QuEChERS (AOAC; Association of Official Analytical Chemists) extraction Method 2007.01 (Lehotav, 2007) and analysed using Gas Chromatography coupled to a tandem quadrupole mass spectrometer (GC-MS/MS).

Dioxins

Freeze-dried samples (5 g; Crios, Cryotec, France) were spiked with a $^{13}C_{12}$ labelled internal standard containing 2,3,7,8-TCDD (TCDD), 1,2,3,7,8-PeCDD (PeCDD), 1,2,3,4,7,8-HxCDD (HxCDD 1), 1,2,3,6,7,8-HxCDD (HxCDD 2), 1,2,3,7,8,9-HxCDD (HxCDD 3), 1,2,3,4,6,7,8-HpCDD (HpCDD), OCDD, 2,3,7,8-TCDF (TCDF), 1,2,3,7,8-PeCDF (PeCDF 1), 2,3,4,7,8-PeCDF (PeCDF 2), 1,2,3,4,7,8-HxCDF (HxCDF 1), 1,2,3,6,7,8-HxCDF (HxCDF 2), 1,2,3,7,8,9-HxCDF (HxCDF 3), 2,3,4,6,7,8-HxCDF (HxCDF 4), 1,2,3,4,6,7,8-HpCDF (HpCDF 1), 1,2,3,4,7,8,9-HpCDF (HpCDF 2), OCDF, 3,39,4,4'-TCB (PCB 77), 3,4,4',5-TCB (PCB 81), 3,3',4,4',5-PeCB (PCB 126) and 3,3',4,4',5,5'-HxCB (PCB 169) (Cambridge Isotope Labs, Andover, MS, USA) and extracted with pressurized liquid extraction (PLE), using a Dionex (Sunnyvale, CA, USA) ASE 200 extractor with hexane (Pestanal, Riedel-de Haen, Seelze, Germany) as a solvent. Hexachlorodisilane (HCDS) columns (FMS Waltham; MA, USA) were used for clean-up before automated multi-column clean-up in a Power-Prep system (FMS). The columns existed of silica columns (4 g acid, 2 g base and 1.5 g neutral), basic alumina (8 g) PX-21

(2 g) carbon columns. The extract (dissolved in 60 mL toluene, Pestanal, Riedel-de Haen, Seelze, Germany) was evaporated to approximately 150µL (Turbocap II Concentration Workstation, Zymark, Hopkinton, MA, USA). The analysis was performed using a GC-HRMS consisting of a MAT95XL HRMS (Finnigan, Bremen, Germany) and a Hewlett-Packard (Palo Alto, CA, USA) 6890 Series GC. A RTX-5SIL-MS (30m x 0.25 mm, 0.25 mm) capillary column (Restek, Evry, France) was used. The carrier gas was Helium (99.9%, Ari Products, Vilvoorde, Belgium).

Quality assurance/quality control: The analysis and QA/QC was performed as descripted in Focant et al. (2001), including more details on the extraction method.

Heptachlor and (trans-/cis-)heptachlorepoxide

These analyses were performed by the Flanders Environment Agency (see 2.4 Analysis of water and sediment samples). Samples (5 g) were spiked with internal standards (^{13}C-heptachlor, Campro Scientific, Germany) dissolved in methanol (>99%, Biosolve) and homogenized in 5 mL of water. A liquid extraction was performed using acetonitrile (>99%, Biosolve). A total of 15 mL acetonitrile (1% acetic acid) and 2mL hexane/DCM (2:1, *v/v*, >99%, Biosolve) was added to the homogenate. After vortexing and centrifugation (Rotofix 32A, Hettich Zentrigugen; 5 min, 4000 rpm), $MgSO_4$ and $CaCl_2$ (Agilent) were added to the supernatant. Further clean-up of the extract was performed by adding a primary-secondary amine sorbent (PSA), Florisil, C-18 sorbent and $MgSO_4$ (all from Agilent). After centrifugation and filtration, the extract was redissolved in iso-octane (>99%, VWR) and DCM before evaporation to a 1 mL iso-octane solution and analysis of the sample using GC-HRMS (idem HCB and HCBD; Waters, USA).

Quality assurance/quality control: For each analyte, the limit of detection (LOD) was calculated as three times the standard deviation of 6 repetitions of samples spiked with the assumed limit of quantification (LOQ). The LOQ was 2 times the LOD. Both LOQ and LOD were defined during method development and verified with every batch of twenty samples (2x10). The quality control was performed by regular analyses of procedural blanks, sample replicates, random injection of standards, spiked samples and solvent blanks. The calibration curves were prepared in hexane (>99%) and ranged from 10 to 200 ng ml^{-1}. Procedural blanks were analysed simultaneously with every batch of twenty samples to check for interferences. An external standard (^{13}C-PCB 28, LGC, UK) was added after extraction. Recoveries for individual compounds ranged between 70-130%.

Polycyclic aromatic hydrocarbons (PAHs)

These analyses were performed by the Flanders Environment Agency (see 2.4 Analysis of water and sediment samples). Freeze-dried mussel tissue (1 g; Virtis genesis 2.0) was spiked with deuterated standards (EPA, LGC, UK) dissolved in hexane (>99%) and extracted using an ASE extraction (Dionex, Thermo). First, the sample was cleaned on the ASE cell topped with 5 g aluminium oxide (Sigma-Aldrich) in 5 g silica (Davison 923, VWR) and 2 g florisil (Merck). The extraction was performed with hexane:dichloromethane (DCM; >99%, Chem-Lab) (2:1, *v/v*) in two extraction cycles. Furthermore, the extract was evaporated (TurboVap 500, Biotage, Sweden) and cleaned using Gel Permeation Chromatography (GPC; Agilent 1100 Variable Wavelength Detector). After another evaporation step, the extract is redissolved in toluene (>99%, VWR) before analysis using a Gas Chromatograph with a single quadrupole mass spectrometer (GC-MS, Agilent 6890N GC, Agilent 5975B Inert XL MS, Agilent Technologies, USA) using a Rxi-5Sil MS column (30m x 0.25 mm, 0.25 µm, Restek, PA, USA).

Quality assurance/quality control: For each analyte, the limit of detection (LOD) was calculated as three times the standard deviation of 6 repetitions of samples spiked with the assumed limit of quantification (LOQ). The LOQ was 2 times the LOD. Both LOQ and LOD were defined during method development and verified with every batch of twenty samples (2x10). The quality control was performed by regular analyses of procedural blanks, sample replicates, random injection of standards, spiked samples and solvent blanks. The calibration curve dissolved in hexane ranged from 1-20 ng ml^{-1}. Procedural blanks were analysed simultaneously with every batch of twenty samples to check for interferences. An external standard (perylene-d12, LGC, UK) was added after extraction. Recoveries of individual compounds ranged between 70-130 %.

Total lipid determination

Freeze-dried tissue (1.5 mg of eel or 5 mg of mussel or perch; Heto PowerDry LL3000, Thermo Scientific) were extracted using the method developed by Bligh and Dyer, 1959. Extraction was performed using a chloroform(≥99%, VWR)/methanol(≥99%, VWR)/MQ water(18.2 mΩ, TOC: 2.0 ppb, Merck Millipore) mixture (2:2:1; 1mL in total) and centrifugation (5 minutes at 13200 rpm; Eppendorf centrifuge 5415 R, VWR). The chloroform phase was then transferred to a glass tube before adding 500 µl of H_2SO_4 (95%, VWR). Samples were then heated at 200°C for 15 minutes (IP 60, LTE Scientific, TCPS, Rotselaar, Belgium). After cooldown, they were diluted using 1 mL of MQ. The extract was then analysed using a spectrophotometer (Ultra microplate reader ELX808IU Bio-Tek Instruments Inc., VT, USA) at 405nm.

Quality assurance/quality control: A calibration solution of 1 mg glycerol tripalmitate (98%, Alfa Aesar, Heysham, Enland) mL^{-1} chloroform was used to create a calibration curve ranging from 25-200 mg mL^{-1}. A good correlation (r^2>0.95) was achieved. A procedural blank was added to every batch of 24 samples (including a reference sample) to check for interferences. Each sample was extracted and analysed in duplicate. The reference material (5 mg, BCR-685 skim milk powder) showed recoveries between 80%-120%.

Table D.1: Sampling locations with waterbody and city. Per location the number of perch (Perca fluviatilis) and eel (Anguilla anguilla) were given as well as the range (and mean) of total length (mm) and weight (g).

No.	Waterbody	City	Sampling year	*Water body type*	*Perca fluviatilis*					*Anguilla anguilla*				
					N	Length (mm)	Weight (g)	Lipid (%)	Dry/wet weight ratio	N	Length (mm)	Weight (g)	Lipid (%)	Dry/wet weight ratio
1	BOVEN-SCHELDE I	Spiere-Helkijn	2015	River	20	86-207 (115)	6.7-121 (19)	0.97	0.22	3	318-634 (454)	61-538 (248)	7.1	0.37
2	DENDER I	Geraardsbergen	2015	River	22	75-213 (126)	4.5-124 (35)	0.75	0.23	3	489-720 (613)	228-708 (450)	13	0.34
3	DEMER VII	Werchter	2015	River	8	98-190 (163)	23-79 (54)	0.77	0.18	3	502-651 (532)	217-763 (443)	9.7	0.32
4	MAAS I+II+III	Kinrooi	2015	River	21	99-228 (142)	9.2-160 (50)	0.87	0.26	4	365-534 (438)	85-234 (146)	2.7	0.25
5	IJZER III	Nieuwpoort	2015	River	19	88-220 (114)	8.3-150 (27)	0.98	0.18	3	385-494 (435)	134-235 (187)	4.6	0.29
6	LEIE I	Wevelgem	2015	River	14	91-222 (137)	8.9-145 (42)	0.98	0.22	3	673-840 (739)	573-979 (727)	23	0.74
7	KANAAL GENT-TERNEUZEN + GENTSE HAVENDOKKEN	Zelzate	2015	Canal	21	111-214 (132)	15-128 (35)	0.98	0.21	0	NA	NA	NA	NA
8	KANAAL GENT-OOSTENDE III	Oostende	2015	Canal	20	90-194 (121)	8.0-89 (25)	0.91	0.21	3	447-700 (544)	183-790 (388)	10	0.45
9	KLEINE NETE I	Retie	2015	Stream	17	140-187 (157)	34-91 (53)	0.87	0.19	3	468-527 (497)	209-344 (266)	9.2	0.27
10	ZEESCHELDE IV	Antwerpen	2015	River (brackish)	0	NA	NA	NA	NA	11	281-645 (424)	46-633 (196)	13	0.38
11	DIJLE I	Oud-Heverlee	2015	River	0	NA	NA	NA	NA	3	450-485 (463)	196-243 (216)	12	0.26
12	IJZER I	Poperinge	2016	River	20	75-103 (91)	4.9-11.8 (7.8)	1.1	0.21	1	622	351	1.9	0.22
13	BLANKENBERGSE VAART + NOORDEDE	Blankenberge	2016	Canal	6	87-239 (133)	6.7-201 (46)	1.0	0.21	3	449-542 (487)	165-275 (213)	12	0.35
14	LEOPOLDKANAAL I	Oostburg	2016	Canal	20	70-92 (80)	3.3-8.1 (5.2)	1.0	0.21	3	465-662 (536)	175-517 (302)	5	0.27
15	BOVEN-SCHELDE IV	Gent	2016	River	18	105-203 (126)	11-106 (29)	0.72	0.20	3	462-495 (479)	177-239 (214)	14	0.34
16	ZEESCHELDE II	Kastel	2016	Estuary (fresh)	3	100-120 (111)	14-21 (17)	0.7	0.20	4	362-431 (400)	78-138 (112)	4.1	0.3
17	ZEESCHELDE III + RUPEL	Hemiksem	2016	River (brackish)	0	NA	NA	NA	NA	3	411-444 (428)	98-154 (117)	6.8	0.26
18	GETIJDEDIJLE-GETIJDEZENNE	Mechelen	2016	River	4	93-135 (110)	11-31 (19)	0.78	0.17	3	395-425 (417)	107-163 (141)	11	0.32
19	HERK + KLEINE HERK	Herk-de-Stad	2016	Stream	0	NA	NA	NA	NA	2	586-611 (599)	295-443 (369)	11	0.36

Table D.1 (continued)

20	MELSTERBEEK I+II	Herk-de-Stad	2016	Stream	0	NA	NA	NA	NA	2	447-594 (521)	156-432 (294)	10	0.3
21	DOMMEL	Neerpelt	2016	Stream	15	135-171 (155)	32-75 (53)	0.88	0.21	2	738-823 (781)	821-1080 (951)	32	0.48
22	DEMER I	Bilzen	2016	Stream	4	86-178 (119)	9.0-78 (29)	1.2	0.22	1	352	83	6.9	0.28
23	KANAAL DUINKERKE-NIEUWPOORT	Koksijde	2017	Canal	20	81-167 (120)	6.1-59 (26)	0.74	0.20	3	424-491 (460)	151-189 (168)	9.1	0.29
24	KANAAL IEPER-IJZER	Ieper	2017	Canal	0	NA	NA	NA	NA	3	422-592 (490)	136-384 (229)	6.2	0.25
25	LEOPOLDKANAAL II	Brugge	2017	Canal	6	137-205 (179)	35-121 (83)	0.79	0.21	3	435-510 (466)	171-294 (221)	25	0.39
26	LEIE III	Deinze	2017	River	10	106-190 (146)	17-95 (47)	0.66	0.20	4	360-583 (459)	112-179 (213)	19	0.38
27	AFLEIDINGSKANAAL van de LEIE/SCHIPDONK-KANAAL I	Nevele	2017	Canal	9	120-200 (146)	25-117 (49)	0.76	0.21	4	392-537 (468)	113-306 (220)	16	0.36
28	BOVEN-SCHELDE II+III	Oudenaarde	2017	River	0	NA	NA	NA	NA	3	465-507 (485)	214-238 (220)	24	0.43
29	BELLEBEEK	Liedekerke	2017	Stream	0	NA	NA	NA	NA	3	490-507 (495)	163-205 (189)	11	0.3
30	ZEEKANAAL BRUSSEL-SCHELDE	Willebroek	2017	Canal	20	97-132 (113)	12-29 (18)	0.60	0.20	3	442-522 (475)	161-295 (218)	7.7	0.28
31	ZENNE II	Zemst	2017	River	7	96-120 (106)	8.2-22 (14)	0.77	0.20	4	467-528 (511)	160-319 (241)	17	0.35
32	GROTE NETE III	Heist-op-den-Berg	2017	River	16	92-169 (130)	10-61 (37)	0.79	0.21	4	432-541 (466)	113-277 (159)	6	0.26
33	MARK (Maas)	Hoogstraten	2017	Stream	18	80-142 (110)	5.7-36 (15)	0.75	0.20	4	372-452 (407)	88-211 (145)	5.6	0.25
34	HAVENGEUL IJZER	Nieuwpoort	2018	Harbour channel	0	NA	NA	NA	NA	2	522-557 (540)	299-332 (315)	16	0.35
35	AFLEIDINGSKANAAL van de LEIE II + KANAAL van EEKLO	Brugge	2018	Canal	20	80-211 (131)	6.2-142 (42)	0.84	0.20	3	480-534 (515)	190-268 (216)	1.9	0.21
36	TOERISTISCHE LEIE	Gent	2018	River	20	75-148 (99)	4.5-31 (14)	0.85	0.20	3	480-541 (514)	205-274 (249)	21	0.37
37	DENDER IV	Aalst	2018	River	20	88-173 (110)	8.2-61 (18)	0.79	0.19	3	445-540 (501)	166-289 (221)	15	0.33
38	DENDER V	Dendermonde	2018	River	20	84-180 (111)	6.8-79 (20)	0.83	0.20	6	420-543 (477)	161-295 (208)	9.1	0.28
39	ZENNE I	Beersel	2018	River	17	106-240 (147)	13-280 (165)	0.91	0.20	0	NA	NA	NA	NA
40	DIJLE IV	Wijgmaal	2018	River	0	NA	NA	NA	NA	3	468-568 (519)	186-308 (247)	15	0.33
41	KLEINE NETE II	Herentals	2018	Stream	0	NA	NA	NA	NA	3	418-687 (576)	120-697 (423)	18	0.35

Table D.1 (continued)

42	KANAAL BOCHOLT-HERENTALS	Mol	2018	Canal	20	91-168 (141)	9.6-69 (36)	0.75	0.20	0	NA	NA	NA	NA
43	ZUID-WILLEMSVAART + KANAAL BOCHOLT-HERENTALS (partly) + KANAAL BRIEGDEN-NEERHAREN	Bocholt	2018	Canal	20	84-135 (97)	6.4-28 (11)	0.72	0.20	3	365-515 (428)	75-252 (152)	5.2	0.25
44	ALBERTKANAAL	Kanne, Riemst	2018	Canal	20	100-160 (116)	8.2-50 (16)	0.79	0.19	2	587-748 (668)	275-856 (566)	16	0.36

Table D.2: Mean concentrations measured in fish (µg kg^{-1} dw) per sampling location.

No.	HCB		HCBD		Hg		∑PBDE		PFOS		HBCD		dicofol		dioxins[a]		heptachlor		tHpClepx		cHpClep		∑PCB	
	Perch	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*
1	<LOQ	9.7	<LOQ	<LOQ	341	199	6.1	285	37	26	4.7	1106	<LOQ		0.004		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	17	182	1677
2	<LOQ	18	<LOQ	<LOQ	604	850	4.4	32.5	24	20	1.8	30	<LOQ		0.009		<LOQ	<LOQ	<LOQ	<LOQ	7.8	42	407	2501
3	0.57	24	<LOQ	<LOQ	531	1035	7.8	34.2	220	25	3.0	58	<LOQ		0.006		<LOQ	<LOQ	<LOQ	<LOQ	1.9	33	103	879
4	0.41	4.4	<LOQ	<LOQ	474	1004	3.1	19.8	52	27	1.3	36	<LOQ		0.002		<LOQ	<LOQ	<LOQ	<LOQ	0.79	2.0	128	1532
5	<LOQ	3.8	<LOQ	<LOQ	698	506	2.5	6.6	159	51	<LOQ	2.6	<LOQ		0.002		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	63	266
6	<LOQ	14	<LOQ	<LOQ	277	322	6.5	21.7	79	8.0	3.8	28	<LOQ		0.008		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.8	293	1475
7	<LOQ		<LOQ		532		6.1		196		1.9		<LOQ		0.008		<LOQ		<LOQ		<LOQ		282	
8	<LOQ	6.0	<LOQ	<LOQ	564	596	4.1	16.3	120	51	2.2	21	<LOQ		0.010		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.3	199	1049
9	<LOQ	12	<LOQ	<LOQ	201	605	3.9	48.3	42	45	1.9	54	<LOQ		0.002		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.4	23	358
10		13		<LOQ		374		56.1		78		20		<LOQ		0.100		<LOQ		<LOQ		16		2889
11		12		<LOQ		1257		16.6		13		57		<LOQ		0.016		<LOQ		<LOQ		42		643
12	<LOQ	0.93	<LOQ	<LOQ	170	1079	0.9	1.2	48	17	<LOQ	<LOQ		<LOQ		0.006	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.7	24	25
13	<LOQ	1.7	<LOQ	<LOQ	651	315	<LOQ	0.9	55	29	1.6	<LOQ	<LOQ		0.003		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.0	49
14	<LOQ	1.5	<LOQ	<LOQ	236	502	<LOQ	2.1	17	27	<LOQ	3.6		<LOQ		0.006	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.2	3.5	61
15	0.59	11	<LOQ	<LOQ	399	407	6.9	195.3	77	51	1.7	217	<LOQ		0.011		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	17	39	2030
16	0.50	5.5	<LOQ	<LOQ	181	333	5.2	97.6	131	67	1.5	42		<LOQ		0.056	<LOQ	<LOQ	<LOQ	<LOQ	1.7	20	100	3038
17		9.1		<LOQ		276		58.7		94		32		<LOQ		0.077		<LOQ		<LOQ		15		4001
18	1.2	11	<LOQ	<LOQ	271	133	8.4	17.6	63	24	2.4	31		<LOQ		0.031	<LOQ	<LOQ	<LOQ	<LOQ	1.6	8.3	161	2446
19		11		<LOQ		312		27.7		23		15		<LOQ		0.019		<LOQ		<LOQ		18		422
20		7.6		<LOQ		534		36.7		220		20		<LOQ		0.021		<LOQ		<LOQ		46		602
21	<LOQ	12	<LOQ	<LOQ	210	179	3.4	43.8	20	14	3.0	92	<LOQ		0.013		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.1	15	240
22	0.93	5.1	<LOQ	<LOQ	164	188	2.8	14.8	28	41	2.3	31	<LOQ		0.005		<LOQ	<LOQ	<LOQ	<LOQ	2.6	13	16	293
23	<LOQ	6.3	<LOQ	<LOQ	176	119	<LOQ	5.3	13	8.2	<LOQ	5.3	<LOQ		0.007		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	22	5.9	168
24		4.6		<LOQ		488		6.5		216		3.7		<LOQ		0.018		<LOQ		<LOQ		17		540
25	<LOQ	3.5	<LOQ	<LOQ	216	83	<LOQ	97.6	47	14	<LOQ	109	<LOQ		0.007		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.1	5.3	2886
26	0.85	18	<LOQ	<LOQ	174	127	3.7	38.3	100	41	1.5	33	<LOQ		0.009		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11	78	1277
27	1.2	16	<LOQ	<LOQ	196	231	3.2	39.2	71	28	1.4	57	<LOQ		0.007		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9.7	62	1397
28		19		<LOQ		222		148.7		13		126		<LOQ		0.055		<LOQ		<LOQ		40		1524
29		7.2		<LOQ		731		16.9		31		10		<LOQ		0.028		<LOQ		<LOQ		23		2272
30	1.0	33	<LOQ	<LOQ	220	332	2.0	20.2	223	124	<LOQ	8.3	<LOQ		0.018		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.5	188	3128
31	<LOQ	13	<LOQ	<LOQ	160	236	2.7	19.9	270	22	<LOQ	19		<LOQ		0.103	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.8	476	3737
32	2.5	25	<LOQ	<LOQ	195	735	1.7	10.6	46	22	<LOQ	18	<LOQ		0.007		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.6	21	735
33	<LOQ	3.8	<LOQ	<LOQ	416	252	1.2	6.7	19	37	<LOQ	2.4	<LOQ		0.006		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.9	21	328
34		3.7		<LOQ		498		5		4.4		2.2		<LOQ		0.023		<LOQ		<LOQ		2.5		273
35	0.62	2.3	<LOQ	<LOQ	417	1526	1.4	5.6	178	94	<LOQ	12	<LOQ		0.002		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	35	425
36	<LOQ	14	<LOQ	<LOQ	230	349	6.3	59.3	51	34	<LOQ	49	<LOQ		0.008		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9.5	180	2692

Table D.2 (continued)

37	<LOQ	13	<LOQ	<LOQ	303	531	5.3	18.8	23	19	1.0	33	<LOQ		0.007		<LOQ	<LOQ	<LOQ	<LOQ	1.5	20	190	1452
38	0.53	5.7	<LOQ	<LOQ	286	274	4.3	26.8	33	29	0.89	18	<LOQ		0.004		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.4	134	1608
39	0.71		3.2		735		6		33		3.2		<LOQ		0.020		<LOQ		<LOQ		<LOQ		786	
40		8.3		<LOQ		465		16.7		6.1		61		<LOQ		0.020		<LOQ		<LOQ		16		1023
41		8.0		<LOQ		662		26.9		10		16		<LOQ		0.033		<LOQ		<LOQ		6.4		982
42	<LOQ		<LOQ		375		<LOQ		46		<LOQ		<LOQ		0.004		<LOQ		<LOQ		<LOQ		82	
43	<LOQ	0.48	4.0	8.4	361	411	1.4	41.7	29	13	<LOQ	8.9	<LOQ		0.005		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.6	160	1630
44	1.4	19	<LOQ	<LOQ	641	672	5.7	23.6	29	18	1.0	25	<LOQ		0.010		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.0	376	1847

[a]concentrations in µg WHO-TEQ_{2005} kg^{-1} dw.

Table D.3: Mean concentrations measured in fish (µg kg^{-1} ww) per sampling location.

No.	HCB		HCBD		Hg		PBDE		PFOS		HBCD		dicofol		dioxins[a]		heptachlor		tHpClepx		cHpClep		PCB	
	Perch	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*
1	<LOQ	3.6	<LOQ	<LOQ	77	74	1.3	106	7.7	9.5	1.1	412	<LOQ		0.0009		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.1	40	624
2	<LOQ	6.3	<LOQ	<LOQ	143	292	0.97	11	5.2	7.0	0.41	10	<LOQ		0.0021		<LOQ	<LOQ	<LOQ	<LOQ	1.9	15	90	858
3	0.10	7.8	<LOQ	<LOQ	94	332	1.4	11	11	8.3	0.53	19	<LOQ		0.0011		<LOQ	<LOQ	<LOQ	<LOQ	0.33	11	18	282
4	0.10	1.1	<LOQ	<LOQ	111	252	0.75	5.0	12	7.1	0.31	9.2	<LOQ		0.0005		<LOQ	<LOQ	<LOQ	<LOQ	0.22	0.5	31	385
5	<LOQ	1.1	<LOQ	<LOQ	132	145	0.47	1.9	30	15	<LOQ	0.74	<LOQ		0.0004		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12	76
6	<LOQ	10	<LOQ	<LOQ	60	238	1.4	16	18	5.9	0.82	21	<LOQ		0.0018		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.8	64	1088
7	<LOQ		<LOQ		114		1.3		42		0.40		<LOQ		0.0016		<LOQ		<LOQ		<LOQ		61	
8	<LOQ	2.7	<LOQ	<LOQ	122	268	0.88	7.3	26	24	0.46	9.3	<LOQ		0.0020		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.8	43	472
9	<LOQ	3.2	<LOQ	<LOQ	39	162	0.76	13	7.8	11	0.37	15	<LOQ		0.0003		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.2	4.4	96
10		5.1		<LOQ		144		22		29		7.7		<LOQ		0.0379		<LOQ		<LOQ		7.9		1110
11		3.1		<LOQ		323		4.3		3.4		15		<LOQ		0.0040		<LOQ		<LOQ		6.9		165
12	<LOQ	0.2	<LOQ	<LOQ	36	232	0.18	0.25	10	3.6	<LOQ	<LOQ		<LOQ		0.0013	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.37	5.1	5.3
13	<LOQ	0.6	<LOQ	<LOQ	134	111	<LOQ	0.33	11	10	0.34	<LOQ	<LOQ		0.0006		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.2	17
14	<LOQ	0.4	<LOQ	<LOQ	50	132	<LOQ	0.56	3.5	5.2	<LOQ	0.94		<LOQ		0.0015	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.62	0.75	16
15	0.12	3.7	<LOQ	<LOQ	80	136	1.4	65	15	17	0.34	73	<LOQ		0.0021		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.7	7.8	681
16	0.10	1.7	<LOQ	<LOQ	36	99	1.0	31	26	20	0.30	13		<LOQ		0.0171	<LOQ	<LOQ	<LOQ	<LOQ	0.33	6.2	20	937
17		2.8		<LOQ		78		18		25		8.9		<LOQ		0.0226		<LOQ		<LOQ		4.6		1141
18	0.20	3.8	<LOQ	<LOQ	46	40	1.4	5.7	11	7.3	0.40	10		<LOQ		0.0097	<LOQ	<LOQ	<LOQ	<LOQ	0.27	2.6	27	771
19		3.9		<LOQ		113		10		8.3		5.3		<LOQ		0.0070		<LOQ		<LOQ		12		152
20		2.3		<LOQ		158		11		65		5.9		<LOQ		0.0062		<LOQ		<LOQ		14		177
21	<LOQ	5.8	<LOQ	<LOQ	44	85	0.71	21	2.4	6.7	0.62	44	<LOQ		0.0028		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.9	3.1	114
22	0.20	1.4	<LOQ	<LOQ	35	52	0.61	4.1	8.1	11	0.50	8.6	<LOQ		0.0011		<LOQ	<LOQ	<LOQ	<LOQ	0.56	3.7	3.5	81
23	<LOQ	1.8	<LOQ	<LOQ	35	35	<LOQ	1.5	2.7	2.4	<LOQ	1.5	<LOQ		0.0014		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.5	1.2	49
24		1.3		<LOQ		117		1.8		52		1.0		<LOQ		0.0046		<LOQ		<LOQ		5.1		153
25	<LOQ	1.4	<LOQ	<LOQ	45	32	<LOQ	38	9.7	5.4	<LOQ	42	<LOQ		0.0015		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.2	1.1	1122
26	0.17	6.9	<LOQ	<LOQ	34	47	0.73	14	20	16	0.29	12	<LOQ		0.0017		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.0	15	461
27	0.25	5.7	<LOQ	<LOQ	40	83	0.66	14	15	9.8	0.29	20	<LOQ		0.0015		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.5	13	498
28		8.5		<LOQ		97		64		5.6		54		<LOQ		0.0241		<LOQ		<LOQ		18		654
29		2.2		<LOQ		217		5.0		9.4		3.0		<LOQ		0.0085		<LOQ		<LOQ		6.9		675
30	0.21	9.4	<LOQ	<LOQ	44	94	0.40	5.7	45	35	<LOQ	2.3	<LOQ		0.0035		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.4	38	885
31	<LOQ	4.6	<LOQ	<LOQ	32	83	0.54	7.0	54	7.9	<LOQ	6.9		<LOQ		0.0361	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.1	94	1321
32	0.52	6.4	<LOQ	<LOQ	41	191	0.34	2.7	9.5	5.6	<LOQ	4.8	<LOQ		0.0013		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.0	4.3	191
33	<LOQ	0.94	<LOQ	<LOQ	82	62	0.24	1.6	3.7	9.1	<LOQ	0.59	<LOQ		0.0013		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.47	4.2	80
34		1.3		<LOQ		174		1.7		1.5		0.78		<LOQ		0.0080		<LOQ		<LOQ		0.86		95
35	0.13	0.48	<LOQ	<LOQ	85	314	0.28	1.1	37	19	<LOQ	2.5	<LOQ		0.0005		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.1	87
36	<LOQ	5.1	<LOQ	<LOQ	45	129	1.2	22	14	13	<LOQ	18	<LOQ		0.0016		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.5	35	992
37	<LOQ	4.2	<LOQ	<LOQ	58	175	1.0	6.2	5.5	6.2	0.20	11	<LOQ		0.0014		<LOQ	<LOQ	<LOQ	<LOQ	0.29	6.6	36	478

Table D.3 (continued)

38	0.11	1.6	<LOQ	<LOQ	57	76	0.86	7.4	7.8	8.1	0.18	5.0	<LOQ		0.0008		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.4	26	450
39	0.14		0.65		148		1.2		6.6		0.65		<LOQ		0.0040		<LOQ		<LOQ		<LOQ		140	
40		2.9		<LOQ		152		5.3		1.8		20		<LOQ		0.0069		<LOQ		<LOQ		5.3		343
41		3.1		<LOQ		220		11		3.4		6.4		<LOQ		0.0117		<LOQ		<LOQ		2.5		370
42	<LOQ		<LOQ		73		<LOQ		9.0		<LOQ		<LOQ		0.0008		<LOQ		<LOQ		<LOQ		16	
43	<LOQ	0.12	0.79	2.1	71	103	0.31	8.0	5.6	3.1	<LOQ	2.2	<LOQ		0.0011		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.4	34	312
44	0.26	7.0	<LOQ	<LOQ	121	243	1.0	8.5	5.6	6.3	0.19	9.0	<LOQ		0.0019		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.1	69	669

[a]concentrations in µg WHO-TEQ_{2005} kg^{-1} ww.

Table D.4: Mean PAH concentrations measured in Dreissena sp. *per sampling location.*

No.	Year of exposure	Benzo(a)pyrene		Fluoranthene	
		µg kg^{-1} dw	µg kg^{-1} ww	µg kg^{-1} dw	µg kg^{-1} ww
1	2015	47	5.2	152	17
2	2015	11	1.3	<LOQ	<LOQ
3	2015	8.9	1.8	54	11
4	2015	41	4.7	147	17
5	2015	5.9	1.0	33	5.6
6	2015	19	2.1	63	6.8
7	2015	157	17	269	29
8	2015	26	2.8	122	13
9	2015	13	1.4	89	10
10	2015	<LOQ[a]	<LOQ[a]	217[a]	28[a]
11	2015	36	4.9	101	14
12	2016	15	2.4	75	12
13	2016	<LOQ[a]	<LOQ[a]	129[a]	22[a]
14	2016	13[a]	2.9[a]	186[a]	41[a]
15	2016	64	8.0	341	43
16	2016	51	6.6	192	25
17	2016	56	7.1	292	37
18	2016	37	5.2	328	46
19	2016	20	3.7	353	67
20	2016	36	6.4	278	50
21	2016	23	2.0	172	15
22	2016	29	4.1	264	37
23	2017	<LOQ[a]	<LOQ[a]	171[a]	29[a]
24	2017	30	2.7	333	30
25	2017	<LOQ[a]	<LOQ[a]	119[a]	19[a]
26	2017	22	2.8	123	16
27	2017	40	5.2	169	22
28	2017	62	8.0	246	32
29	2017	12	2.0	89	15
30	2017	87	11	87	11
31	2017	270	27	1073	107
32	2017	20	2.8	129	18
33	2017	<LOQ	<LOQ	114	16
34	2018	NA	NA	NA	NA
35	2018	23	3.0	345	45
36	2018	34	4.4	201	26
37	2018	15	1.6	169	18
38	2018	33	4.0	198	24
39	2018	35	4.9	207	29
40	2018	9.3	1.4	173	26
41	2018	<LOQ	<LOQ	85	11
42	2018	21	3.5	54	9
43	2018	75	12	113	18
44	2018	94	13	289	40
REF1	2015; (2015-2018)[a]	< LOQ (<LOQ)[a]	< LOQ (<LOQ)[a]	<LOQ (76)[a]	<LOQ (12)[a]
REF2	2016	<LOQ	<LOQ	153	21
REF3	2017; 2018	<LOQ	<LOQ	<LOQ	<LOQ

[a] Exposure of *Corbicula fluminea* instead of *Dreissena sp.* Reference locations where mussels were collected: REF1: Blaarmeerse, REF2: Drinkwater reservoir (Waterlink) Duffel, REF3: Nekker.

Table D.5: Abiotic characteristics in sediment and water per location (geometric Mean). Individual sediment pH (-), O_2 (mg L^{-1}), conductivity (EC20; µS cm^{-1}), TOC (g C kg^{-1} dw), Clay (%), and water parameters pH (-), O_2 (mg L^{-1}), conductivity (EC20; µS cm^{-1}), DOC (mg C L^{-1}), nitrate (mg N L^{-1}) and nitrite (mg N L^{-1}) are given.

No.	Sediment					Water					
	pH	O_2	EC20	TOC	Clay (%)	pH	O_2	EC20	DOC	Nitrate	Nitrite
1				26	13	7.8	7.7	781	5.6	4.9	0.16
2	8.7	11	1030	15	13	7.9	8.6	883	7.0	2.3	0.11
3	7.9	13	1494	6.9	5.5	7.7	8.0	864	6.0	3.3	0.1
4				31	4.4	7.9	10	466	4.1	3.3	0.03
5				14	28	8.3	10	1590	9.4	2.2	0.06
6	7.8	7.3	845	11	6.7	7.8	7.7	914	6.2	4.2	0.18
7				18	16	7.8	8.0	4610	6.5	4.7	0.12
8	7.9	8.9	1690	50	30	8.0	8.9	2225	8.9	3.7	0.09
9	7.0	8.2	316	17	1.7	7.3	8.5	339	6.8	0.9	0.03
10	7.9	9.3	10770	6.6	9.3	7.9	8.7	13830	5.9	2.4	0.01
11				3.1	2.1	8.0	8.6	690	4.4	6.5	0.17
12				28	32	8.1	8.2	933	7.5	3.6	0.1
13				26	25	8.3	9.5	4167	12	0.4	0.01
14	8.2	13	2890	21	10	7.9	8.1	4994	12	0.8	0.02
15				5.2	4.0	7.8	7.9	800	6.2	5.0	0.14
16	7.6	4.9	826	4.1	4.9	7.9	8.4	839	6.8	4.5	0.02
17	7.4	4.9	1162	2.9	5.2	7.8	7.5	1513	6.8	4.1	0.02
18	7.7	8.6	1217	10	8.8	7.8	7.5	1104			
19				13	6.7	8.1	10	817	6.3	3.3	0.12
20				9.5	13	8.0	9.4	895	5.8	3.6	0.15
21	6.8	7.0	744	3.8	1.8	7.1	8.1	738	6.3	3.1	0.09
22	7.8	8.8	546	14	8.0	8.0	9.6	599	6.3	2.0	0.06
23				14	9.5	8.2	10	3173	11	1.7	0.05
24	8.5	13	540	45	35	7.9	7.9	550	10	1.2	0.08
25	7.9	11	2020	4.1	3.0	7.9	8.5	2521	14	1.1	0.06
26	7.9	8.8	935	1.8	2.8	7.8	7.0	899	7.2	4.4	0.19
27				4.2	6.1	7.7	6.9	885	6.5	4.7	0.18
28				16	11	7.8	7.9	810	6.4	4.9	0.16
29	8.0	9.8	434	12	7.0	7.9	7.9	703	7.1	2.5	0.12
30				5.4	7.0	8.0	8.5	801	5.3	3.9	0.03
31	7.4	2.8	1225	20	5.2	7.5	4.4	1085	7.9	2.4	0.26
32				5.6	6.1	7.5	8.2	1187	7.3	1.6	0.04
33				28	5.7	7.3	7.9	442	13	3.9	0.1
34				15	31	8.0	8.9	16233	6.3	0.91	0.02
35				7.7	5.4	8.1	9.2	779	9.5	2.4	0.07
36	7.9	10	902	27	18	7.8	7.0	880	7.0	3.8	0.14
37	9.1	11	847	5.9	4.4	7.9	9.5	793	7.0	2.2	0.09
38	8.1	11	848	19	18	7.7	8.4	825	8.1	2.3	0.09
39				11	4.6	7.8	6.7	745		3.4	0.21
40	8.2	8.9	857	9.3	3.5	8.0	8.6	753	5.5	6.3	0.18
41	7.2	8.8	381	3.3	5.5	7.3	9.0	569	7.5	1.1	0.04
42	8.5	12	425	45	36	8.0	10.5	464	4.3	2.6	0.01
43	7.9	7.6	257	1.2	1.8	7.9	8.9	457	3.9	3.1	0.03
44	7.8	5.1	254	19	21	8.0	9.0	454	3.7	3.0	0.03

Table D.6: Geometric means of compounds measured in the water column per location (ng L^{-1}).

No.	HCB	HCBD	Hg	PFOS	heptachlor	cHpChlepx	tHpChlepx	∑PCB	Benzo(a) pyrene[a]	Fluor-anthene[a]
1	1.3	<LOQ	22	3.7	<LOQ		<LOQ	4.8	30	73
2	<LOQ		19	1.7	<LOQ		<LOQ	<LOQ	4.1	6.8
3	<LOQ		14	2.6	<LOQ	<LOQ	<LOQ	<LOQ		17
4	<LOQ	<LOQ	6	1.9	<LOQ	<LOQ	<LOQ	<LOQ	6.6	22
5	<LOQ		14	2.0	<LOQ		<LOQ	<LOQ	1.4	4.8
6	1.2		39	7.3	<LOQ		<LOQ	5.8	5.5	13
7	<LOQ		12	10	<LOQ		<LOQ	5.0	7	29
8	<LOQ		12	6.7	<LOQ		<LOQ	<LOQ	<LOQ	
9			6							
10	<LOQ		30	14	<LOQ		<LOQ	4.8		
11	<LOQ		14	1.1	<LOQ	<LOQ	<LOQ	<LOQ	14	15
12	<LOQ		17	1.2	<LOQ		<LOQ	<LOQ	<LOQ	<LOQ
13	<LOQ		16	2.3	<LOQ		<LOQ	<LOQ	<LOQ	<LOQ
14	<LOQ		37	1.4	<LOQ		<LOQ	<LOQ	<LOQ	3.5
15	<LOQ		21	4.4	<LOQ		<LOQ	<LOQ		
16	1.3		65	6.2	<LOQ		<LOQ	7.4	23	43
17	<LOQ		61	9.4	<LOQ		<LOQ	7.2	39	67
18										
19	<LOQ		7		<LOQ		<LOQ			
20			7							
21	<LOQ		21	1.4	<LOQ		<LOQ	<LOQ	1.7	10
22			7							
23	<LOQ		14	2.2	<LOQ		<LOQ	<LOQ	<LOQ	
24			41							
25	<LOQ		16	2.7	<LOQ		<LOQ	<LOQ	1.3	7.1
26	<LOQ		40		<LOQ		<LOQ			
27	<LOQ		37	7.5	<LOQ		<LOQ	<LOQ	16	43
28			47							
29	<LOQ		29		<LOQ		<LOQ			
30			6							
31			13						29	
32	<LOQ		40	3.9	<LOQ		<LOQ	4.7	3.5	12
33	<LOQ		17	0.80	<LOQ		<LOQ	<LOQ	<LOQ	5.2
34	<LOQ		20	2.5	<LOQ		<LOQ	<LOQ	<LOQ	<LOQ
35	<LOQ		15	6.4	<LOQ		<LOQ	<LOQ	<LOQ	5.2
36			28							
37			21							
38	<LOQ		12	3.5	<LOQ		<LOQ	5.3	1.7	8.8
39										
40	<LOQ		14		<LOQ		<LOQ	<LOQ		
41			6							
42			9							
43			8							
44	<LOQ		18	1.5	<LOQ		<LOQ	<LOQ	11	22

[a] concentrations were calculated for duration of exposure of mussels.

Table D.7: Geometric means of compounds measured in the sediment per location (µg kg^{-1} dw).

No.	HCB	HCBD	Hg	∑PBDE	PFOS	HBCD	dicofol	cHpChlepx	tHpChlepx	∑PCB	Benzo(a)pyrene[a]	Fluoranthene[a]
1	0.30	<LOQ	474	2.0	0.49	<LOQ	7.7	<LOQ	<LOQ	97	1300	1800
2	0.11	<LOQ	181	1.0	0.27	<LOQ	<LOQ	<LOQ	<LOQ	7.7	760	1500
3	<LOQ	<LOQ	26	0.61	0.17	<LOQ	<LOQ	<LOQ	<LOQ	3.0	<LOQ	<LOQ
4	0.93	<LOQ	220	0.15	0.61	<LOQ	<LOQ	<LOQ	<LOQ	55		
5	<LOQ	<LOQ	55	0.26	0.22	<LOQ	<LOQ	<LOQ	<LOQ	3.8	130	230
6	0.13	<LOQ	183	1.2	0.27	<LOQ	6.4	<LOQ	<LOQ	27	90	270
7	0.50	<LOQ	413	1.5	0.52	<LOQ	7.2	<LOQ	<LOQ	25		
8	0.74	<LOQ	684	2.8	5.1	<LOQ	15	<LOQ	<LOQ	81	2800	7500
9	<LOQ	<LOQ	48	0.46	0.25	<LOQ	<LOQ	<LOQ	<LOQ	2.9	65	<LOQ
10	0.11	<LOQ	97	0.74	0.79	<LOQ	<LOQ	<LOQ	<LOQ	5.5	210	360
11	<LOQ	<LOQ	42	0.38	0.07	<LOQ	<LOQ	<LOQ	<LOQ	3.0	<LOQ	<LOQ
12	0.29	<LOQ	119	1.0	0.37	<LOQ	<LOQ	<LOQ	<LOQ	6.4	100	<LOQ
13	<LOQ	<LOQ	40	<LOQ	1.1	<LOQ	<LOQ	<LOQ	<LOQ	1.4	300	900
14	<LOQ	<LOQ	61	0.26	0.51	<LOQ	<LOQ	<LOQ	<LOQ	1.1	120	210
15	0.12	<LOQ	121	4.1	<LOQ	<LOQ	19	<LOQ	<LOQ	13	90	430
16	<LOQ	<LOQ	91	2.2	0.29	<LOQ	3.7	<LOQ	<LOQ	5.9	190	310
17	0.13	<LOQ	72	0.36	<LOQ	<LOQ	12	<LOQ	<LOQ	3.0	170	380
18	0.22	<LOQ	167	1.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	24	90	220
19	<LOQ	<LOQ	48	0.66	<LOQ	<LOQ	5.9	<LOQ	<LOQ	5.5	<LOQ	<LOQ
20	0.21	<LOQ	53	0.68	1.2	<LOQ	<LOQ	<LOQ	<LOQ	11	<LOQ	<LOQ
21	<LOQ	<LOQ	206	0.31	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.0	<LOQ	<LOQ
22	<LOQ	<LOQ	39	0.63	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.3	<LOQ	300
23	<LOQ	<LOQ	60	0.05	<LOQ	<LOQ	<LOQ			2.1	310	420
24	0.83	<LOQ	2404	2.1	8.0	<LOQ	<LOQ			76	515	950
25	<LOQ	<LOQ	42	0.69	<LOQ	<LOQ	<LOQ			8.3	<LOQ	<LOQ
26	<LOQ	<LOQ	66	1.1	<LOQ	<LOQ	<LOQ			6.6	<LOQ	<LOQ
27	0.13	<LOQ	76	1.7	<LOQ	<LOQ	<LOQ			8.8	<LOQ	<LOQ
28	0.17	<LOQ	169	8.3	<LOQ	<LOQ	<LOQ			31	295	530
29	0.12	<LOQ	168	0.75	<LOQ	<LOQ	<LOQ			32	120	<LOQ
30	<LOQ	<LOQ	49	0.19	<LOQ	<LOQ				31	170	300

Table D.7 (continued)

31	1.4	<LOQ	665	2.5		<LOQ				318	2300	4800
32	0.13	<LOQ	438	0.97	<LOQ	<LOQ				1.6	<LOQ	<LOQ
33	<LOQ	<LOQ	51	1.5	<LOQ	<LOQ				12	80	<LOQ
34	<LOQ	<LOQ	54	0.25	0.62	<LOQ	<LOQ	<LOQ	<LOQ	2.8	100	210
35	<LOQ	<LOQ	136	0.72						11	<LOQ	<LOQ
36	0.64	<LOQ	747	4.1	0.79	<LOQ	14	<LOQ	<LOQ	83	370	900
37	<LOQ	<LOQ	89	0.71	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.7	140	280
38	0.15	<LOQ	264	6.9	0.90	<LOQ	<LOQ	<LOQ	<LOQ	44	290	460
39	<LOQ		200	0.49						55	71	170
40	<LOQ	<LOQ	75	0.73	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.2	390	900
41	<LOQ	<LOQ	21	0.06	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.8	<LOQ	<LOQ
42	0.71	2.7	1709	1.6	0.67	<LOQ	<LOQ	<LOQ	<LOQ	80	490	730
43	<LOQ	<LOQ	69	0.15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
44	1.4	1.7	521	1.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	43	550	1000

[a] concentrations were calculated for duration of exposure of mussels.

Table D.8: Pearson correlation test performed on abiotic characteristics of water and sediment (r^2; p-value). Values in bold indicate r^2-values of significant correlations ($p < 0.05$).

		Sediment (S)					Water (W)					
		O_2	pH	EC20	TOC	Clay (%)	O_2	pH	EC20	nitrate	nitrite	DOC
S	O_2	*	**0.62** (<0.001)	0.11 (0.61)	0.32 (0.12)	0.36 (0.08)	**0.42** (<0.05)	0.28 (0.18)	0.12 (0.57)	-0.37 (0.08)	-0.19 (0.38)	0.35 (0.10)
	pH		*	0.05 (0.81)	0.31 (0.13)	**0.42** (<0.05)	0.33 (0.10)	**0.73** (<0.001)	0.05 (0.81)	-0.04 (0.85)	0.02 (0.93)	0.09 (0.67)
	EC20			*	-0.11 (0.62)	-0.05 (0.82)	0.01 (0.97)	0.15 (0.46)	**0.99** (<0.001)	-0.10 (0.65)	-0.22 (0.31)	0.09 (0.67)
	TOC				*	**0.74** (<0.001)	0.13 (0.41)	0.18 (0.25)	-0.03 (0.87)	-0.17 (0.28)	-0.09 (0.57)	0.20 (0.21)
	Clay (%)					*	0.27 (0.08)	**0.44** (<0.005)	0.27 (0.08)	-0.26 (0.09)	-0.25 (0.11)	0.12 (0.45)
W	O_2						*	**0.49** (<0.001)	0.09 (0.54)	-0.26 (0.09)	**-0.64** (<0.001)	-0.06 (0.72)
	pH							*	0.21 (0.17)	-0.02 (0.92)	-0.17 (0.28)	0.04 (0.82)
	EC20								*	**-0.31** (<0.05)	**-0.32** (<0.05)	0.08 (0.61)
	Nitrate									*	**0.56** (<0.001)	**-0.48** (<0.001)
	Nitrite										*	-0.12 (0.45)
	DOC											*

Table D.9: LOQs for measurements in biota (µg kg^{-1} ww), sediment (µg kg^{-1} dw) and water (ng L^{-1}).

LOQ	Biota	Sediment	Water
HCB	0.1	0.36	2.5
HCBD	0.5	1	126
Hg	0.1	10	5
∑PBDE[b]	0.3	0.06	NA
PFOS	0.1	0.5	0.5
HBCD	0.3	150	NA
Dicofol	20	5	NA
Dioxins	0.0003[a]	NA	NA
heptachlor	0.25	NA	1
tHpClepx	0.5	2	2
cHpClepx	0.25	1	1
∑PCB[b]	0.9	1.4	8
Benz(a)pyrene	1	60	5
Fluoranthene	5	200	10

[a] dioxins in biota were indicated in µg WHO-TEQ$_{2005}$ kg^{-1} ww. [b] this LOQ was calculated as the sum of LOQs of each congener.

Table D.10: Details of multiple regression analyses based on the effect of environmental concentrations and physical/chemical characteristics on the bioaccumulated concentrations of persistent pollutants. Parameters in the table were included in the significant model after stepwise deletion (Table 5.3).

Compound	Response variable	Explanatory variable	t-value	Estimate ± SE	*p*-value
HCB	Log(eel)	*(Intercept)*	3.203	11.62244 ± 3.62845	0.00280
		Log(sediment)	3.127	0.52630 ± 0.16831	0.00343
		TOC	-3.152	-0.04363 ± 0.01384	0.00321
		pH	-2.179	-1.01484 ± 0.46571	0.03577
PFOS	Log(perch)	*(Intercept)*	12.178	3.1387 ± 0.2577	<0.001
		Log(water)	3.963	0.8036 ± 0.2028	<0.001
	Log(eel)	*(Intercept)*	10.553	3.333 ± 0.3158	<0.001
		Log(water)	3.455	0.5940 ± 0.1719	0.00237
		Conductivity	-2.742	-0.0001 ± 0.00004	0.01223
		Nitrite	-2.362	-6.041 ± 2.558	0.02793
∑PBDE	Log(perch)	*(Intercept)*	6.669	2.15829 ± 0.32362	<0.001
		Log(sediment)	3.339	0.29043 ± 0.08699	0.00239
		DOC	-3.584	-0.14791 ± 0.04127	0.00127
	Log(eel)	*(Intercept)*	11.160	5.03022 ± 0.45076	<0.001
		Log(sediment)	4.249	0.47808 ± 0.11252	<0.001
		Clay	-4.013	-0.06026 ± 0.01502	<0.001
		DOC	-2.632	-0.15689 ± 0.05962	0.012433
∑PCB	Log(perch)	*(Intercept)*	8.197	5.31965 ± 0.64900	<0.001
		Log(sediment)	3.293	0.39874 ± 0.12108	0.002767
		DOC	-4.433	-0.28732 ± 0.06481	<0.001
	Log(eel)	*(Intercept)*	25.189	6.29788 ± 0.25002	<0.001
		Log(sediment)	6.199	0.70843 ± 0.11428	<0.001
		TOC	-5.673	-0.07742 ± 0.01365	<0.001
Benzo(a)pyrene	Log(mussel)	*(Intercept)*	9.841	2.2769 ± 0.2314	<0.001
		Log(water)	4.496	0.5832 ± 0.1297	<0.001
	Log(*Dreissena*)	*(Intercept)*	9.816	2.6184 ± 0.2667	<0.001
		Log(water)	3.341	0.4521 ± 0.1353	0.00415

Table D.11: Details of linear regression analyses based on the (extrapolation) effect of accumulated concentrations in perch on the accumulated concentrations in eel of persistent compounds (Table 5.4).

Compound	Response variable	Explanatory variable	t-value	Estimate ± SE	*p*-value
PFOS	Log(perch)	*(Intercept)*	3.356	1.6788 ± 0.4996	0.00228
		Log(eel)	3.373	0.4127 ± 0.1224	0.00219
Hg	Log(perch)	*(Intercept)*	1.181	1.7401 ± 1.4729	0.24738
		Log(eel)	2.842	0.7624 ± 0.2577	0.00827
HBCD	Log(perch)	*(Intercept)*	10.298	2.6764 ± 0.2599	<0.001
		Log(eel)	2.537	1.1469 ± 0.4520	0.0173
∑PBDE	Log(perch)	*(Intercept)*	6.095	1.9004 ± 0.3118	<0.001
		Log(eel)	4.435	1.0754 ± 0.2425	<0.001
∑PCB	Log(perch)	*(Intercept)*	7.187	3.9054 ± 0.5434	<0.001
		Log(eel)	5.395	0.6764 ± 0.1254	<0.001

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Appendix E: Chapter 6

Table E.1: Mean accumulated concentrations measured in fish and mussels (µg kg^{-1} ww) and MMIF score per sampling location. Concentrations of dicofol, heptachlor and trans-heptachlor epoxide were below their respective LOQs (20, 0.25 and 0.5 µg kg^{-1} ww) in all sample locations for both species and were not included in this table.

No.	MMIF	HCB		HCBD		Hg		∑PBDE		PFOS		HBCD		Dioxins[a]		Cis - Heptachlor epoxide		∑PCB		Fluoranthene			Benzo(a)pyrene		
		Perch	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*D.p.*	*D.b.*	*All*	*D.p.*	*D.b.*	*All*
LOQ		0.1	0.1	0.5	0.5	0.1	0.1	0.3	0.3	0.1	0.1	0.3	0.3	0.0003	0.0003	0.25	0.25	0.9	0.9	5	5	5	1	1	1
1	0.35	<LOQ	3.6	<LOQ	<LOQ	77	74	1.3	106	7.7	9.5	1.1	412	0.0009		<LOQ	6.1	40	624	17		17	5.2		5.2
2	0.5	<LOQ	6.3	<LOQ	<LOQ	143	292	0.97	11	5.2	7.0	0.41	10	0.0021		1.9	15	90	858	<LOQ		<LOQ	1.3		1.3
3	0.45	0.10	7.8	<LOQ	<LOQ	94	332	1.4	11	11	8.3	0.53	19	0.0011		0.33	11	18	282	11		11	1.8		1.8
4	0.41	0.10	1.1	<LOQ	<LOQ	111	252	0.75	5.0	12	7.1	0.31	9.2	0.0005		0.22	0.5	31	385	17		17	4.7		4.7
5	0.3	<LOQ	1.1	<LOQ	<LOQ	132	145	0.47	1.9	30	15	<LOQ	0.74	0.0004		<LOQ	<LOQ	12	76	5.6		5.6	1.0		1.0
6	0.4	<LOQ	10	<LOQ	<LOQ	60	238	1.4	16	18	5.9	0.82	21	0.0018		<LOQ	2.8	64	1088	6.8		6.8	2.1		2.1
7	0.35	<LOQ		<LOQ		114		1.3		42		0.40		0.0016		<LOQ		61		29		29	17		17
8	0.25	<LOQ	2.7	<LOQ	<LOQ	122	268	0.88	7.3	26	24	0.46	9.3	0.0020		<LOQ	2.8	43	472	13		13	2.8		2.8
9	0.75	<LOQ	3.2	<LOQ	<LOQ	39	162	0.76	13	7.8	11	0.37	15	0.0003		<LOQ	1.2	4.4	96	10		10	1.4		1.4
10			5.1		<LOQ		144		22		29		7.7		0.0379		7.9		1110			28[b]			<LOQ[b]
11	0.55		3.1		<LOQ		323		4.3		3.4		15		0.0040		6.9		165	14		14	4.9		4.9
12	0.75	<LOQ	0.2	<LOQ	<LOQ	36	232	0.18	0.25	10	3.6	<LOQ	<LOQ		0.0013	<LOQ	0.37	5.1	5.3	12		12	2.4		2.4
13	0.15	<LOQ	0.6	<LOQ	<LOQ	134	111	<LOQ	0.33	11	10	0.34	<LOQ	0.0006		<LOQ	<LOQ	1.2	17			22[b]			<LOQ[b]
14	0.4	<LOQ	0.4	<LOQ	<LOQ	50	132	<LOQ	0.56	3.5	5.2	<LOQ	0.94		0.0015	<LOQ	0.62	0.75	16			41[b]			2.9[b]
15	0.35	0.12	3.7	<LOQ	<LOQ	80	136	1.4	65	15	17	0.34	73	0.0021		<LOQ	5.7	7.8	681	59	26	43	9.5	6.5	8.0
16		0.10	1.7	<LOQ	<LOQ	36	99	1.0	31	26	20	0.30	13		0.0171	0.33	6.2	20	937	30	20	25	7.7	5.5	6.6
17	0.15		2.8		<LOQ		78		18		25		8.9		0.0226		4.6		1141	53	21	37	8.3	5.8	7.1
18		0.20	3.8	<LOQ	<LOQ	46	40	1.4	5.7	11	7.3	0.40	10		0.0097	0.27	2.6	27	771	46		46	5.2		5.2
19	0.45		3.9		<LOQ		113		10		8.3		5.3		0.0070		12		152	67		67	3.7		3.7
20	0.7		2.3		<LOQ		158		11		65		5.9		0.0062		14		177	50		50	6.4		6.4
21	0.8	<LOQ	5.8	<LOQ	<LOQ	44	85	0.71	21	2.4	6.7	0.62	44	0.0028		<LOQ	2.9	3.1	114	14	15	15	1.8	2.1	2.0
22	0.7	0.20	1.4	<LOQ	<LOQ	35	52	0.61	4.1	8.1	11	0.50	8.6	0.0011		0.56	3.7	3.5	81	37		37	4.1		4.1
23	0.65	<LOQ	1.8	<LOQ	<LOQ	35	35	<LOQ	1.5	2.7	2.4	<LOQ	1.5	0.0014		<LOQ	6.5	1.2	49			29[b]			<LOQ[b]
24	0.6		1.3		<LOQ		117		1.8		52		1.0		0.0046		5.1		153		30	30		2.7	2.7
25	0.25	<LOQ	1.4	<LOQ	<LOQ	45	32	<LOQ	38	9.7	5.4	<LOQ	42	0.0015		<LOQ	1.2	1.1	1122			19[b]			<LOQ[b]
26	0.35	0.17	6.9	<LOQ	<LOQ	34	47	0.73	14	20	16	0.29	12	0.0017		<LOQ	4.0	15	461		16	16		2.8	2.8
27	0.3	0.25	5.7	<LOQ	<LOQ	40	83	0.66	14	15	9.8	0.29	20	0.0015		<LOQ	3.5	13	498		22	22		5.2	5.2
28	0.4		8.5		<LOQ		97		64		5.6		54		0.0241		18		654		32	32		8.0	8.0
29	0.6		2.2		<LOQ		217		5.0		9.4		3.0		0.0085		6.9		675		15	15		2.0	2.0
30	0.3	0.21	9.4	<LOQ	<LOQ	44	94	0.40	5.7	45	35	<LOQ	2.3	0.0035		<LOQ	2.4	38	885		11	11		11	11
31	0.15	<LOQ	4.6	<LOQ	<LOQ	32	83	0.54	7.0	54	7.9	<LOQ	6.9		0.0361	<LOQ	3.1	94	1321		107	107		27	27
32	0.5	0.52	6.4	<LOQ	<LOQ	41	191	0.34	2.7	9.5	5.6	<LOQ	4.8	0.0013		<LOQ	2.0	4.3	191		18	18		2.8	2.8
33	0.8	<LOQ	0.94	<LOQ	<LOQ	82	62	0.24	1.6	3.7	9.1	<LOQ	0.59	0.0013		<LOQ	0.47	4.2	80		16	16		<LOQ	<LOQ

Table E.1 (continued)

34			1.3		<LOQ		174		1.7		1.5		0.78		0.0080		0.86		95				
35	0.4	0.13	0.48	<LOQ	<LOQ	85	314	0.28	1.1	37	19	<LOQ	2.5	0.0005		<LOQ	<LOQ	7.1	87	45	45	3.0	3.0
36	0.35	<LOQ	5.1	<LOQ	<LOQ	45	129	1.2	22	14	13	<LOQ	18	0.0016		<LOQ	3.5	35	992	26	26	4.4	4.4
37	0.55	<LOQ	4.2	<LOQ	<LOQ	58	175	1.0	6.2	5.5	6.2	0.20	11	0.0014		0.29	6.6	36	478	18	18	1.6	1.6
38	0.5	0.11	1.6	<LOQ	<LOQ	57	76	0.86	7.4	7.8	8.1	0.18	5.0	0.0008		<LOQ	2.4	26	450	24	24	4.0	4.0
39	0.7	0.14		0.65		148		1.2		6.6		0.65		0.0040		<LOQ		140		29	29	4.9	4.9
40	0.5		2.9		<LOQ		152		5.3		1.8		20		0.0069		5.3		343	26	26	1.4	1.4
41	0.8		3.1		<LOQ		220		11		3.4		6.4		0.0117		2.5		370	11	11	<LOQ	<LOQ
42	0.55	<LOQ		<LOQ		73		<LOQ		9.0		<LOQ		0.0008		<LOQ		16		9	9	3.5	3.5
43	0.5	<LOQ	0.12	0.79	2.1	71	103	0.31	8.0	5.6	3.1	<LOQ	2.2	0.0011		<LOQ	1.4	34	312	18	18	12	12
44	0.4	0.26	7.0	<LOQ	<LOQ	121	243	1.0	8.5	5.6	6.3	0.19	9.0	0.0019		<LOQ	1.1	69	669	40	40	13	13

[a] concentrations in µg WHO-TEQ_{2005} kg^{-1} ww. [b] *Corbicula fluminea* (Asian clam). D.p.: *Dreissena polymorpha* (zebra mussel); D.b.: *Dreissena bugensis* (quagga mussel); All: all mussel species combined. LOQ: limit of quantification.

Table E.2: MMIF scores and accumulated concentrations of pollutants included in the present study measured in fish (µg kg^{-1} ww) per sampling location and standardised on 5% lipid content or 26% dry weight (for PFOS and Hg).

No.	MMIF	Hg		∑PBDE		PFOS		HBCD		Dioxins[a]		∑PCB	
		Perch	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*	*Perch*	*Eel*
LOQ		0.1	0.1	0.3	0.3	0.1	0.1	0.3	0.3	0.0003	0.0003	0.9	0.9
1	0.35	91	52	6.7	75	9.1	6.7	5.7	290	0.005		206	439
2	0.5	162	223	6.7	4.3	5.9	5.4	2.7	3.9	0.014		600	330
3	0.45	136	270	9.1	5.7	16	6.7	3.2	9.6	0.007		117	145
4	0.41	111	262	4.6	9.3	12	7.4	1.7	17	0.003		178	713
5	0.3	191	130	2.6	2.1	43	13	0.77	0.76	0.002		61	83
6	0.4	71	84	7.1	3.5	21	2.1	4.1	4.5	0.009		327	237
7	0.35	141		6.6		52		2.0		0.008		311	
8	0.25	151	155	4.9	3.7	32	14	2.7	4.7	0.011		236	236
9	0.75	53	156	4.6	7.0	11	11	2.3	7.9	0.002		23	52
10			99		8.5		20		3.0		0.015		427
11	0.55		323		1.8		3.4		6.3		0.002		69
12	0.75	45	274	0.91	0.79	12	4.3	0.68	0.39		0.003	23	13
13	0.15	166	82	1.0	0.13	14	7.4	1.7	0.06	0.003		5.0	7.1
14	0.4	62	127	1.0	0.60	4.3	5.0	0.75	0.94		0.002	5.0	16
15	0.35	104	104	9.7	23	20	13	2.4	26.0	0.015		56	243
16		47	86	7.1	37	34	17	2.1	16.0		0.021	143	1143
17	0.15		78		13		25		6.5		0.017		839
18		70	33	9.0	2.6	17	5.9	2.6	4.5		0.004	173	350
19	0.45		82		4.5		6.0		2.4		0.003		69
20	0.7		137		5.4		56		3.0		0.003		89
21	0.8	54	46	4.0	3.3	3.0	3.6	3.5	6.9	0.016		17	18
22	0.7	41	48	2.5	3.0	9.6	10	2.1	6.2	0.005		17	59
23	0.65	46	31	1.4	0.88	3.5	2.2	1.0	0.82	0.009		6.8	27
24	0.6		122		1.5		54		8.1		0.004		123
25	0.25	56	21	1.3	7.6	12	3.6	0.95	8.5	0.009		6.3	224
26	0.35	44	32	5.3	3.6	26	11	2.2	3.1	0.013		114	121
27	0.3	50	60	4.6	4.4	19	7.1	1.9	6.3	0.01		86	156
28	0.4		59		13		3.4		11		0.005		136
29	0.6		188		2.3		8.1		1.4		0.004		307
30	0.3	57	87	3.3	3.7	59	33	1.3	1.5	0.029		317	575
31	0.15	42	62	3.2	2.1	70	5.9	0.97	2.0		0.011	610	389
32	0.5	51	191	1.9	2.3	12	5.6	0.95	4.0	0.008		25	159
33	0.8	107	64	2.0	1.4	4.8	9.5	1.0	0.53	0.009		27	71
34			129		0.53		1.1		0.24		0.003		30
35	0.4	111	389	1.7	2.9	48	24	0.89	6.6	0.003		42	229
36	0.35	59	91	7.1	5.2	18	9.1	0.88	4.3	0.009		206	236
37	0.55	79	138	6.3	2.1	7.5	4.9	1.3	3.7	0.009		228	159
38	0.5	74	71	5.2	4.1	10	7.5	1.1	2.7	0.005		157	247
39	0.7	192		6.6		8.6		3.6		0.022		769	
40	0.5		120		1.8		1.4		6.7		0.002		114
41	0.8		163		3.1		2.5		1.8		0.003		103
42	0.55	95		1.0		12		1.0		0.005		107	
43	0.5	92	107	2.2	7.7	7.3	3.2	1.0	2.1	0.008		236	300
44	0.4	166	176	6.3	2.7	8.6	3.7	1.2	2.8	0.012		437	209

[a]concentrations in µg WHO-TEQ$_{2005}$ kg^{-1} ww.

Table E.3: Overview of MMIF scores per sampling location (No. as indicated in Table D.1) between 2013 and 2019. Lines indicated in bold were used for analysis.

No.	date sampling	sampling year	MMIF
1	2014-06-18	2014	0.45
1	**2015-06-23**	**2015**	**0.35**
1	2016-06-27	2016	0.4
1	2017-07-18	2017	0.35
1	2018-06-26	2018	0.35
1	2019-07-22	2019	0.3
2	2014-06-20	2014	0.4
2	**2015-07-29**	**2015**	**0.5**
2	2016-07-20	2016	0.5
2	2017-08-31	2017	0.5
2	2018-07-06	2018	0.55
2	2019-07-26	2019	0.6
3	**2015-04-30**	**2015**	**0.45**
3	2017-04-06	2017	0.4
3	2020-08-06	2020	0.5
4	NA	2014	0.4
4	**NA**	**2015**	**0.4**
4	NA	2016	0.3
5	**2016-09-27**	**2016**	**0.3**
5	2019-07-10	2019	0.2
6	**2013-12-17**	**2013**	**0.4**
6	2018-12-10	2018	0.4
7	2014-10-09	2014	0.35
7	**2015-08-31**	**2015**	**0.35**
7	2016-10-28	2016	0.3
7	2017-10-05	2017	0.25
7	2018-07-20	2018	0.35
7	2019-08-28	2019	0.25
8	**2013-05-27**	**2013**	**0.25**
8	2018-10-17	2018	0.2
8	2019-07-04	2019	0.2
9	**2016-10-03**	**2016**	**0.75**
9	2019-10-18	2019	0.9
11	**2016-08-25**	**2016**	**0.55**
11	2020-08-06	2020	0.55
12	**2018-10-11**	**2018**	**0.75**
13	**2018-12-11**	**2018**	**0.15**
13	**2018-11-06**	**2018**	**0.1**
14	2013-11-06	2013	0.45
14	2014-06-06	2014	0.45
14	2015-09-30	2015	0.3
14	**2016-09-12**	**2016**	**0.4**
14	2017-09-26	2017	0.35
14	2018-07-18	2018	0.4
15	2014-06-26	2014	0.55
15	2015-06-24	2015	0.5
15	**2016-06-29**	**2016**	**0.35**
15	2017-07-24	2017	0.45
15	2018-06-28	2018	0.35
15	2019-07-25	2019	0.4
15	2019-11-20	2019	0.4
17	**2018-06-12**	**2018**	**0.15**
19	2014-04-30	2014	0.5
19	**2017-05-29**	**2017**	**0.45**
19	2020-05-07	2020	0.55
20	2014-04-30	2014	0.6
20	**2017-07-17**	**2017**	**0.7**
21	**2016-09-13**	**2016**	**0.8**
21	2018-10-23	2018	0.55
22	**2016-08-01**	**2016**	**0.7**
22	2018-07-10	2018	0.85
22	2020-10-07	2020	0.8
23	**2018-10-17**	**2018**	**0.65**
24	2015-08-19	2015	0.55
24	**2018-10-15**	**2018**	**0.6**
25	**2017-07-04**	**2017**	**0.25**
25	2019-05-16	2019	0.3
26	2014-07-03	2014	0.3
26	**2016-06-29**	**2016**	**0.35**
26	2019-08-20	2019	0.35
27	2014-10-16	2014	0.45
27	2015-08-27	2015	0.35
27	2016-09-27	2016	0.35
27	**2017-10-06**	**2017**	**0.3**
27	2018-08-29	2018	0.55

Table E.3 (continued)

27	2019-08-29	2019	0.4
28	**2016-06-28**	**2016**	**0.4**
28	2019-07-24	2019	0.35
29	2015-07-22	2015	0.6
29	**2017-03-29**	**2017**	**0.6**
30	**2018-08-01**	**2018**	**0.3**
31	**2016-05-30**	**2016**	**0.15**
31	2019-09-24	2019	0.25
32	2014-09-10	2014	0.5
32	2016-09-13	2016	0.5
32	2019-10-09	2019	0.5
33	**2016-10-05**	**2016**	**0.8**
33	2019-10-24	2019	0.8
35	**2017-09-20**	**2017**	**0.4**
36	2016-06-29	2016	0.3
36	**2019-08-19**	**2019**	**0.35**
37	2014-06-25	2014	0.45
37	**2017-09-01**	**2017**	**0.55**
38	2014-06-25	2014	0.4
38	2015-07-28	2015	0.35
38	2016-07-20	2016	0.35
38	2017-09-07	2017	0.4
38	**2018-07-09**	**2018**	**0.5**
38	2019-07-19	2019	0.5
39	**2018-08-08**	**2018**	**0.7**
40	2016-08-18	2016	0.35
40	**2019-10-16**	**2019**	**0.5**
41	2016-09-30	2016	0.75
41	**2019-09-06**	**2019**	**0.8**
42	2014-05-20	2014	0.8
42	**2017-05-31**	**2017**	**0.55**
42	2020-06-02	2020	0.6
43	**2016-05-23**	**2016**	**0.5**
44	2016-05-23	2016	0.3
44	**2018-06-11**	**2018**	**0.4**

*Table E.4: Threshold values (µg kg^{-1} ww) for PAHs based on the 95th percentile and 90th quantile regression approaches and significant regression models. For each compound, the European environmental quality standard for biota (EQS$_{biota}$) was given. Calculations were performed for zebra mussel (*D. polymorpha*), quagga mussel (*D. bugensis*) separately and for all* Dreissena *spec. individuals together as well as including* Corbicula fluminea *(mussels).*

Compound	95th percentile threshold value				EQS$_{biota}$
	D. polymorpha	***D. bugensis***	***Dreissena* spec.**	***mussels***	
Fluoranthene	46.8	27.0	46.1	46.1	**30**
Benzo(a)pyrene	6.06	4.57	5.98	5.98	**5**
	90th quantile regression model				
Fluoranthene	ns	y= -0.013[flu] + 0.997 (p=0.029)	ns	ns	/
Benzo(a)pyrene	ns	y=-0.037[benzo] + 0.877 (p=0.013)	y=-0.027[benzo] + 0.814 (p=0.004)	y=-0.027[benzo] + 0.814 (p=0.008)	/
	Threshold value based on regression model				
Fluoranthene	P=0.42	22.8	P=0.48	P=0.47	**30**
Benzo(a)pyrene	P=0.22	4.78	4.22	4.22	**5**

ns: no threshold value could be calculated because no significant ($p<0.05$) quantile regression model was found.

Table E.5: Results of the 90th quantile regression models for concentrations normalized for lipid content (or dry weight content for PFOS or Hg). In case of a significant model ($p < 0.05$) an equation was constructed.

Compound	90th quantile regression model	
	Perch	***eel***
PFOS	EQR = -0.009[PFOS] + 0.844 (p<0.001)	ns (p=0.81)
Hg	ns (p=0.79)	ns (p=0.74)
HBCD	ns (p=0.49)	ns (p=0.11)
Dioxins[a]	ns (p=0.79)	ns (p=0.49)
∑PBDE	ns (p=0.33)	ns (p=0.17)
∑PCB	ns (p=0.12)	EQR = -0.0006 [∑PCB] + 0.810 (p<0.001)

[a] concentrations in µg WHO-TEQ$_{2005}$ kg^{-1} ww. ns: the quantile regression model was not significant ($p > 0.05$).

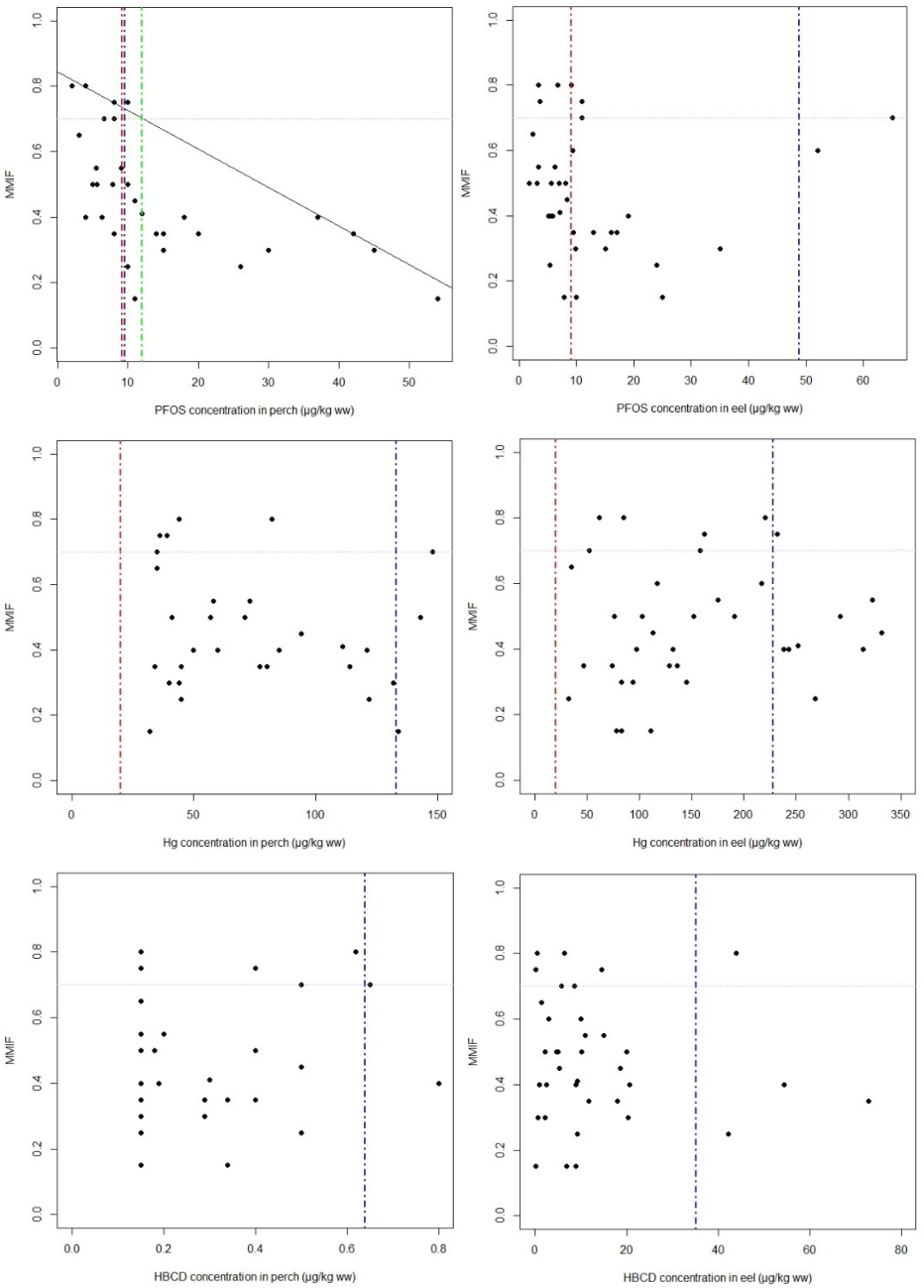

Figure E.1: Scatterplots of the relationship between accumulated concentrations of persistent compounds in fish and the ecological quality calculated as the MMIF. The blue line indicates the threshold concentration calculated with the 95th percentile. The green line indicates the threshold concentration based on the 90th quantile regression model. The red line indicates the current EQS$_{biota}$. The horizontal dotted line indicates an MMIF (EQR) value of 0.7, the threshold value for a good ecological quality. Regression lines were only indicated when the quantile regression model was significant.

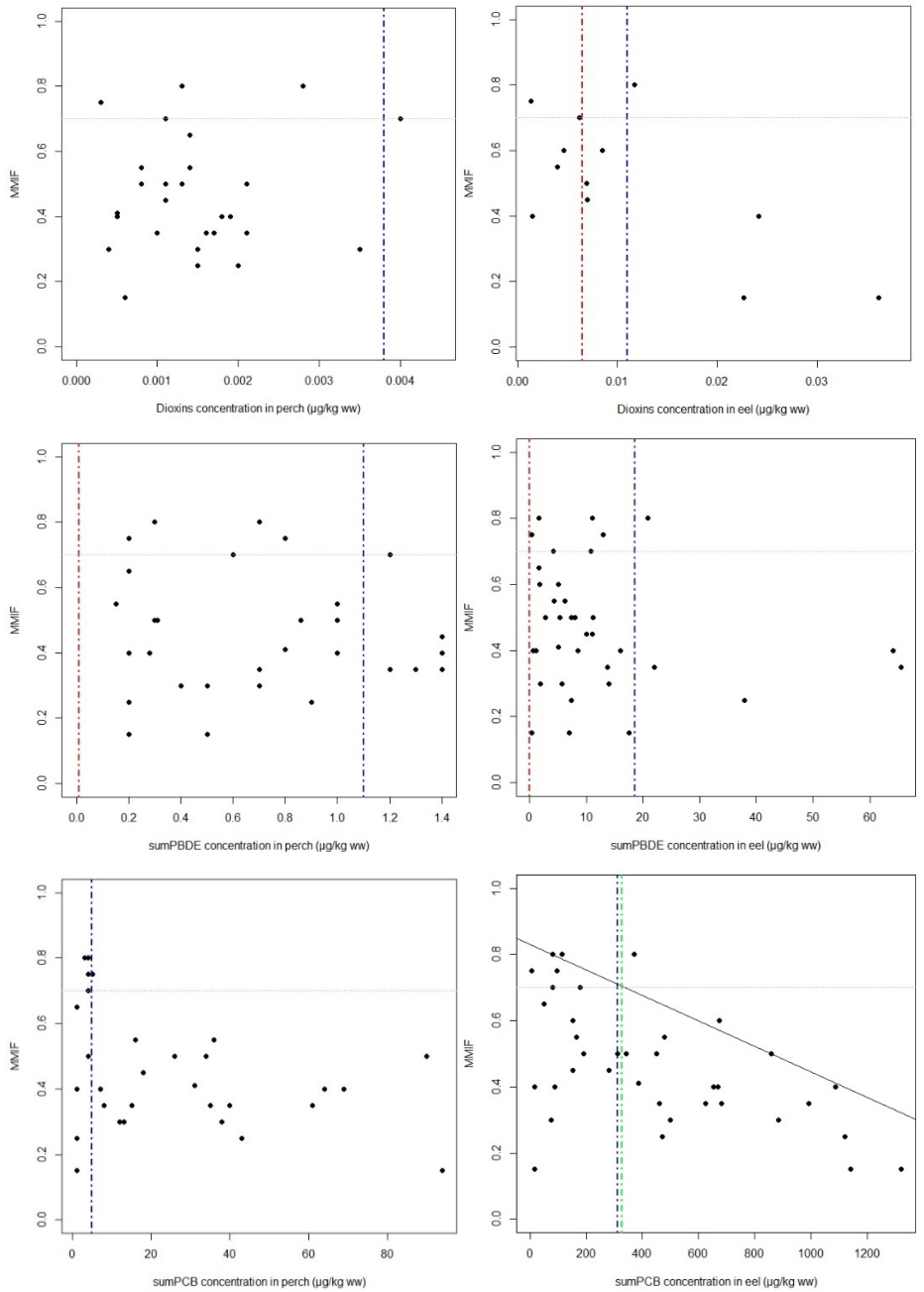

Figure E.1 (continued). Scatterplots of the relationship between accumulated concentrations of persistent priority compounds in fish and the ecological quality calculated as the MMIF. The blue line indicates the threshold concentration calculated with the 95th percentile The green line indicates the threshold concentration based on the 90th quantile regression model. The red line indicates the current EQS$_{biota}$. The horizontal dotted line indicates an MMIF (EQR) value of 0.7, the threshold value for a good ecological quality. Regression lines were only indicated when the quantile regression model was significant.

Curriculum Vitae

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2013-2015	Master of Science in Biology: Evolutionary and Behavioural Biology (Great Distinction) Thesis: "*Het belang van macro- en microklimaat op geprefereerde overwinteringsplaatsen voor vleermuizen in de fortengordel rond Antwerpen."*

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2010-2013	Bachelor of Science, Biology (Distinction) Thesis: "*Studie van enkele Rhabdocoela uit het noorden van Lanzarote.*"

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Conferences

5/10/2016	Poster Presentation 2nd Biology Research Day (Antwerp, Belgium): *"Biota quality standard for mercury: Global exceedance or too strict?"*
7/05/2017-11/05/2017	Student Volunteer SETAC Europe 27th Annual Meeting (Brussels, Belgium)
31/08/2017-2/09/2017	Poster Presentation 15th International Conference on Environmental Science And Technology (Rhodes, Greece): *"Mercury accumulation in two freshwater fish species in Flanders (Belgium). Internal distribution and effects of length, weight and sex."*
2/10/2017	Oral Presentation 3rd Biology Research Day (Antwerp, Belgium): *"Assessing the quality of Flemish waterbodies using Biota Quality Standards."*
13/5/2018-17/5/2018	Poster Presentation SETAC Europe 28th Annual Meeting (Rome, Italy): "*Effect of environmental characteristics on the bioavailability of hydrophobic organic compounds to fresh water organisms from natural aquatic systems.*"
29/10/2018	Poster Presentation 4th Biology Research Day (Antwerp, Belgium): "*Effect of environmental characteristics on the bioavailability of hydrophobic organic compounds to fresh water organisms from natural aquatic systems.*"

26/05/2019-30/5/2019	Poster Presentation SETAC Europe 29th Annual Meeting (Helsinki, Finland): "*Ecological relevance of the Biota Quality Standards."*
8/09/2019-13/09/2019	Poster Presentation 14th International Conference on Mercury as a Global Pollutant (Krakow, Poland): "*Relevance of active biomonitoring of mercury using freshwater bivalves.*"
29/11/2019	Poster Presentation 5th Biology Research Day (Antwerp, Belgium): "*Ecological relevance of the Biota Quality Standards."*