



Comparison of contaminant levels in liver/filet of large flounder (*P. flesus*) and in smaller whole-flounder

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Comparison of contaminant levels in liver/filet of large flounder (*P. flesus*) and in smaller whole-flounder

Vergelijking tussen contaminant gehalten in lever/filet van grote bot (*P. flesus*) en in kleine, hele vis

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Graphical abstract

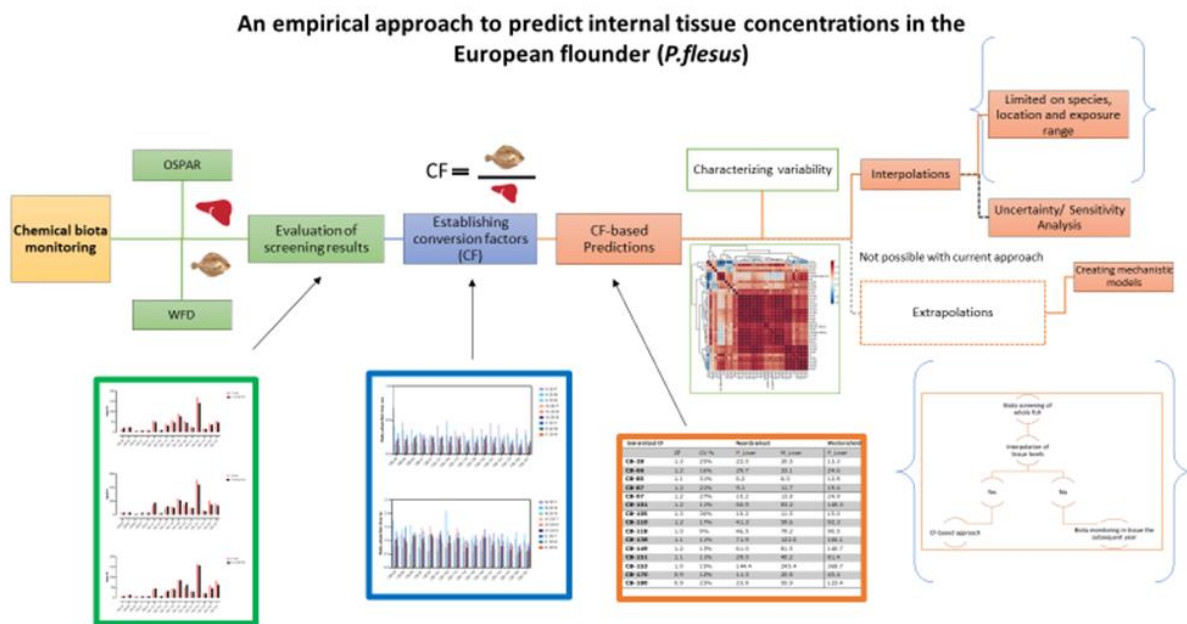


Figure 1 Graphical Abstract of the present project

Executive summary

Background:

The ultimate protection goal of regulatory directives applying fish monitoring is to prevent adverse effects posed by chemical pollution on human and animal health as such, and on the ecosystem health. Fish monitoring applies instrumental analysis to determine the internal concentration of well-known hazardous chemicals within organisms and provides the means to measure the risk posed by these environmental contaminants that bioaccumulate in species.

In the Netherlands, a whole suite of prioritized organic and inorganic compounds are screened in the flatfish species flounder (*P. flesus*) from three locations in Dutch coastal and estuarine waters, commissioned by Rijkswaterstaat (RWS). To meet the protection goals, tissue (liver and muscle (only for mercury)) and whole-fish samples of flounder are simultaneously collected under the umbrella of two different directives, the OSPAR and WFD, respectively. Due to the non-harmonized sample strategies within fish monitoring, the biological matrices (whole body vs. tissue samples) as well as body weight, size and sex differ. These non-harmonized standards result in higher cost and time intensive monitoring and data processing as well as the use of a larger amount of fish, which hampers the common ethical goal of reducing, replacing, or refining (3 R's) of animal use. Therefore, RWS raised the question whether concentrations of contaminants in the two sample types (whole body and tissue samples) are correlated, and if so, can one be predicted from data of the other sample type.

Study goal:

The goal of the present study was to understand how chemical levels detected in tissue (liver or fillet) and whole-fish relate to one-other by creating conversion factors (CF) (Part 1), and whether the analysis of only whole-fish is sufficient to predict levels in tissues found in the flounder by applying CF (Part 2)

Results and discussions:

This reports shows an in-depth look for differences and parallels between levels of contaminants (organic compounds such as PCBs, PBDEs, PFOS, OCPs and the metals mercury, lead, zinc, copper and cadmium) in livers, fillets of flounder (females, body size 15-30 cm), and those in whole-fish of flounder (non-sexed, body size 15-20 cm), sampled at the same location (three locations; Noordzeekust, Westerschelde and Eems-Dollard) and time (in 2017, 2018, and 2019).

- We compared first the differences and parallels of average chemical concentrations detected in tissue or whole-fish on wet weight and lipid weight or on a dry weight basis per location. Since PCBs (polychlorinated biphenyls) are stored preferably in lipids, the ratio of distribution between the whole body and tissues if given in wet weight represents the differences in the distribution of lipids in these two matrices. Therefore, as the effect of data normalization on lipid weight is evident, the average concentrations become comparable between tissue and whole-body burden for PCBs. Though limited data was available due to very low concentrations, the same effects were observed for two BDE's (brominated diphenyl ether, brominated flame retardants) within the two matrices.

For metals, the differences between concentrations in tissue/whole-fish differ for each metal. The normalization on dry weight mainly increases the concentration levels. Similar to metals, the dry weight normalization did not affect the margin of differences between liver and whole-fish samples for PFAS.

- We then calculated the whole-body-to-tissue ratios for each location and year based on average concentrations to better understand the effect of data normalization and derive possible conversion factors. As the behaviour of all PCB congeners was similar on lipid weight, a ratio of almost one was observed in all locations (1.02). The margin of observed variability for this ratio is comparable for the wet weight and lipid weight results. The studied BDE's (with concentrations above quantification limits) act similarly to PCBs. For BDE100, however, with levels very close to quantification limits, the ratios are showing high variability.

For metals, as the distribution over body parts is different for the metals studied, the ratios of whole-fish / liver (or filet in case of mercury) are calculated individually. Though a large variation of ratio's determined over the years and across the sampling locations was observed, there is consistency in the ratio for each metal.

- These calculated ratios create the baseline for predictive conversion factors from whole-fish to tissue (liver/fillet). To test and validate the predictive power of these conversion factors, we based the CFs on data from 2017-2018 and used these CFs to predict, from the levels in whole-fish of 2019, the level of contaminants in fish tissues caught in 2019 over three distinct locations. The results show the

applicability of a conversion factor for all chemicals in future studies, with a margin of a factor 3 to take into account the intraspecies variability. However, some drawbacks of an empirical approach as such presented within this project and its limitations need to be considered. Such empirical approaches do not count for any mechanistic relationship of the biology of species as well as properties of chemicals. This, in turn, hampers the way of understanding which parameters/factors influence the accumulation (uptake, distribution, metabolism, and elimination) of chemicals within organisms, hence, influence the predictive power of mathematical tools.

Conclusions:

The main goal of this approach was to investigate the possibility to replace the analytical screening efforts of the OSPAR directive for liver tissue with estimations of liver concentrations based on whole body burden (whole-fish) determined for WFD. The predictive conversion factors can be applied to estimate the liver/fillet concentrations based on whole body burden of chemicals (while considering a safety factor of minimum 3 to take into account characterized intraspecies and spatial variability). Thus with limited predictive power, interpolations are possible but extrapolations (to other contaminants, fishes etc) cannot be done without more advanced modelling approaches

Data presented within this study is the first step towards superseding the need for monitoring different matrices (e.g., whole-fish and liver). To date, no predictive approaches are applied within biota monitoring. How to deal with the uncertainties as well as implication of such CF needs to be considered and discussed with experts.

Uitgebreide samenvatting

Achtergrond:

Het uiteindelijke doel van vis monitoring is om negatieve effecten van chemische vervuiling op de gezondheid van mens en dier, en daarmee ook het milieu, te voorkomen. Het monitoren van vis, waarbij de interne concentraties van schadelijke contaminanten analytisch worden bepaald, is noodzakelijk om de risico's van deze contaminanten, die bioaccumuleren, te bepalen.

In Nederland wordt een breed scala aan contaminanten, zowel organische als anorganische, gemeten in de platvis Bot (*P. flesus*). Dit vindt plaats in drie locaties, kust zone en estuarium, op verzoek van RWS. Om de gehalten met de normen te vergelijken worden zowel filet en lever als in hele vis geanalyseerd voor twee verschillende regelingen, De OSPAR en de kader richtlijn water. De monsternamen zijn niet geharmoniseerd, naast het verschil in matrix (filet, lever en hele vis) is er ook verschil in grootte en geslacht van de bemonsterde vissen. Dit kost dus meer inspanning en draagt niet bij aan de gewenste beperking van dierproeven (de drie R's: reducing, replacing, of refining).

RWS heeft daarom de vraag gesteld of de gehalten van contaminanten in deze verschillende monstertypes gecorreleerd zijn, en zo ja, of door het meten van de ene matrix de gehalten in de andere matrix kan worden voorspeld.

Doel:

Het doel van deze studie was om te doorgronden of de gehalten van contaminanten in weefsels (lever, filet) gerelateerd zijn aan de gehalten in de hele vis en of een conversiefactor kan worden bepaald (deel I). Met die conversiefactor moet dan met de analyseresultaten van alleen hele vis ook de gehalten in lever en filet worden geschat (deel II).

Resultaten en discussie:

Dit rapport laat in detail zien waar de verschillen en parallellen zijn tussen de gehalten in lever en filet van bot; vrouwen, 15-30 cm en de gehalten in hele vis; geslacht onbekend, 15-30 cm lengte, bemonsterd op dezelfde locaties (drie locaties Noordzeekust, Westerschelde en Eems-Dollard) en tijd (in 2017, 2018, and 2019). Vijf gepoolde monsters (bestaande uit vijf vissen elk) werden onderzocht per matrix (en per locatie, per jaar).

We hebben eerst de concentraties van contaminanten in weefsels in hele vis vergeleken, op natgewicht basis en verrekend naar hoeveelheid vet. Omdat PCBs bij voorkeur in vet zijn opgeslagen geeft de vergelijking van gehalten op natgewicht meer de verschillen in vetgehalten tussen de weefsels aan (levers zijn vetter dan andere weefsels). Het grote effect van normalisatie op vet was daarom verwacht; de gehalten PCBs werden vergelijkbaar tussen de weefsel en de hele vis. Ofschoon er maar weinig data was voor de BDEs, werd hetzelfde effect waargenomen voor twee verschillende BDEs.

Voor metalen zijn de verschillen tussen gehalten in weefsels en hele vis per metaal anders. Normalisatie op drooggewicht zorgt alleen voor hogere gehalten, de verschillen blijven. Ook bij PFOS, een organische stof die geen voorkeur heeft voor vet, werden de verschillen tussen de weefsels niet anders door normalisatie op droog gewicht (of vetgewicht).

Hierna zijn de hele-vis/ weefsel ratio's berekend voor elk jaar en elke locatie, gebaseerd op gemiddelde concentraties van de 5 monsters per locatie per jaar om het effect van data normalisatie te zien en om mogelijke conversie factoren (hele-vis naar weefsel) te bepalen. Het gedrag van alle PCBs is sterk vergelijkbaar, een ratio van 1 (gemiddeld 1.02) is op alle locaties geconstateerd. De mate van variabiliteit van deze ratio is vergelijkbaar voor de natgewicht en vetgewicht data. De BDEs (met gehalten boven de bepalingsgrens) vertonen hetzelfde gedrag als de PCBs. De variabiliteit van de ratio's van BDE100 was erg groot, de gemeten gehalten lagen erg dicht bij de bepalingsgrens.

Bij de metalen is de verdeling over weefsels van de vis verschillend per metaal. De ratio's hele-vis/weefsel zijn daarom voor elk metaal apart berekend. Er is een grote variatie in de ratio's geconstateerd, over de jaren en over de locaties, deze waarden zijn wel eenduidig voor elk metaal (gehalten altijd hoger in de ene matrix dan in de andere).

De berekende ratio's vormen de basis voor de conversiefactoren (CFs), die uit de gehalten van de hele-vis de gehalten in weefsels (lever, filet) voorspellen. Om de voorspellende waarde van deze CFs te testen en te valideren, baseerden we de CFs op data uit 2017 en 2018. Deze CFs werden dan gebruikt, met de gemeten waarden van hele-vis in 2019, om de waarden in weefsels in 2019 op de drie locaties te voorspellen en te vergelijken met de echt gemeten gehalten. Het resultaat toont dat de conversiefactor toegepast kan worden, maar in het voorspelde gehalte heeft een fouten marge van

een factor 3 vanwege de intra-species variabiliteit. De empirische aanpak in dit project heeft beperkingen en deze verdienen aandacht. De mechanistische relatie van de contaminanten met de biologie van de vis, noch de specifieke eigenschappen van de contaminanten worden nemeegenomen. Hierdoor wordt niet duidelijk welke parameters, factoren de accumulatie van contaminanten in vis beïnvloeden (opname, verdeling over weefsels, afbraak en uitscheiding) en hiermee de voorspellende kracht van de mathematische tools.

Conclusies

Het hoofddoel van dit project was te onderzoeken of de gehalten in lever en filet die voor de OSPAR moeten worden gemeten kunnen worden geschat met de metingen in hele-vis voor de KRW. De voorspellende CFs kunnen inderdaad worden toegepast om de gehalten in lever en filet te schatten uit de gehalten in hele-vis. De juistheid van deze voorspelde waarde is niet groot (een marge van factor 3 vanwege de intra species and spatiele variabiliteit). Interpolatie is mogelijk, maar extrapolatie (naar andere vissen, stoffen en gehalten) is niet mogelijk zonder gebruik van geavanceerde modellen.

De data in dit rapport zijn een eerste stap naar een nieuwe aanpak van monitoring wat verschillende matrices betreft (hele-vis, lever, filet). Tot nu toe worden voorspellende aanpakken nog niet toegepast in biota-monitoring. Hoe de onzekerheden, als ook de toepassing van de CFs moeten worden aangepakt zal in een brede groep met experts overwogen en besproken moeten worden.

1 Introduction

Man-made release and enrichment of chemicals in marine ecosystems is a global phenomenon. Life-time accumulation of even trace levels of chemical pollutants in biota can cause immunosuppression, reduced fertility, and permanent genetic changes, all of which can lead to an increased vulnerability of organisms, populations, and ecosystems¹. Therefore, biota monitoring is an inevitable approach to regulate the risk posed by aquatic pollution. The ultimate protection goal of regulatory directives applying fish monitoring is to prevent adverse effects posed by aquatic chemical pollution on human and animal health as such, and on the ecosystem health²⁻⁴. Therefore, bioaccumulation of environmental contaminants in species and biomagnification throughout the food web needs to be carefully monitored⁵.

Due to their aquatic habitat, fish species are particularly vulnerable through direct or secondary poisoning. Two main exposure pathways of contaminants are considered in fish species; 1) directly from the water through the gills, and 2) indirectly by feeding on small fish, invertebrates, and/or aquatic vegetation^{4,5}. Humans, in turn, are exposed to contaminants via consumption of some portion of muscle in fish (= fillet). Generally, lipophilic contaminants pre-dominantly bioaccumulate in metabolic active and fatty tissue such as the liver (e.g., PCBs)⁶. Inorganic compounds preferably accumulate in liver (e.g., Cd) and/or protein-rich tissues such as fillet (e.g., mercury)⁷. Adverse health effects become apparent if chemical and species specific toxicity thresholds are exceeded in the specific tissue or whole body⁸. Lipophilic compounds can trigger more specific biological perturbations (e.g., receptor mediated pathways) than generic toxicity triggered by metals (e.g., cytotoxicity or oxidative stress)^{3,7,9}.

The European flounder, *Platichthys flesus* (Linnaeus 1758), is an ecologically important flatfish species omnipresent in coastal and estuarine waters throughout Western Europe¹⁰. By occupying the benthic position in the food web, *P. flesus* is an ideal bioindicator species to monitor spatial and temporal trends of chemical pollutants in coastal and estuarine environments, and an early warning species for biomagnification of chemicals throughout the aquatic food web¹¹. However, the selection of ideal species and sample matrix for biota monitoring in fish is a widely discussed and controversial topic, which is mirrored in discrepancies in sample strategies, and is investigated throughout the world¹². Hence, long term fish monitoring results are often not comparable due to the use of different species as well as matrices¹².

In the Netherlands, a whole suite of prioritized organic and inorganic compounds are screened in *P. flesus* from three locations in Dutch coastal and estuarine waters, commissioned by Rijkswaterstaat (RWS). Many of the analysed priority compounds are well known to bioaccumulate with age and through the food web. To meet the protection goals, tissue (liver and muscle(only for mercury)) and whole-fish samples of *P. flesus* are simultaneously collected under the umbrella of two different directives, the OSPAR and WFD, respectively. Due to the non-harmonized sample strategies within fish monitoring, the biological matrices (whole body vs. tissue samples) as well as body weight and body size differ. Such non-harmonized standards can result in unnecessary cost and time intensive monitoring and data processing as well as the use of a large amount of fish, which hampers the common ethical goal of reducing, replacing, or refining (3 R's) of whole animal use in (environmental) toxicology¹³.

Therefore, RWS raised the question whether concentrations in two sample types (whole body and tissue samples) are correlated, and therefore can be predicted from data of only one matrix. For this, RWS has requested an in-depth look for differences and parallels between levels of contaminants in livers of *P. flesus* (adult age group, females, body size ranging 15-30 cm), and those in whole-fish of *P. flesus* (non-sexed sub-adults age group, size 15-20 cm), sampled at the same location and time.

2 Materials and Methods

2.1 Sampling

Over 3 years (2017-2019), the European flounder (*P. flesus*) was sampled annually at 3 locations in coastal and estuarine waters in the Netherlands. Figure 1 shows the three sampling sites as the focus of investigation: Noordzeekust (N), Westerschelde (W) and Eems-Dollard (E).

For OSPAR, a total of 50 female *P. flesus* were caught for each year and sampling site (n=450 in total) with body size ranging from 15-30 cm of length. Per location and year, the liver samples of all animals and fillet samples of 25 animals were screened. The organic compounds were analysed in livers of 25 fish, pooled in 5 samples, each pool consisting of livers from 5 animals. The inorganic analyses (Cd, Cu, Pb, Zn) were carried out on the livers of the other 25 fish, also pooled in 5 samples (5 animals per pool). In the fillet of the same 25 animals, mercury (Hg) was analysed by pooling the same 5 animals per sample.

For the WFD, another 25 animals of shorter length (15-20cm) and undefined sex, simultaneously caught at the same location each year, were used as whole-fish to prepare 5 samples of 5 pooled fish. Those 5 samples undergo a whole body burden screening for all inorganic and organic compounds analysed conform OSPAR requirements.

The main differences between both sampling strategies (OSPAR and WFD) were 1) body size of the animals 2) all-female samples versus non-defined sex and 3) sample type (liver or fillet vs. whole body)



Figure 2 Locations of the three study sites (Noordzeekust, Westerschelde, and Eems-Dollard) in which *P. flesus* were annually sampled (2017-2019).

2.2 Statistics

D'Agostino-Pearson test confirmed the normal distribution of data. A two-way ANOVA was run using GraphPad™ (Version 8.2.1; San Diego, CA, USA) for statistical differences. Pearson's correlation coefficient was calculated to understand the impact of variables on the concentration levels measured in fish from different locations. The required level of statistical significance was set at $p < 0.05$. Linear regressions were generated using GraphPad™ (Version 8.2.1; San Diego, CA, USA).

2.3 Analysis

The analysis of the flounder samples was performed routinely as described in the report Biomonitoring 2019-Deel II. To compare both sample types, the same analytical methods were used. For economic reasons HBCD was not analyzed in the small whole-fish. This enabled the analysis of BDE's together with the PCB's, instead of a separate analysis for PCB's and one for BDE's plus HBCD.

3 Project approach

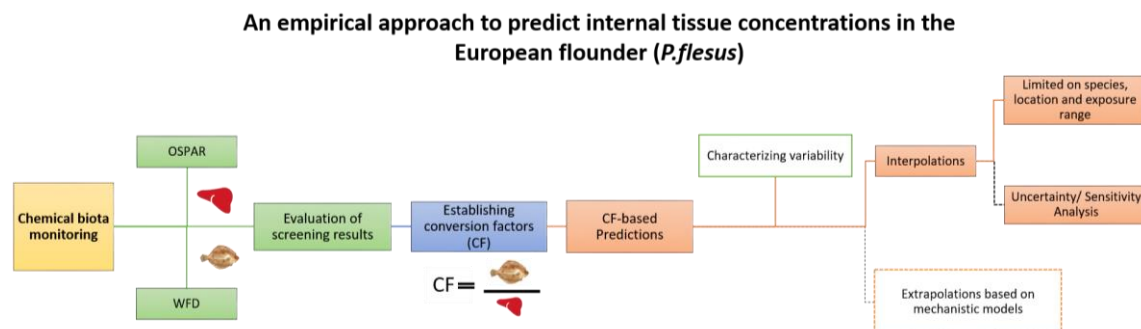


Figure 3.1 The conceptual framework of the empirical study approach to predict internal tissue concentration in the European flounder (*P. flesus*)

The goal of the study is to understand how chemical levels detected in tissue (liver or fillet) and whole-fish relate to one another by creating conversion factors (CF) (Part 1), and whether the analysis of one whole-fish is sufficient to predict levels in tissues found in the *P. flesus* by applying CF (Part 2) (Figure 3.1).

- Firstly, ('Evaluation of screening results', Figure 3.1) we compare the differences and parallels of average chemical concentrations detected in tissue or whole-fish. Organic compounds were compared on wet versus lipid weight and metals on wet versus dry weight basis per location (Chapter 4.1–4.3).
-
- Secondly, ('Establishing conversion factors', Figure 3.1) to better understand the effect of data normalization and derive possible conversion factors, we calculate the whole-body-to-tissue ratios for each location and year based on average concentrations detected. These calculated ratios based on chemical screening, in turn, create the baseline for predictive conversion factors from whole-fish to tissue (liver/fillet) (Chapter 4.4).
- Thirdly, in Chapter X ('CF-based predictions', Figure 3.1) we applied the conversion factors to predict concentrations in liver tissue for organic and inorganic chemicals, and in fillet for mercury, to test the goal of the study. Next to this, the variability within the data set is characterised as well as the uncertainties in regards to assessment of environmental quality standards are addressed (Chapter 5.1-5.2).

Lastly, we finish the report with conclusions and future recommendations (Chapter 6 and 7), and focus on how to overcome uncertainties within predictive approaches. We explain the difference between empirical approaches, which allows us to interpolate to exposure scenarios we based our CF factors on, and mechanistic approaches to extrapolate to exposure scenarios outside of concentration ranges the CF factors are based on. We specifically clarify the limitations of our approach and propose future research efforts to continue to implement this empirical predictions in real biota monitoring efforts.

4 Results and Discussion (Part 1)

The results of the data are reported with a decimal point (.) instead of a comma (,) (in derogation of the Dutch SI). The analytical results stated in this report, and the conclusions based on those, only apply to the samples as they have been received and analysed. Raw data of all samples within this project can be found in the Appendix (Chapter 11) Tables 1 to Table 6.

4.1 Average PCB levels in liver and whole-fish

Polychlorinated biphenyls (PCBs) are omnipresent in our environment, posing a serious health threat to aquatic and humans life. Although PCBs have been banned in Western countries, their persistent, bioaccumulative and toxic properties cause an everlasting concern. Within chemical monitoring, the non-planar congeners number 28, 52, 101, 138, 153, and 180 are commonly selected as the focus of investigation^{3, 9}. Dioxin-like activity through the AhR-mediated pathway has been shown for twelve PCBs, namely the non-ortho CBs number 77, 81, 126, and 169, as well as the mono-ortho CBs number 105, 114, 118, 123, 156, 157, 167 and 189. Generally, an increase in chlorination grade is concomitant with an increase in lipophilicity and, to a lesser extent, toxicity¹⁴. Generally, different tissues can serve as a surrogate for bioaccumulation of specific contaminants in the body. For example, PCBs are preferably accumulated and stored in body lipids, hence, in fatty tissue such as the liver¹⁶.

PCBs that are detected in all three sample locations over the three consecutive sampling years (2017-2019) are presented. The average of 15 samples (5 per year) and the concentrations of 16 PCB compounds (or congeners) in the liver tissue (OSPAR) and whole-fish (WFD) for *P. flesus* caught in three different study locations are given in Figure 4.1 on a wet weight basis. Figure 4.2 illustrates the same results on a lipid weight basis. The PCB concentrations are highly different between tissue and whole-body burden if compared on a wet weight basis by showing a large difference in absolute concentrations. Such results are shown in previous studies conducted on the same topic in other species and locations in Europe^{2, 8, 12, 15-18}. Since PCBs are stored in lipids the ratio of distribution between the whole body and tissues if given in wet weight represents the differences in the distribution of lipids in these two matrices.

However, the effect of data normalization on lipid weight is evident in Figure 4.2, where the average concentrations become comparable between tissue and whole-body burden for PCBs. The levels of small PCBs (low chlorinated, and therefore relatively low molecular weight) in whole-fish are similar or even slightly higher than in the liver of *P. flesus*, which corresponds to a chemical equilibrium state. This equilibrium is not expected nor seen for the large PCBs (highly chlorinated, high molecular weight PCBs). The levels of these PCBs are higher in liver of the larger and older flounder. These first results indicate that the PCBs in the liver normalized on a lipid weight basis in large fish can be applied as a predictive mean if lipid content in the whole small fish is co-screened. Similar observations have been done for selected compounds, however, the present project represents the most comprehensive analysis of such comparison, and can serve as a basis for other studies and directives^{12, 15, 16}. Overall, the PCB concentrations will be given in wet weight as well as lipid weight as data normalization makes these values comparable.

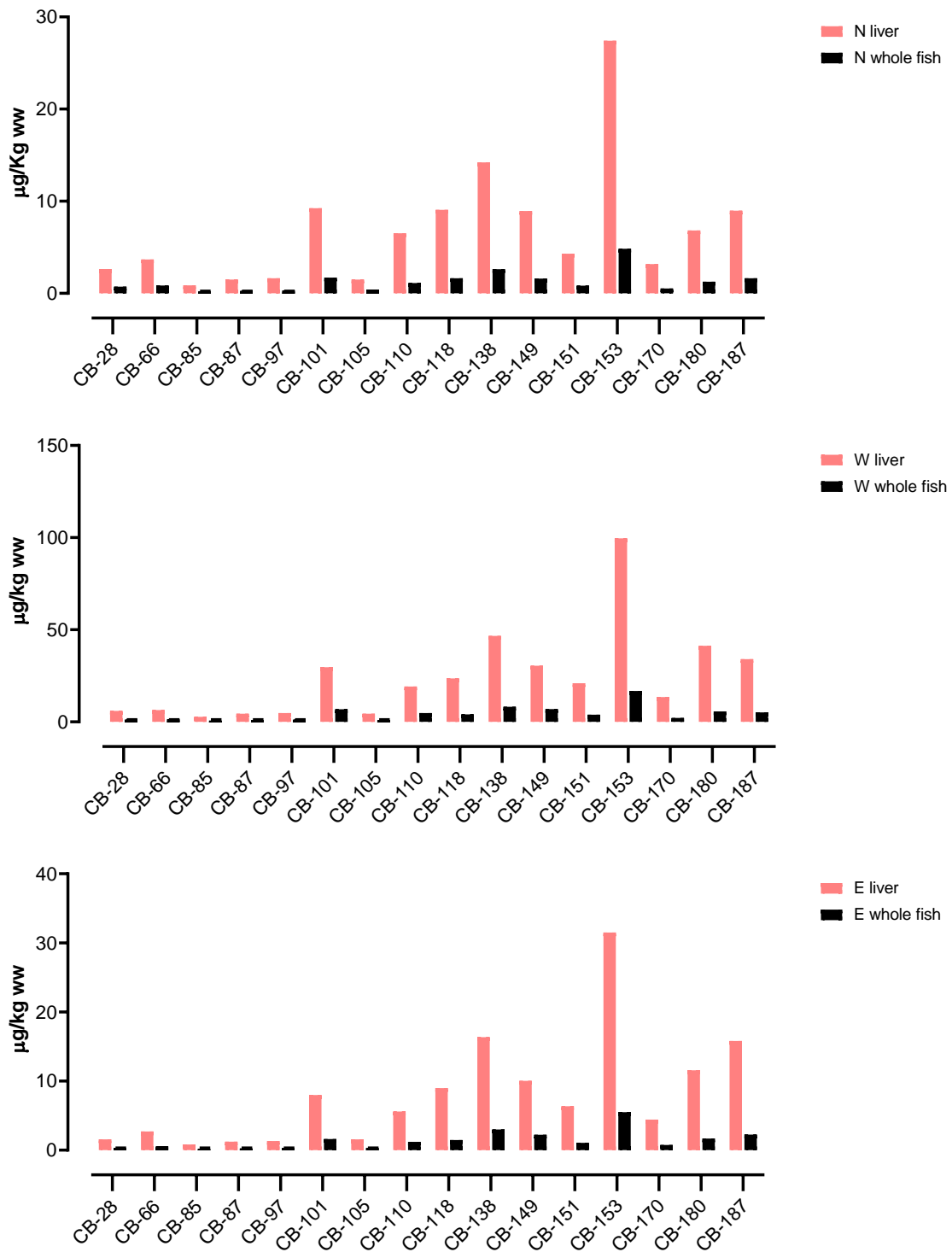


Figure 4 Mean concentrations of PCBs µg/kg wet weight (ww) detected in the liver (red) and whole-fish (black) of *P. flesus* sampled annually over three years (2017-2019) at three locations in Dutch waters (Noordzeekust (N), Westerschelde (W), Eems-Dollard (E)).

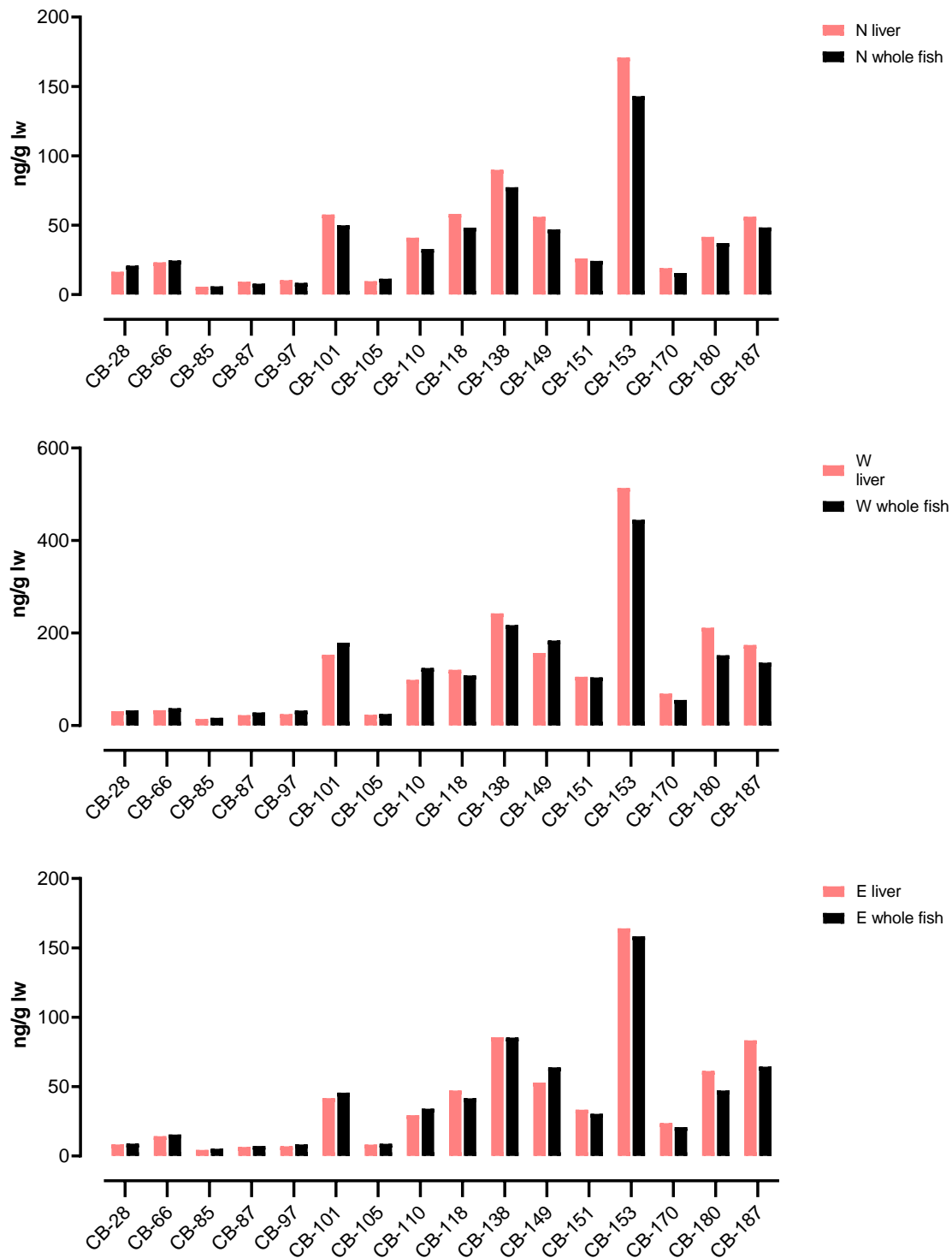


Figure 4.2 Mean concentrations of PCBs ng/g lipid weight (lw) detected in the liver (red) and whole-fish (black) of *P. flesus* sampled annually over three years (2017-2019) at three locations in Dutch waters (Noordzeekust (N), Westerschelde (W), Eems-Dollard (E)).

4.2 Average non-essential and essential inorganic levels in the liver, filet (only mercury), and whole-fish

The chemical properties of the majority of inorganic elements allow that they are readily absorbed by living organisms. Once in the body, they have been found to attach to cellular components, such as enzymes, or structural proteins, and influence their functionality⁵. When the concentrations of these substances reach a toxic threshold, they can trigger responses from the organism, causing adverse health effects^{4, 5, 7, 14}.

Metallic elements can be divided into those which have an unknown biological function (mercury (Hg), cadmium (Cd), and lead (Pb)), and those compounds with a known biological role (copper (Cu) and zinc (Zn)). Therefore, Hg, Cd, Pb toxicity raises with increasing tissue and body burden¹⁴. In contrast, Zn and Cu toxicity are triggered either due to increasing concentrations or due to concentration deficiencies^{4, 7}. In the case of mercury, fillet samples of *P.flesus* are analysed as a commonly used sample matrix because mercury concentrates within protein-rich tissues carrying functional sulfhydryl group. For Cd, for example, the liver is the first target tissue for accumulation and detoxification via reversible protein binding, and therefore, the tissue of interest ^{4, 8, 12, 14}.

The average of 15 samples (5 per year) and the concentrations of three non-essential and two essential metals are given on a wet weight basis and dry weight basis in Figures 4.3-4.4 and 4.5-4.6, respectively. In contrast to lipophilic compounds, metal data normalization based on dry weight is not evident as the effect of lipid normalization for PCBs, by which, the concentrations become comparable. The normalization on dry weight mainly increases the concentration levels, however, the difference between tissue/whole-fish remains high. Due to the fact that the threshold values are in wet weight, the metal conversion factors and predictions will be given only in wet weight.

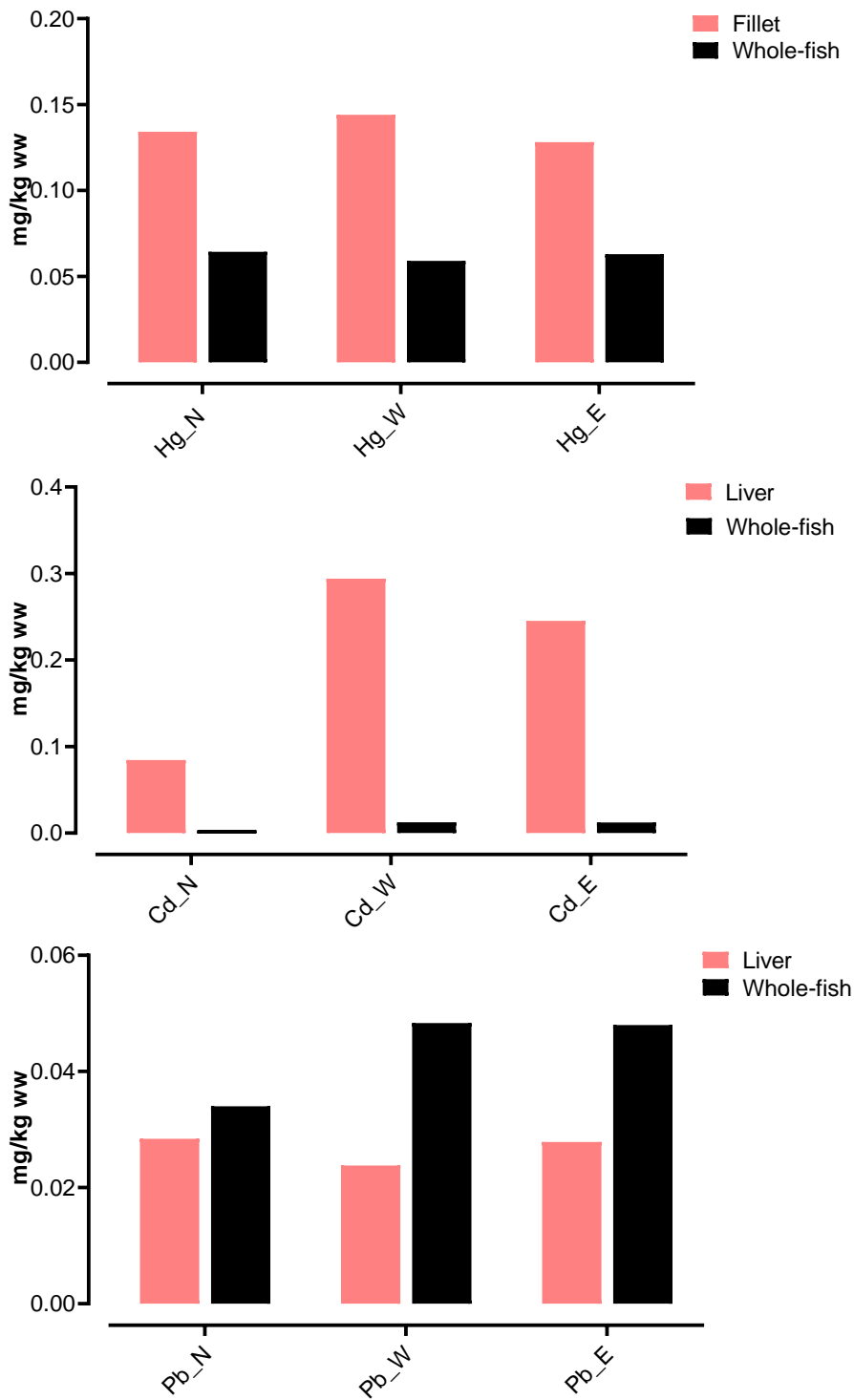


Figure 4.3 Mean metal concentrations with non-essential mercury (Hg), cadmium (Cd), and lead (Pb) given in wet weight (ww) detected in the liver (red) or fillet (only for mercury) and whole-fish (black) of *P. flesus* sampled annually over three years (2017-2019) at three locations in Dutch waters (Noordzeekust (N), Westerschelde (W), Eems-Dollard (E)).

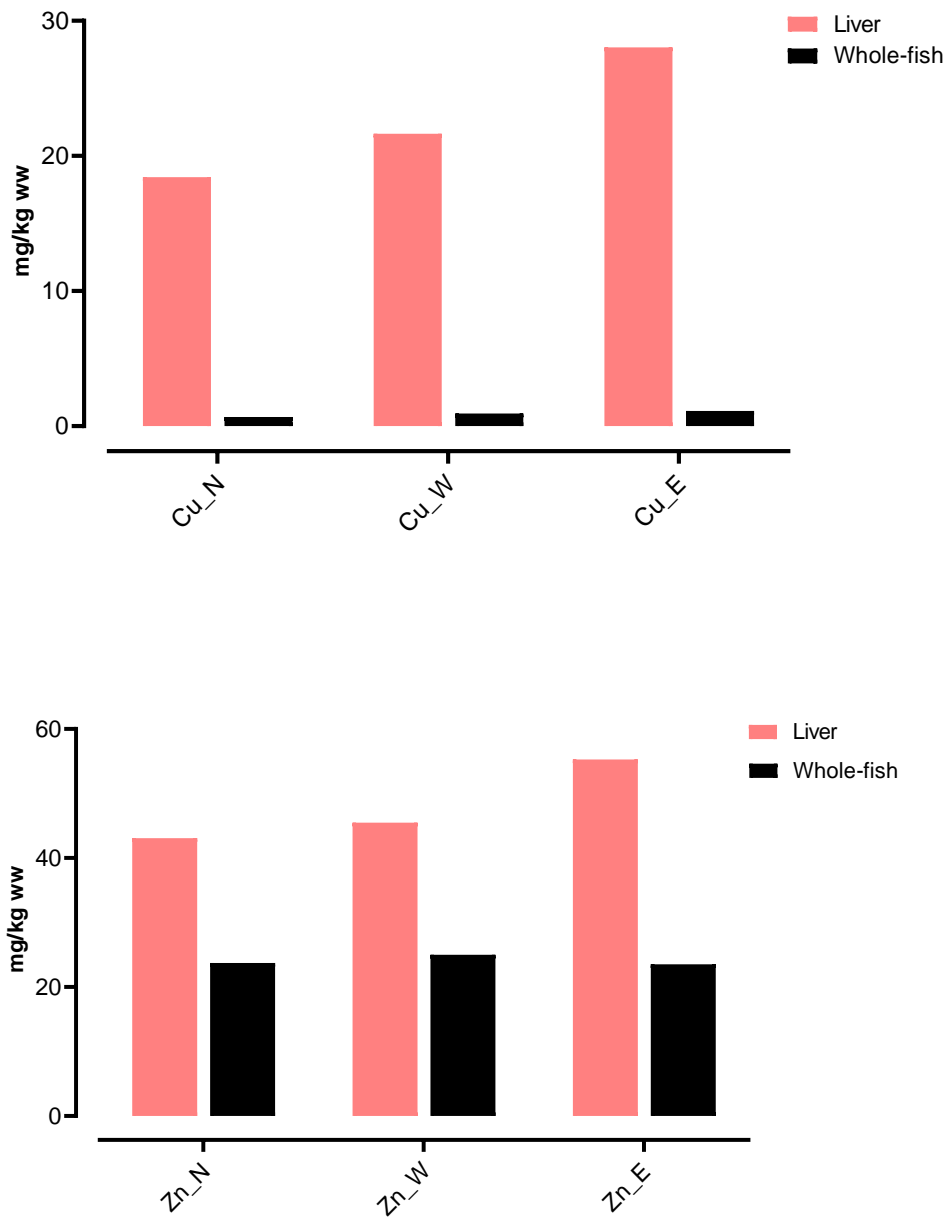


Figure 4.4 Mean metal concentrations with essential biological function copper (Cu) and zinc (Zn) given in wet weight (ww) detected in the liver (red) and whole-fish (black) of *P. flesus* sampled annually over three years (2017-2019) at three locations in Dutch waters (Noordzeekust (N), Westerschelde (W), Eems-Dollard (E)).

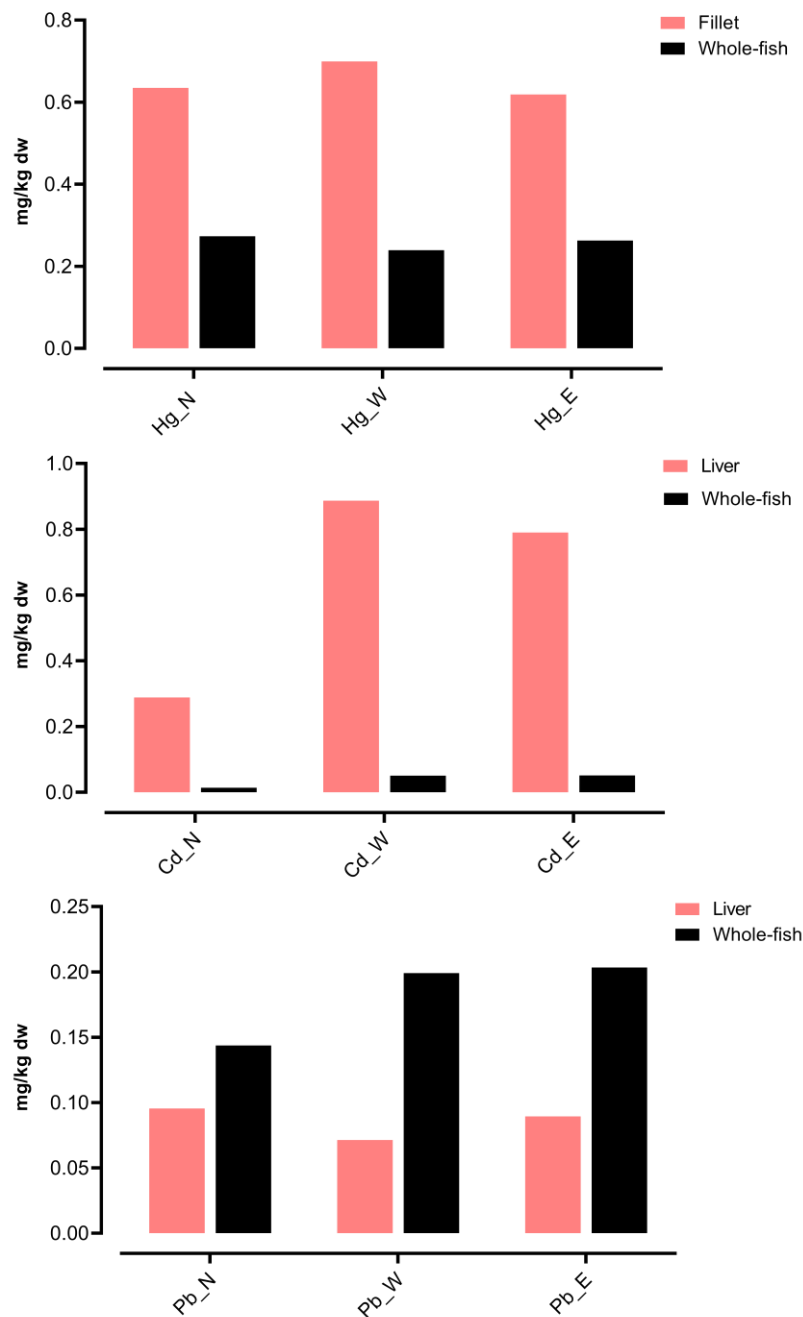


Figure 4.5 Mean metal concentrations with non-essential mercury (Hg), cadmium (Cd), and lead (Pb) given in dry weight (dw) detected in the liver (red) or fillet (only for mercury) and whole-fish (black) of *P. flesus* sampled annually over three years (2017-2019) at three locations in Dutch waters (Noordzeekust (N), Westerschelde (W), Eems-Dollard (E)).

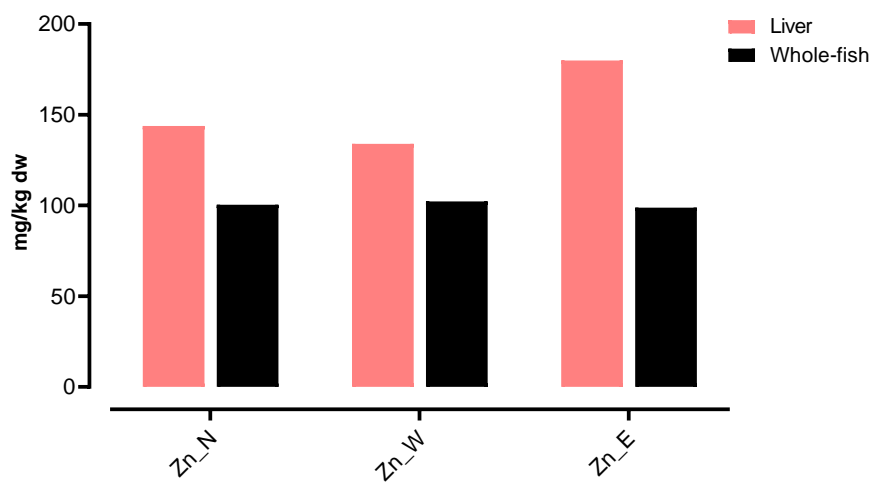
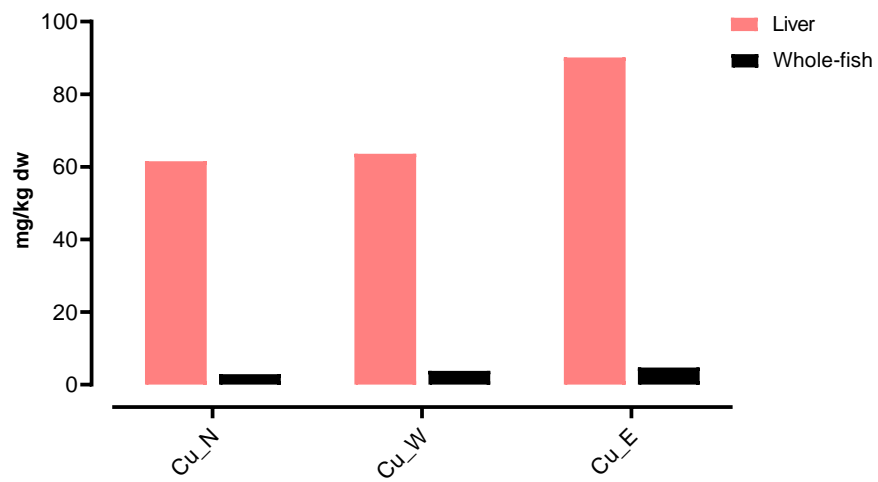


Figure 4.6 Mean metal concentrations with essential biological function copper (Cu) and zinc (Zn) given in dry weight (dw) detected in the liver (red) and whole-fish (black) of *P. flesus* sampled annually over three years (2017-2019) at three locations in Dutch waters (Noordzeekust (N), Westerschelde (W), Eems-Dollard (E)).

4.3 Average PFOS and BDE 47, BDE 100 in the liver and whole-fish

Concentrations of brominated flame retardants (HCB and PBDEs), perfluoroalkylated substances (PFAS), and organochlorine pesticides, were often not quantified (below limit of quantification). This occurred predominantly at the study site 'Noordzeekust' and in lesser extent at 'Eems-Dollard'. The few compounds (BDE 47, BDE 100, and PFAS), for which relationships could be established are discussed below.

Similar to metals, the dry weight normalization did not affect the margin of differences between liver and whole-fish samples for PFAS (Figure. 4.7). Therefore, PFAS will be given only in wet weight within the next steps of the discussion. Similar to PCBs, the normalization of the two BDE's on lipid weight affects the levels within the two matrices, the concentrations become comparable.

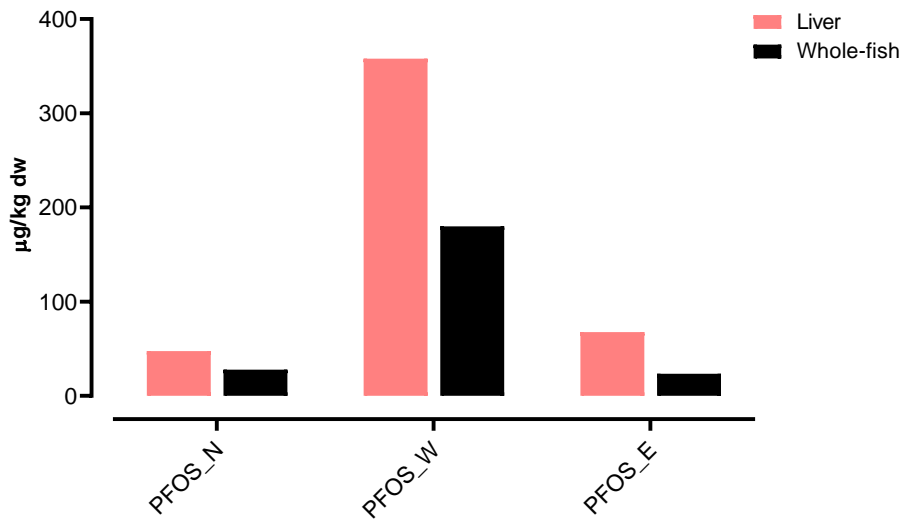
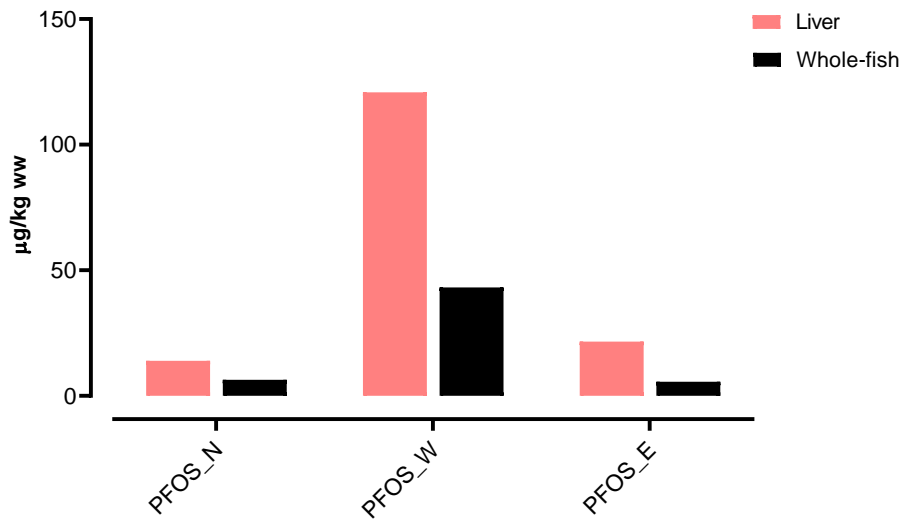


Figure 4.5 Mean PFOS levels given in wet weight (ww) and dry weight (dw) detected in the liver (red) and whole-fish (black) of *P. flesus* sampled annually over three years (2017-2019) at three locations in Dutch waters (Noordzeekust (N), Westerschelde (W), Eems-Dollard (E)).

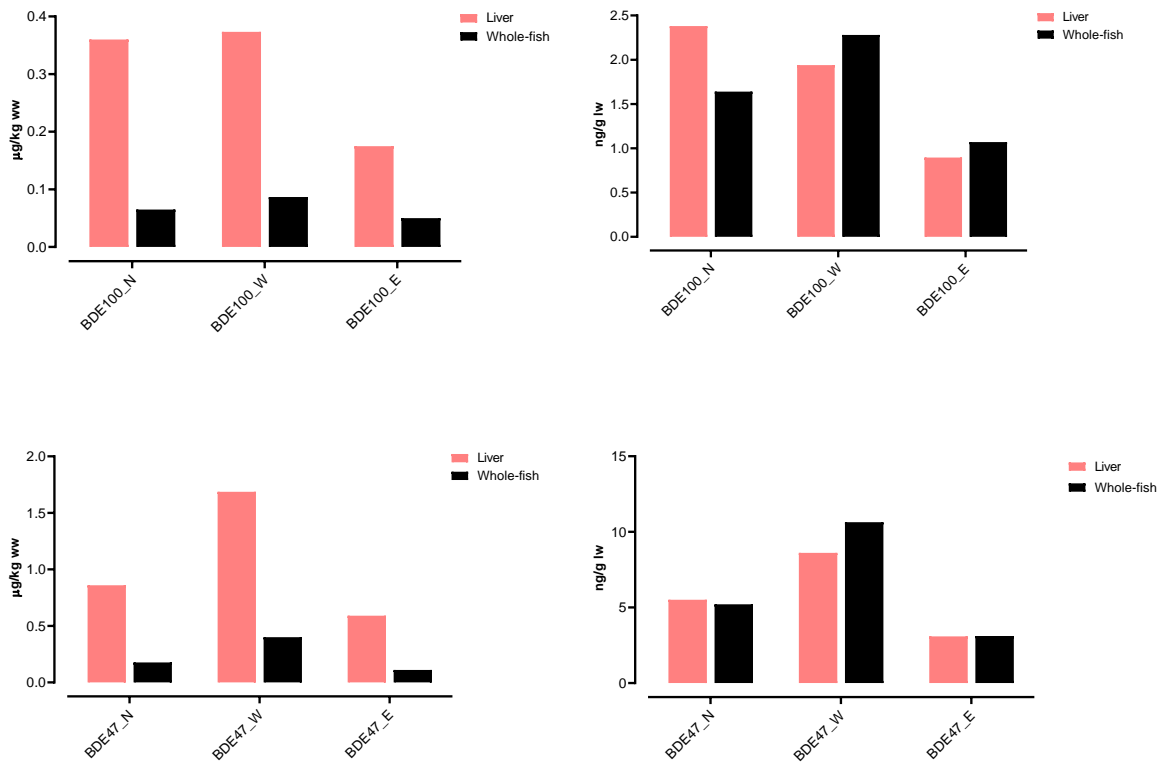


Figure 4.6 Mean BDE100 (upper graphs) and BDE47 (lower graphs) concentrations given in wet weight and lipid weight in the liver (red) and whole-fish (black) of *P. flesus* sampled annually over three years (2017-2019) at three locations in Dutch waters (Noordzeekust (N), Westerschelde (W) and Eems-Dollard (E)).

4.4 Establishing conversion factors

The PCB's analysed in these samples, have different chemical properties, and show in general a different level of accumulation with fish species. Small PCBs (low chlorinated, low molecular weight) tend to be quickly in equilibrium in both small and large fish, while the large PCBs (highly chlorinated, high molecular weight) are taken up so slow that equilibrium (if any) is only obtained in older fish. This must be taken into account when extrapolating PCB levels from whole fish (15-20 cm) to livers of larger (older) fish (15-30 cm).

To check whether these phenomena occur the ratios of all PCB congeners, between whole-fish samples and liver samples, were calculated. The ratio of levels in whole-fish/liver for PCB congeners are on wet and lipid weight similar for all PCBs and all locations (Figure 4.7). Yet, sometimes a large variation can be observed (*e.g.*, Noordzeekust). As the behaviour of all PCB congeners was similar, the ratio's of sum-PCB are shown in Figure 4.8. On lipid weight, a ratio of almost one was observed in all locations. The sum of ratio's for all PCBs is 1.02 in lw and 0.22 in ww. The margin of observed variability for this ratio for the wet weight and lipid weight results are comparable.

For metals, as the distribution over body parts is different for the metals studied, the ratio's of whole-fish / liver (or fillet in case of mercury) are depicted individually. These Figures (4.8 –4.10) show again that distribution of metals over body parts can be very different. Still, despite the observed large variation of ratio's determined over the years and across the sampling locations, there is consistency in the ratio for each metal.

For example, mercury levels are higher in fillet samples than in whole-fish. Similar observations have been done in fish species from Germany ¹². In this literature study, part of the fillet has been removed from the same organisms, and linear correlations were drawn. This resulted in significant linear relationships between tissue and whole-body burden of mercury. The calculated fillet-to-whole-body conversion ratios are in a similar range as those observed within the literature (around 0.7)¹².

For BDE's, the ratios have been established only for locations, where concentrations were above detection limits. Some locations/years have sparsely represented data. BDE 47 acts similarly like PCB's. For BDE100, however, at levels close to quantification limits, the ratios are showing high variability. Predictions based on minimal data points and such high fluctuating ratios are less reliable. Generally, a predictive approach is as good as the underlying data of validation.

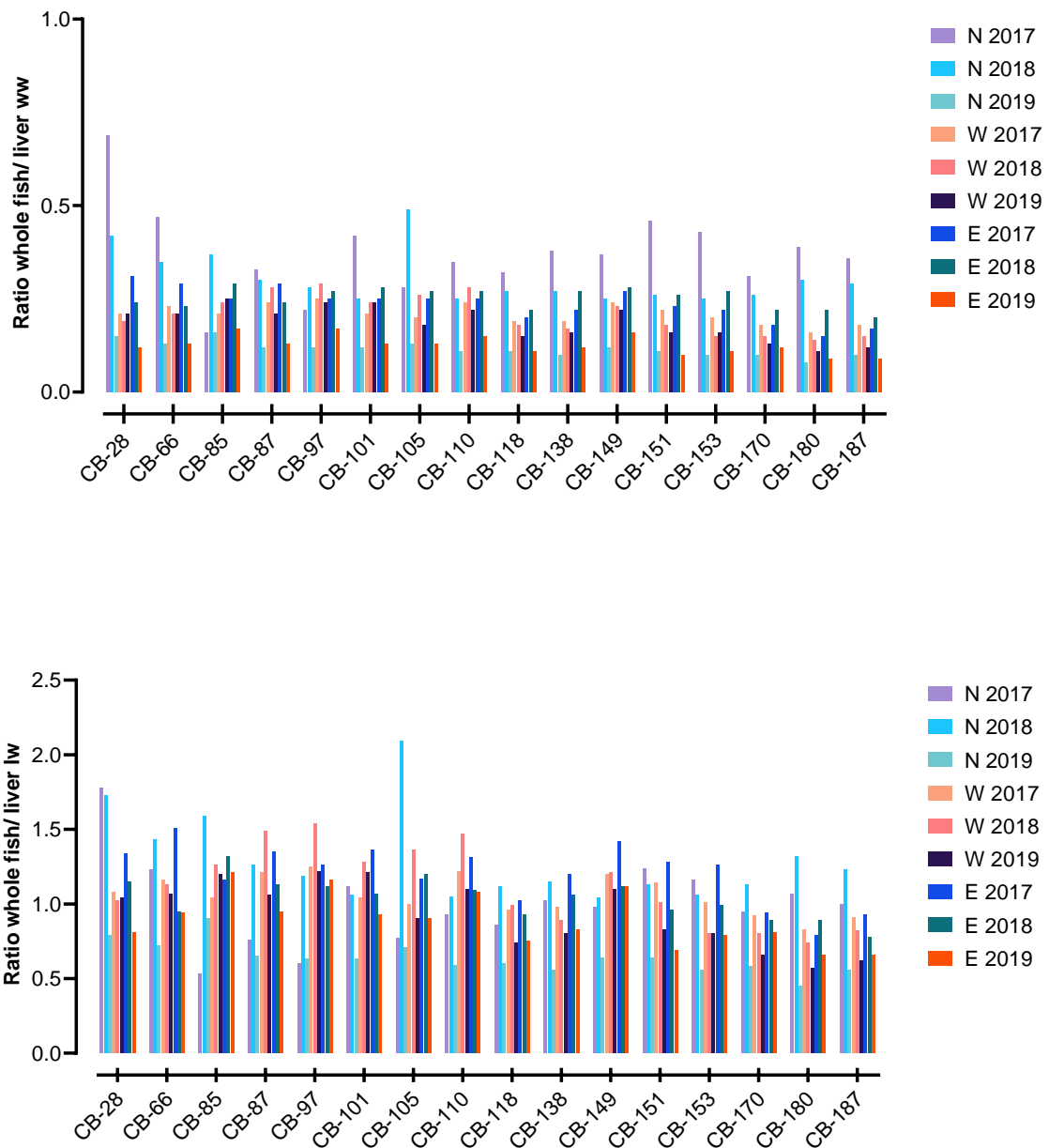


Figure 4.7 Whole-body-to-tissue ratios (whole-fish/liver) for PCBs given in wet weight (ww, top) and lipid weight (lw, bottom) for all three habitats over all three sampling years. (lower concentrations in whole-fish < 1, equal concentrations =1, higher concentrations in whole-fish >1).

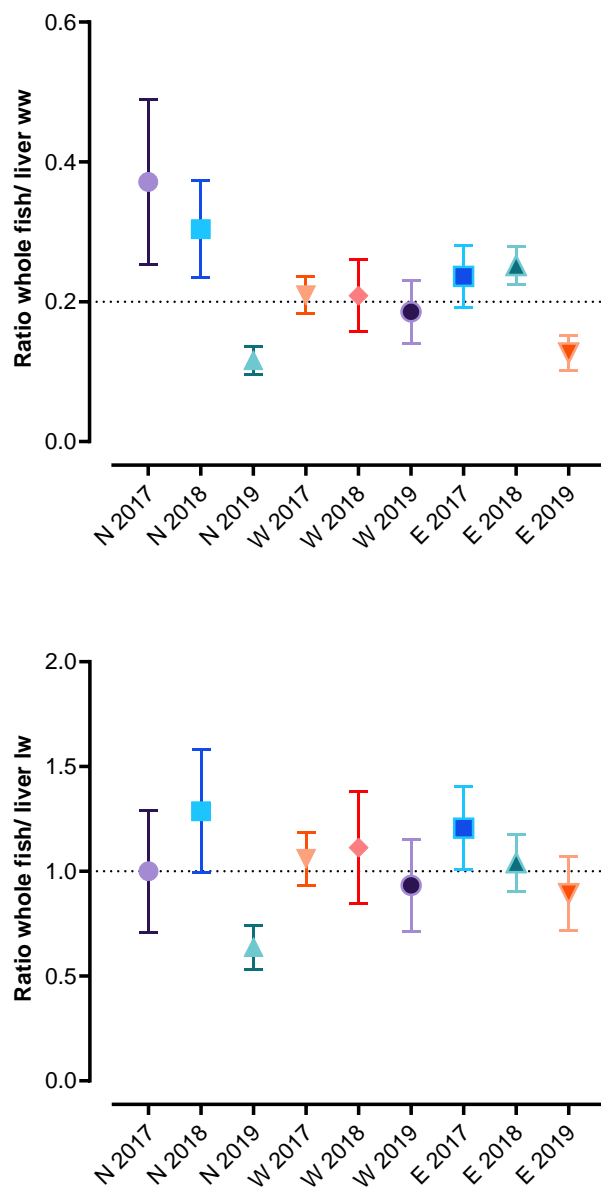


Figure 4.8 Whole-body-to-tissue ratios (whole-fish/liver) for sum-PCB given in wet weight (ww, top) and lipid weight (lw, bottom) for all three habitats over all three sampling years. Mean ratio for all PCBs are indicated through a dashed line. (lower concentrations in whole-fish < 1, equal concentrations = 1, higher concentrations in whole-fish > 1).

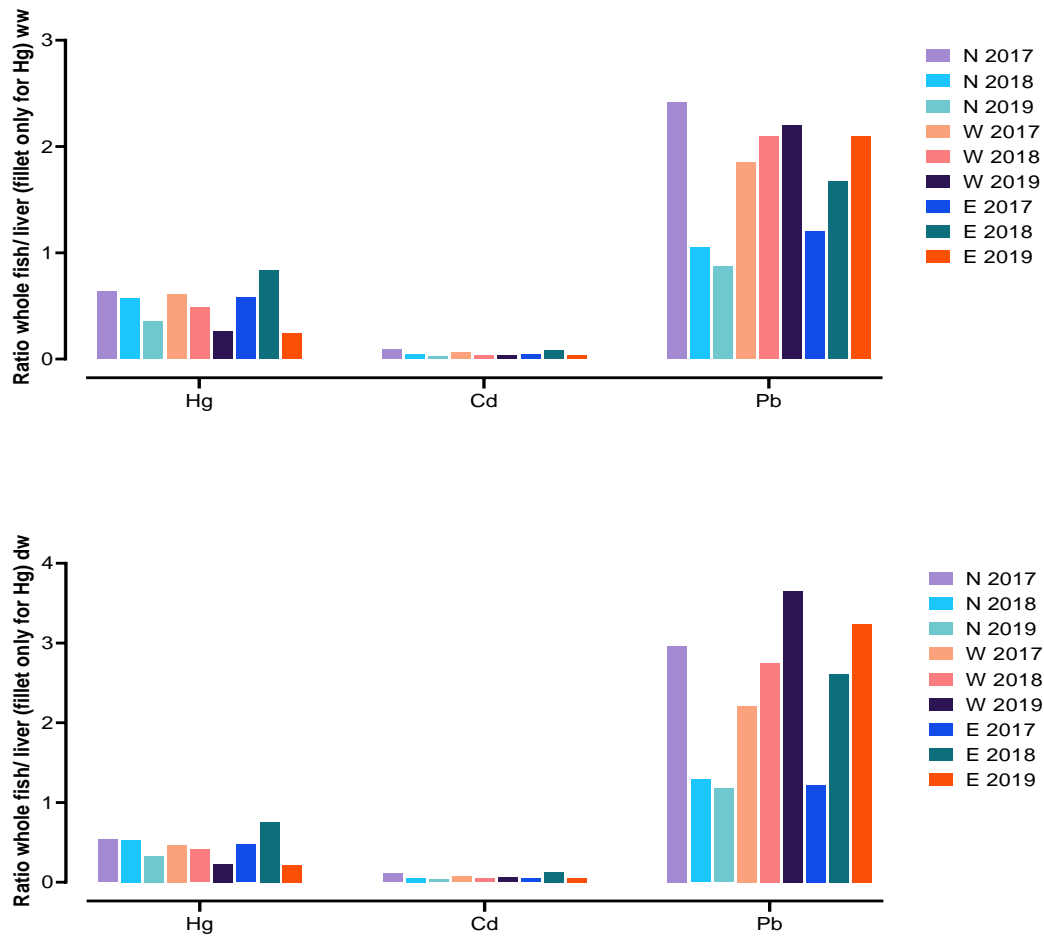


Figure 4.9 Whole-body-to-tissue ratios (whole-fish/liver) for cadmium (Cd) and lead (Pb), and whole-fish/fillet for mercury (Hg) given in wet weight (ww, top) and dry weight (dw, bottom) for all three habitats over all three sampling years (lower concentrations in whole-fish < 1, equal concentrations =1, higher concentrations in whole-fish >1)

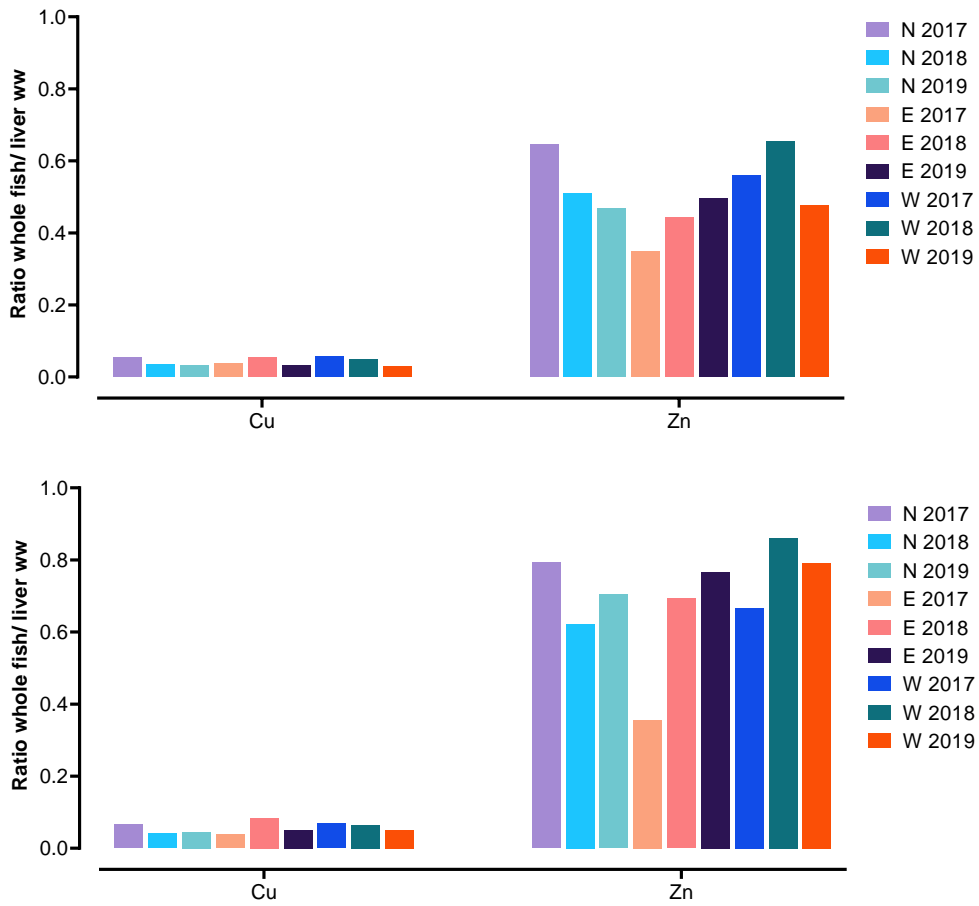


Figure 4.10 Whole-body-to-tissue ratios (whole-fish/liver) for copper (Cu) and zinc (Zn) given in wet weight (ww, top) and dry weight (dw, bottom) for all three habitats over all three sampling years (lower concentrations in whole-fish < 1, equal concentrations =1, higher concentrations in whole-fish >1).

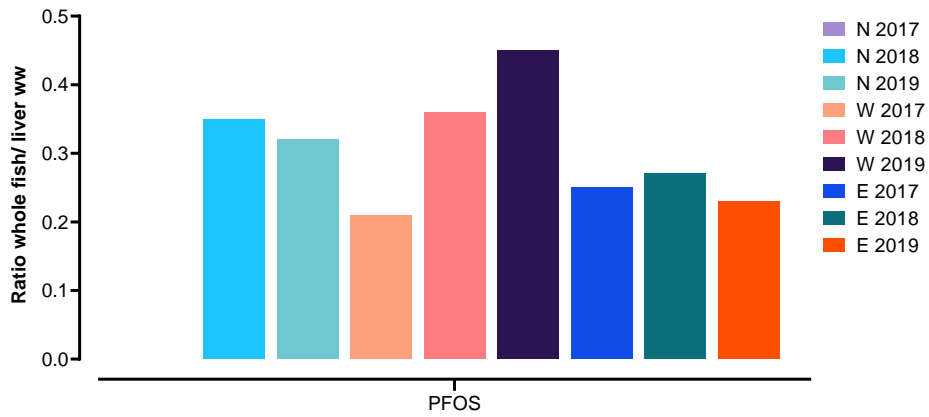


Figure 4.11 Whole-body-to-tissue ratios (whole-fish/liver) for PFOS given in wet weight for all three habitats over all three sampling years (lower concentrations in whole-fish < 1, equal concentrations =1, higher concentrations in whole-fish >1)

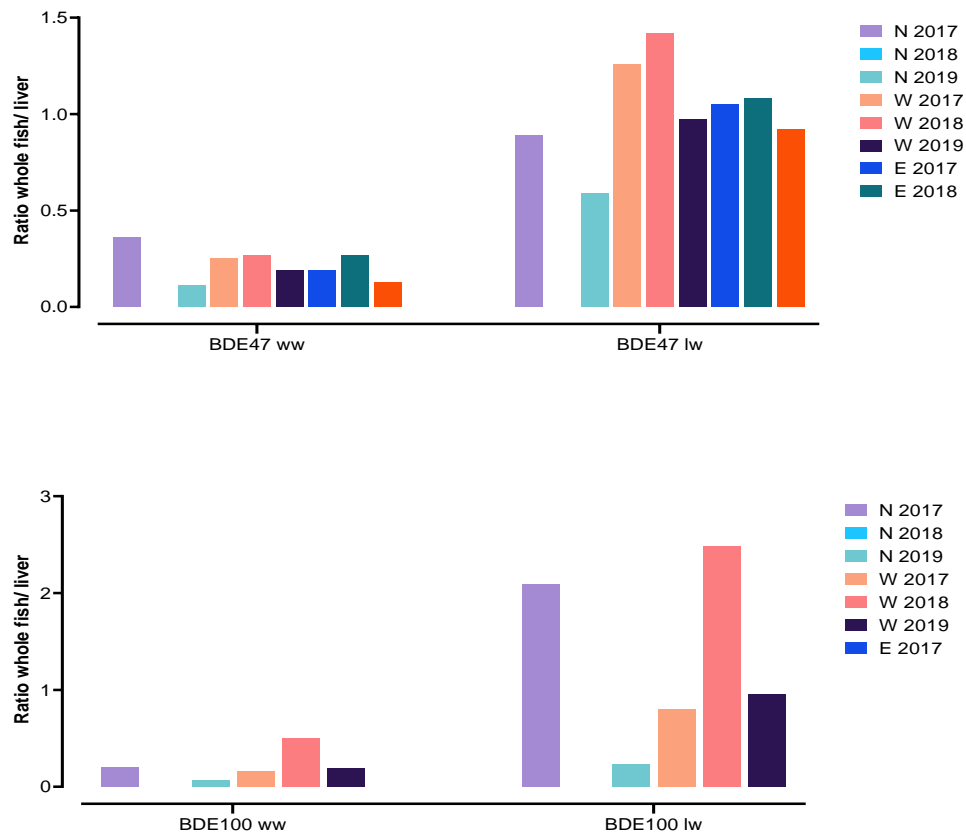


Figure 4.12 Whole-body-to-tissue ratios (whole-fish/liver) for BDE 47 and BDE 100 given in wet weight (ww, left) and lipid weight (lw, right) for all three habitats over all three sampling years. (lower concentrations in whole-fish < 1, equal concentrations =1, higher concentrations in whole-fish >1).

5 Results and discussions (Part 2)

5.1 Conversion factor based predictions from whole-fish to liver

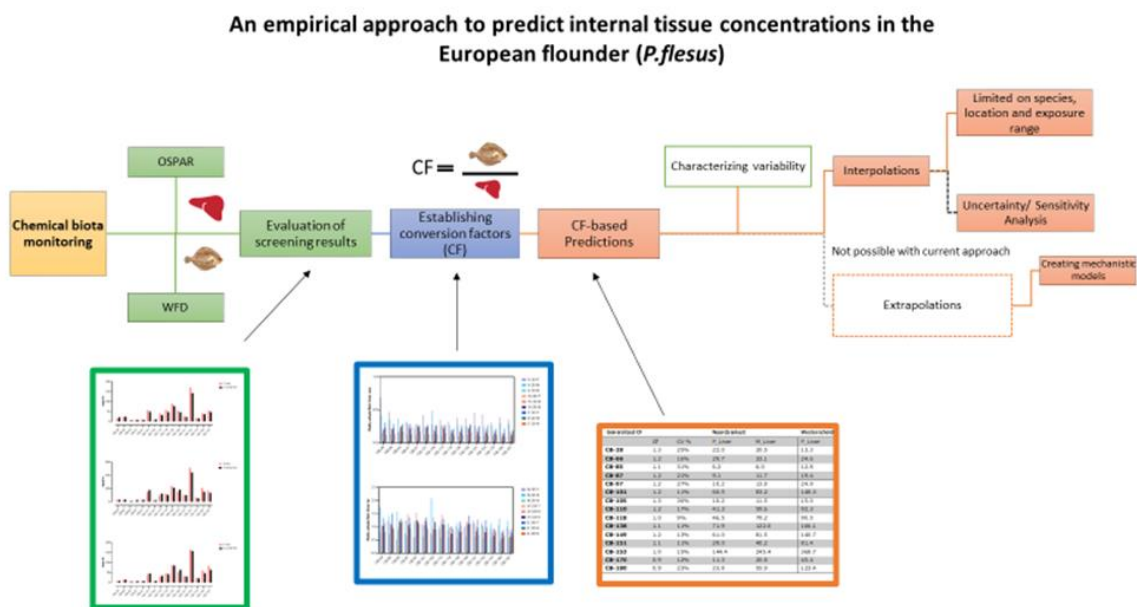


Figure 5.1 The conceptual framework of the empirical study approach to predict internal tissue concentration in the European flounder (*P.flesus*)

In the previous steps we derived the ratio's between whole-fish to liver (or fillet for mercury). Such ratios have the potential to be applied as a possible conversion factor for predictions of concentrations in liver tissue in future studies (Figure 5.1). The major goal of this approach is to be able to replace the analytical screening efforts of the OSPAR directive for liver tissue with estimations of liver concentrations based on whole body burden (whole-fish) determined for WFD. However, some drawbacks of an empirical approach as such presented within this project and its limitations need to be considered.

To test and validate the predictive power of this conversion factors, we used the data sets for fish caught in 2019 and based the CFs only on data from 2017-2018. Table 1 illustrates the variability of the derived conversion factors across habitats, if the conversion factors are determined for each location. In location N, the variability (% CV) is higher than in the other two locations. By averaging the ratios across all habitats, which is shown in Table 2, the variability of the conversion factor is lower for each chemical and predictions are improved (Factor 3 without generalized CF, Factor 2 with generalized CF). This generalization step enables to take into account intraspecies and spatial variability, and strengthens the robustness of the CF. However, a further discussion of how to take into account for variability within such predictions will be discussed in future recommendations. Overall, a generalized conversion factor is recommended. As expected, the ratios for PCBs if lipid normalized are around 1 (average for all PCBs 1.02 conversion factor from Table 2).

Similar to PCBs, inorganic compounds also show the applicability of a conversion factor in future studies by applying a factor of 3 to take into account the intraspecies variability. Such relationships as drawn within this study are the first of its kind. Studies applying similar approaches mainly focused on mercury and extrapolations from fillet to whole-fish. Table 3 and 4 summarize the results of individual conversion factors and the predictions based applied for the data set in 2019.

Similar to the other compound groups, conversion factors for PFOS can be applied while taking into account a factor of 3 for predictions, based on our data presented in table 5. For BDE47, however, the study area Noordzeekust could not be predicted due to the insufficient amount of data as too many concentrations were below quantification limits. For BDE 100 only data for Westerschelde could be used for predictions. Therefore, only generalized CFs has been applied (Table 6). The CF of BDE47 was closer to that of PCBs with lipid weight 1.2 and in wet weight 0.25. BDE 100, in contrast, has higher conversion factors with lipid weight 1.6 and wet weight 0.30. Due to the lack of data, the uncertainties regarding BDE's are increased.

Overall, the approach for predictions presented here is empirical. We concluded that creating generalized (location non-specific) conversion factors results in the lowest variability of the ratios, as with a higher number of samples the variability decreases. Hence, only exposure concentrations (ranges found in whole fish, see appendix Table 1 to 6), can be applied for predictions at only the studied three locations and within the same species. For extrapolations to different exposure scenarios, more complex modelling approaches need to be applied (Figure 5.2, dashed lines). Another option for applying such data is to develop mechanistic models, that can range from simple toxicokinetic models to complex physiologically based toxicokinetic modelling approaches¹⁸. Such models take into account the physiology (e.g., blood flow, lipid levels, etc.) of the species of interest as well as chemical properties (e.g., blood/tissue partition coefficients), which can simulate the fate of the compound within the body and explain the underlying biological processes. The empirically observed values can serve as data to validate such models and quantify the variability. The uncertainty within such models can be determined easier with well-established approaches. A statistical supplementation of mechanistic models can mathematically suggest reliable probabilities and credibility intervals (Monte Carlo simulations or Bayesian statistics). Most importantly, such models simulate if animals are in an equilibrium state. Overall, linking processes quantitatively linked would provide an in-depth understanding of bioaccumulation (external to internal concentrations). Therefore, predictions can be done not only from one internal tissue to another, it can also be applied to predict external concentrations. Further, such models can be extrapolated to other species, and connected with quantitative knowledge of toxic effects triggered within the species of interest.

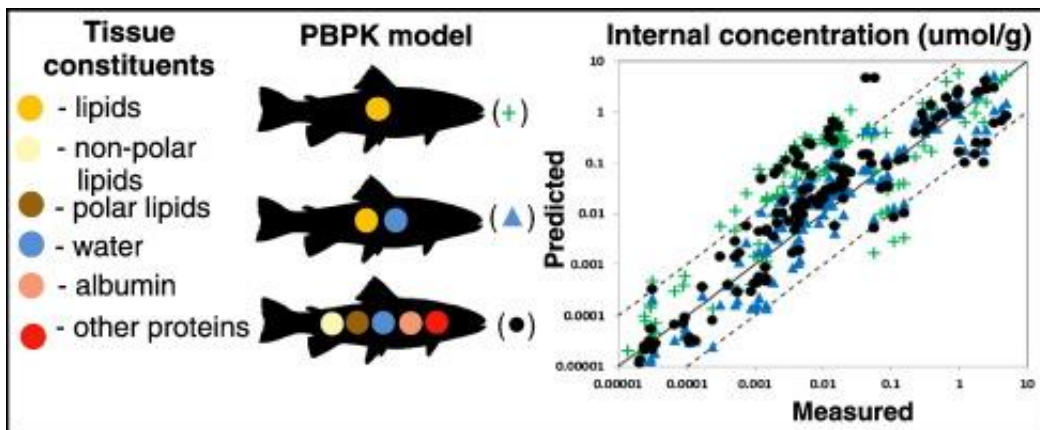


Figure 5.2 Illustrations of a mechanistic modelling to simulate the absorption, distribution, metabolism and elimination of compounds within fish species to ultimately determine internal concentrations (Source: Salmina et al. 2016)

Table 1a Predictions for PCB level in liver tissue in 2019 based on wet weight conversion factors (CF) determined per location (N, W, and E) averaged for year 2017-2018. Using the measured whole-fish PCB concentrations, we predict the concentrations of PCB in the liver tissue (P_liver) and compare the results to measured concentrations in the liver (M_liver)

	Noordzeekust				Westerschelde				Eems-Dollard			
	CF	CV %	P_Liver	M_Liver	CF	CV %	P_Liver	M_Liver	CF	CV %	P_Liver	M_Liver
CB-28	0.42	64%	1.4	4.1	0.20	5%	3.7	3.6	0.22	45%	1.1	2.1
CB-66	0.32	54%	2.8	6.6	0.22	4%	5.8	6.0	0.22	36%	2.3	3.7
CB-85	0.23	51%	0.9	1.2	0.23	9%	2.7	2.5	0.24	25%	0.8	1.1
CB-87	0.25	45%	1.1	2.3	0.24	15%	4.0	4.7	0.22	37%	1.0	1.6
CB-97	0.20	41%	1.6	2.8	0.26	9%	4.7	5.0	0.23	24%	1.1	1.6
CB-101	0.26	58%	7.3	16.6	0.23	9%	31.4	29.5	0.22	35%	6.7	11.2
CB-105	0.30	60%	1.0	2.3	0.21	20%	3.8	4.5	0.21	35%	1.2	2.0
CB-110	0.24	51%	5.4	11.8	0.25	12%	18.5	20.8	0.22	28%	4.9	7.2
CB-118	0.23	47%	7.5	15.8	0.17	13%	21.4	25.2	0.18	35%	7.5	12.6
CB-138	0.25	55%	10.0	24.4	0.18	9%	41.8	45.0	0.20	38%	13.8	23.8
CB-149	0.24	51%	7.9	16.2	0.23	3%	30.0	30.9	0.24	28%	9.0	13.4
CB-151	0.28	63%	3.4	8.6	0.19	16%	20.3	23.6	0.19	43%	5.1	10.0
CB-153	0.26	62%	19.5	49.0	0.17	15%	94.8	100.0	0.20	41%	26.9	48.5
CB-170	0.22	48%	2.0	4.3	0.15	16%	11.6	13.6	0.17	29%	3.3	4.7
CB-180	0.26	62%	3.6	11.3	0.14	18%	35.4	42.9	0.16	40%	8.4	13.7
CB-187	0.25	53%	6.7	16.2	0.15	19%	30.6	37.8	0.16	35%	13.4	22.1

Table 1b Predictions for PCB level in liver tissue in 2019 based on lipid normalized conversion factors (CF) determined per location (N, W, and E) averaged for year 2017-2018. Using the measured whole-fish PCB concentrations, we predict the concentrations of PCB in the liver tissue (P_liver) and compare the results to measured concentrations in the liver (M_liver)

	Noordzeekust				Westerschelde				Eems-Dollard			
	CF	CV %	P_Liver	M_Liver	CF	CV %	P_Liver	M_Liver	CF	CV %	P_Liver	M_Liver
CB-28	1.8	64%	9.3	20.5	1.1	3%	17.1	17.3	1.2	24%	6.4	9.9
CB-66	1.3	54%	18	33.1	1.1	4%	27.2	28.4	1.2	29%	13.6	17.9
CB-85	1.1	51%	5.1	6	1.2	10%	12.5	12.2	1.2	6%	4.9	5
CB-87	1	45%	7.5	11.7	1.3	17%	18.6	21.9	1.2	17%	6.1	7.9
CB-97	0.9	41%	9.9	13.8	1.3	13%	21.5	23.7	1.2	6%	7.4	7.5
CB-101	1.1	58%	48.1	83.2	1.2	10%	145.2	141.8	1.2	19%	40.9	53.4
CB-105	1.4	60%	5.7	11.5	1.1	22%	17.5	21.1	1.2	15%	7.4	9.7
CB-110	1	51%	35.4	59.6	1.3	15%	85.8	98.8	1.2	11%	31.1	34.6
CB-118	1	47%	47.9	79.2	0.9	15%	98.6	119.3	1	16%	46.4	60.7
CB-138	1.1	55%	62.8	122	0.9	10%	196.2	217	1.1	18%	84.1	114.9
CB-149	1	51%	52.1	81.5	1.2	5%	139.6	148.4	1.3	14%	56.9	64.5
CB-151	1.2	63%	21.8	40.2	1	16%	92.7	110.9	1.1	30%	29.8	48.6
CB-153	1.1	62%	124.3	245.4	0.9	14%	443.3	481.9	1.1	23%	163.2	233.5
CB-170	1	48%	11.6	20.8	0.8	17%	53.8	64.2	0.9	7%	20.6	23.1
CB-180	1.2	62%	21.1	55.9	0.7	18%	163.4	202.5	0.8	15%	52.3	66.6
CB-187	1.1	53%	40.9	81.1	0.8	19%	141.7	179	0.9	17%	83.3	107.2

Table 2a Predictions for PCB level based on wet weight conversion factors (CF) determined averaged for year 2017-2018 and across sampling locations. Using the measured whole-fish concentrations, we predict the PCB concentrations in the liver tissue (P_liver) and compare the results with concentrations measured in liver (M_Liver) to evaluate the predictive power on an independent data set

Generalized CF	Noordzeekust		Westerschelde		Eems-Dollard		
	CF	P_Liver	M_Liver	P_Liver	M_Liver	P_Liver	M_Liver
CB-28	0.28	2.1	4.1	2.7	3.6	0.9	2.1
CB-66	0.25	3.5	6.6	5.1	6.0	2.0	3.7
CB-85	0.23	0.9	1.2	2.7	2.5	0.8	1.1
CB-87	0.24	1.2	2.3	4.1	4.7	0.9	1.6
CB-97	0.23	1.4	2.8	5.3	5.0	1.1	1.6
CB-101	0.24	8.1	16.6	30.4	29.5	6.2	11.2
CB-105	0.24	1.2	2.3	3.3	4.5	1.1	2.0
CB-110	0.24	5.4	11.8	19.5	20.8	4.7	7.2
CB-118	0.20	8.9	15.8	19.2	25.2	6.9	12.6
CB-138	0.21	11.9	24.4	35.1	45.0	13.3	23.8
CB-149	0.24	8.1	16.2	29.1	30.9	9.0	13.4
CB-151	0.22	4.3	8.6	17.5	23.6	4.5	10.0
CB-153	0.21	24.0	49.0	77.2	100.0	25.6	48.5
CB-170	0.18	2.4	4.3	9.8	13.6	3.0	4.7
CB-180	0.18	5.0	11.3	26.9	42.9	7.1	13.7
CB-187	0.19	8.9	16.2	25.1	37.8	11.2	22.1

Table 1b Predictions for PCB level based on lipid normalized conversion factors (CF) determined averaged for year 2017-2018 and across sampling locations. Using the measured whole-fish concentrations, we predict the PCB concentrations in the liver tissue (P_liver) and compare the results with concentrations measured in liver (M_Liver) to evaluate the predictive power on an independent data set

Generalized CF	Noordzeekust			Westerschelde		Eems-Dollard	
	CF	P_Liver	M_Liver	P_Liver	M_Liver	P_Liver	M_Liver
CB-28	1.3	22	20.5	13.3	17.3	5.9	9.9
CB-66	1.2	29.7	33.1	24.6	28.4	13.5	17.9
CB-85	1.1	6.2	6	12.8	12.2	5.3	5
CB-87	1.2	9.1	11.7	19.4	21.9	6.3	7.9
CB-97	1.2	10.2	13.8	24.9	23.7	7.5	7.5
CB-101	1.2	60.5	83.2	148.3	141.8	43.1	53.4
CB-105	1.3	10.2	11.5	15	21.1	6.9	9.7
CB-110	1.2	41.3	59.6	92.3	98.8	31.7	34.6
CB-118	1	46.5	79.2	90.5	119.3	46.3	60.7
CB-138	1.1	71.9	122	166.1	217	90.6	114.9
CB-149	1.2	61	81.5	140.7	148.4	62.2	64.5
CB-151	1.1	29	40.2	81.4	110.9	29.7	48.6
CB-153	1	144.4	245.4	368.7	481.9	175.4	233.5
CB-170	0.9	11.3	20.8	45.4	64.2	20	23.1
CB-180	0.9	23.8	55.9	123.4	202.5	46.8	66.6

Table 2 Predictions for metals in liver tissue based on wet weight conversion factors (CF) determined per location (N, W, and E) averaged for year 2017-2018. Using the measured whole-fish concentrations, we predict the concentrations in the liver tissue (P_liver) and compare the results to measured concentrations in the liver

	Noordzeekust				Westerschelde				Eems-Dollard			
	CF	CV %	P_Liver	M_Liver	CF	CV %	P_Liver	M_Liver	CF	CV %	P_Liver	M_Liver
Hg	0.6	8%	0.11	0.19	0.55	15%	0.09	0.19	0.71	26%	0.05	0.16
Cd	0.1	47%	0.05	0.12	0.05	34%	0.19	0.27	0.06	40%	0.17	0.31
Cu	0	32%	15.2	19.91	0.05	12%	14.1	25.08	0.05	25%	20.9	29.51
Pb	1.7	56%	0.02	0.03	1.97	9%	0.03	0.02	1.43	23%	0.05	0.04
Zn	0.6	17%	39.28	48.53	0.61	11%	41.5	52.99	0.4	17%	68.45	54.71

Table 3 Predictions for metals based on wet weight conversion factors (CF) determined averaged for year 2017-2018 and across sampling locations. Using the measured whole-fish concentrations, we predict the concentrations in the liver tissue (P_liver) and compare the results with concentrations measured in liver (M_Liver) to evaluate the predictive power on an independent data set

Generalized CF	Noordzeekust			Westerschelde			Eems-Dollard		
	CF	P_Liver	M_Liver	P_Liver	M_Liver	P_Liver	M_Liver		
Hg	0.62	0.08	0.19	0.08	0.19	0.06	0.16		
Cd	0.06	0.15	0.27	0.15	0.27	0.18	0.31		
Cu	0.05	15.83	25.08	15.83	25.08	20.09	29.51		
Pb	1.71	0.03	0.02	0.03	0.02	0.05	0.04		
Zn	0.53	47.83	52.99	47.83	52.99	51.5	54.71		

Table 4 Predictions for PFOS based on wet weight conversion factors (CF) determined averaged for year 2017-2018 and across sampling locations. Using the measured whole-fish concentrations, we predict the concentrations in the liver tissue (P_liver) and compare the results with concentrations measured in liver (M_Liver) to evaluate the predictive power on an independent data set

Generalized CF	Noordzeekust				Westerschelde		Eems-Dollard	
	CF	CV %	P_Liver	M_Liver	P_Liver	M_Liver	P_Liver	M_Liver
PFOS	0.29	22%	17.0	15.6	217.4	140.0	16.3	21.0

Table 5 Predictions for BDEs based on lipid normalized conversion factors (CF) determined averaged for year 2017-2018. Data only for Westerschelde due to the lack of data (below detection limits at other locations). Using the measured whole-fish concentrations, we predict the concentrations in the liver tissue (P_liver) and compare the results with concentrations measured in liver (M_Liver) to evaluate the predictive power on an independent data set

Generalized CF	Westerschelde			Eems-Dollard	
	CF	P_Liver	M_Liver	P_Liver	M_Liver
BDE100 ww	0.33	1.2	1.6	0.39	0.75
BDE100 lw	1.6	5.8	7.3	2.7	3.6
BDE47 ww	0.25	0.26	0.44		
BDE47 lw	1.2	1.2	2.1		

5.2 Characterizing variability

The biometric information of data collected in fish under the umbrella of the two directives (OSPAR and WFD) was characterized. This step is necessary to characterize the possible impact of covariates on contamination levels (whole-fish body length or body weight, lipid content) and the interaction thereof (weight whole-fish vs weight liver, body length, and body weight, body length and lipid content). This approach reveals that the biological variability impacts the contaminant levels within individual or grouped organism, hence, may cause uncertainties for future extrapolation efforts. However, conversion factors do not include any mechanistic information such as how the biology (growth), physiology (metabolism or adaptive stress response), and the chemical properties (partitioning from blood to tissues) reveal this ratio derived within an empirical approach. Therefore the presented CFs can only be applied within this specific biota monitoring approach. To quantitatively understand how these biological, physiological or chemical traits can affect the CFs or concentrations found within tissue or whole-fish, such relationships need to be implemented within a modelling approach. Generally, a balance within a predictive approach is to keep it as simple as possible, and as complex as necessarily. Below, possible biological factors that can affect the data are discussed.

Many contaminants screened during the present biomonitoring project are known to have long biogeological half-lives. Hence, organisms are chronically exposed to such chemicals over time and gradually accumulate these chemicals internally. PCBs, for example, are highly stable, hydrophobic, and persistent, hence, bioaccumulate in species¹⁹. Correlations of PCB levels with increasing body size have been shown in the literature²⁰. Such relationships have been also observed for metals, for example, mercury^{16, 21}. Therefore, it is important to characterize variables, which can impact the data collected in this present project, and as such, impact potential application of predicted values within biomonitoring studies.

The body size and body length distribution of *P. flesus* caught for both monitoring efforts are significantly different as shown in Figure 5.3. The lipid content in the sample matrices for the both directives (liver for OSPAR, Mercury only in fillet and whole-fish for the WFD) are significantly different as well (Figure 5.4). As the body size and hence the age of the fish caught for monitoring may differ, these covariates can affect the data due to the nature of biological variability and differences within different individuals of species (intra). This can, in turn, cause uncertainties on extrapolation efforts. However, quantification of this uncertainty based on CFs is not possible.

Many chemicals screened within this project accumulate within lipid rich tissues such as the liver¹⁵. As mentioned previously, under WFD the whole-fish samples are screened for environmental quality assessment. Lipid content of whole-fish does not indicate a strong body size dependency. In contrast, liver lipid content is related to body size of the animals (Figure 5.5). Therefore, the variability caused by body size might be more prevalent in liver samples. Importantly, the sensitivity of such covariates and relationships thereof can cause variability in observed extrapolation ratios for the aim of this study and needs to be considered in future monitoring efforts. A well-established curve between body weight and body size relation of all samples show a very conservative spatial difference (Figure 5.4 b, $R^2=0.96$).

To better understand the impact of body size and lipid content, that is, the biological variability within a pooled data set, we use the prominent compound PCB 153 as a case-study chemical for an in-

depth discussion of concentrations found in liver and whole-fish per location and year (Figure 5.6). For PCB153, all data are shown in Figure 5.6. The levels clearly differ each year at each location, both on wet weight as lipid weight and for liver (large fish, only mercury in fillet) and whole-fish (smaller fish). Further, the mathematical significance (Appendix, Table 7) disappears after lipid normalization for the majority of samples (n=16 significantly different on a wet weight basis and n=1 significantly different after lipid normalization of concentrations) and makes the values comparable, which, in turn, is an additional indication of the importance of co-determining the exact lipid content of matrices in fish.

Finally, we looked at possible correlation of those confounding factors on the levels detected. The graphical illustration of a Pearson correlation matrix (Figure 5.7) shows the complexity of the interaction of biological information (lipid content, body size and average body weight) as well as the concentration of PCBs found in whole-fish/liver. For example, the lipid content is strongly affecting concentrations of CB-52, CB-101, CB-153, CB-138, closely to a linear relationship. As shown prior for PCB153 in detail (see above) such correlations of biological properties of sampling matrix can influence data detected and cause intra- and inter-species variability. Hence, conversion factors derived from real-world exposure scenarios, as such in the present study, can be impacted by such interactions. Within the present project, the chemical-chemical interactions, that can influence the kinetics (uptake, distribution, metabolism, and elimination) within an organism, are not closely investigated.

Overall, we could clearly show that factors like body size or lipid content can influence the levels observed within the data. However, without conducting a sensitivity or uncertainty analysis on the ratios established within this study, which was beyond the scope of the project, it cannot be determined which of those characterized biological or chemical parameters impact the predictions and the extent significantly. Such empirical approaches do not count for any mechanistic relationship of the biology of species as well as properties of chemicals. Future studies need to focus on ways how to determine such impacts and uncertainty in-depth.

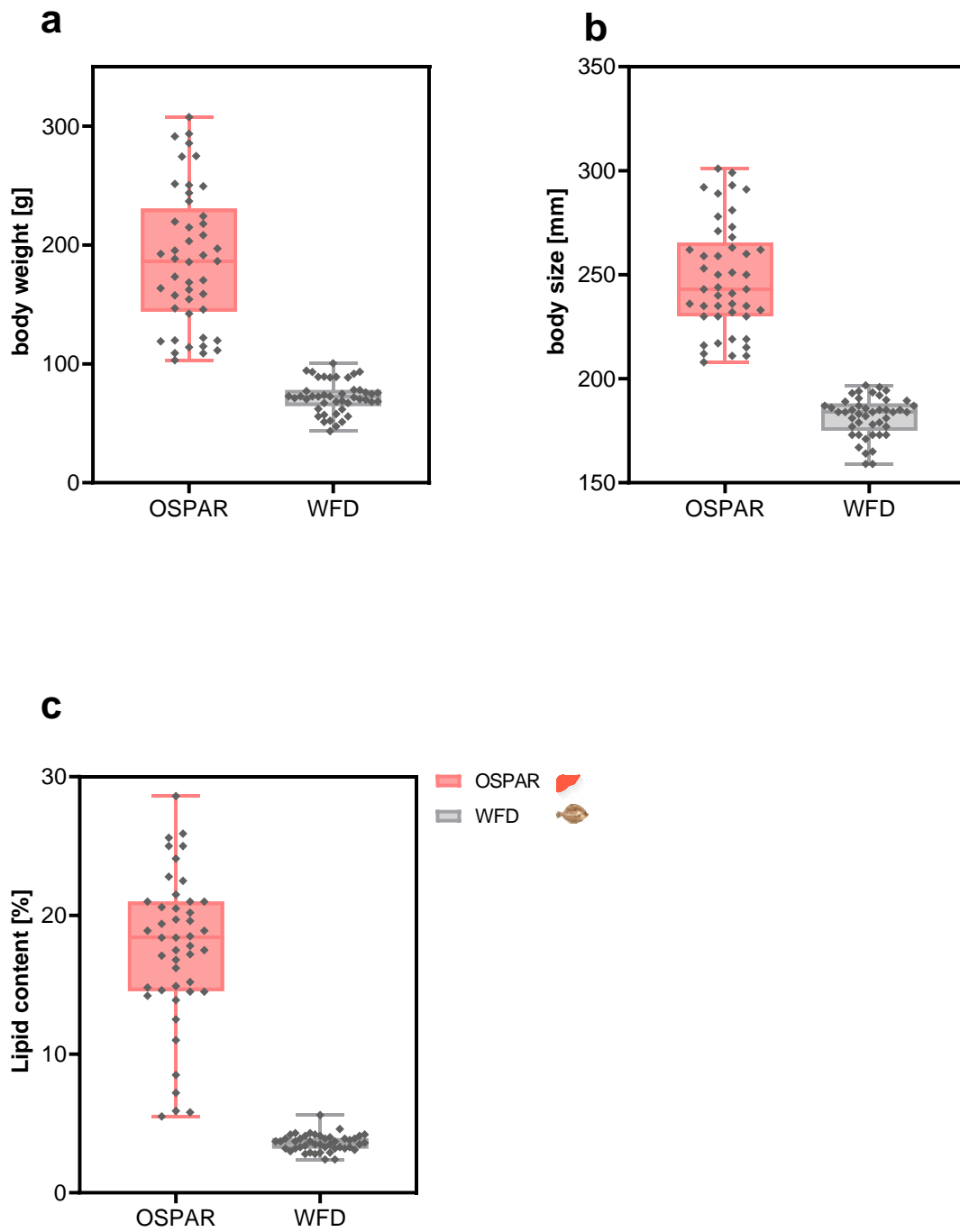


Figure 5.3 Bodyweight (g; a) and body size (mm; b) of *P. fesus*, and lipid content (%) in liver and whole-fish distributions of *P. fesus* caught under the umbrella of the two different sampling strategies (red=OSPAR and grey= WFD). Whiskers-boxes= 1st and 3rd quartiles, line = median, and grey dots= data from minimum to maximum.

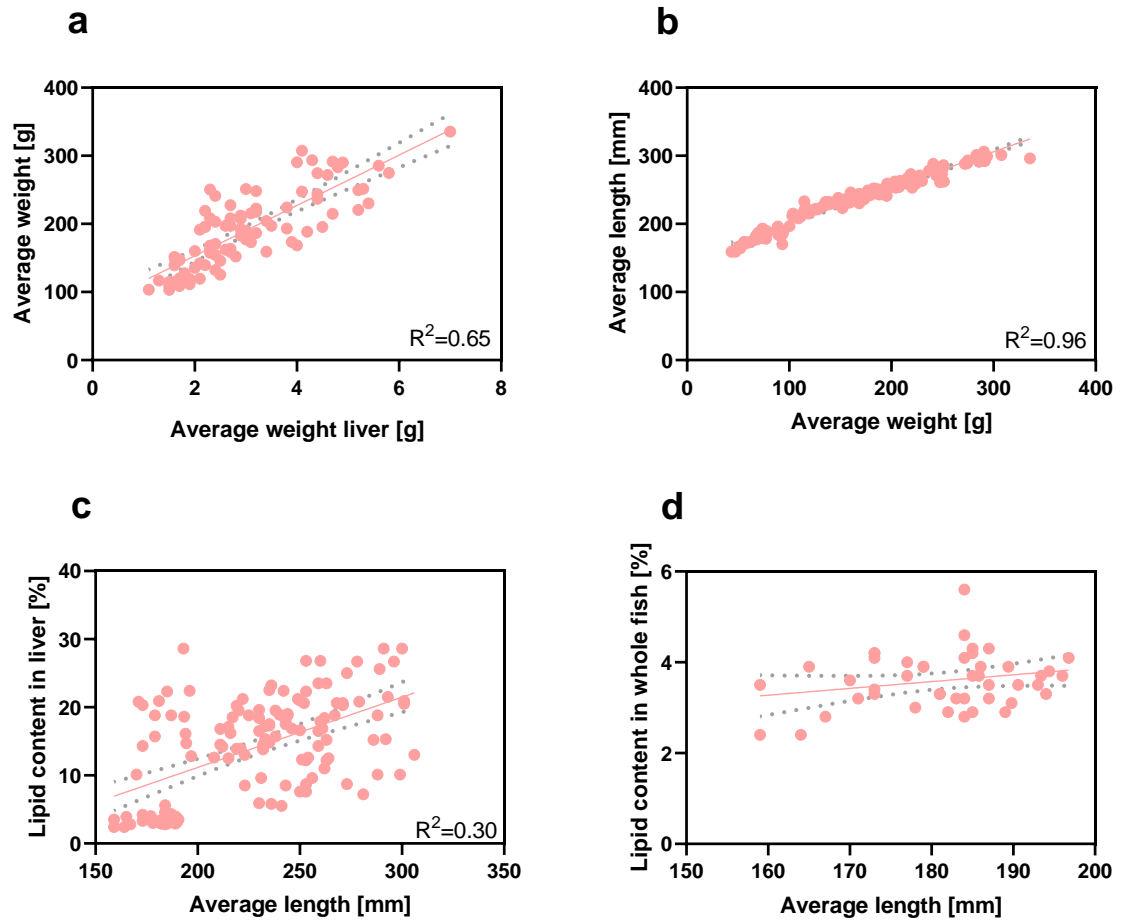


Figure 5.4 The observed linear correlations (indicated with r^2) of biometric information collected for biomonitoring. Significant relationships could be observed between covariates such as weight of liver(g) and whole-fish (g) samples (OSPAR)[a]; the total body weight(g) [b] and body size correlation for samples caught for both monitoring efforts, OSPAR and WFD; the body length and lipid content in liver (%) for OSPAR [c], and relationship between average length of fish and lipid content of whole-fish for WFD (%) [d]

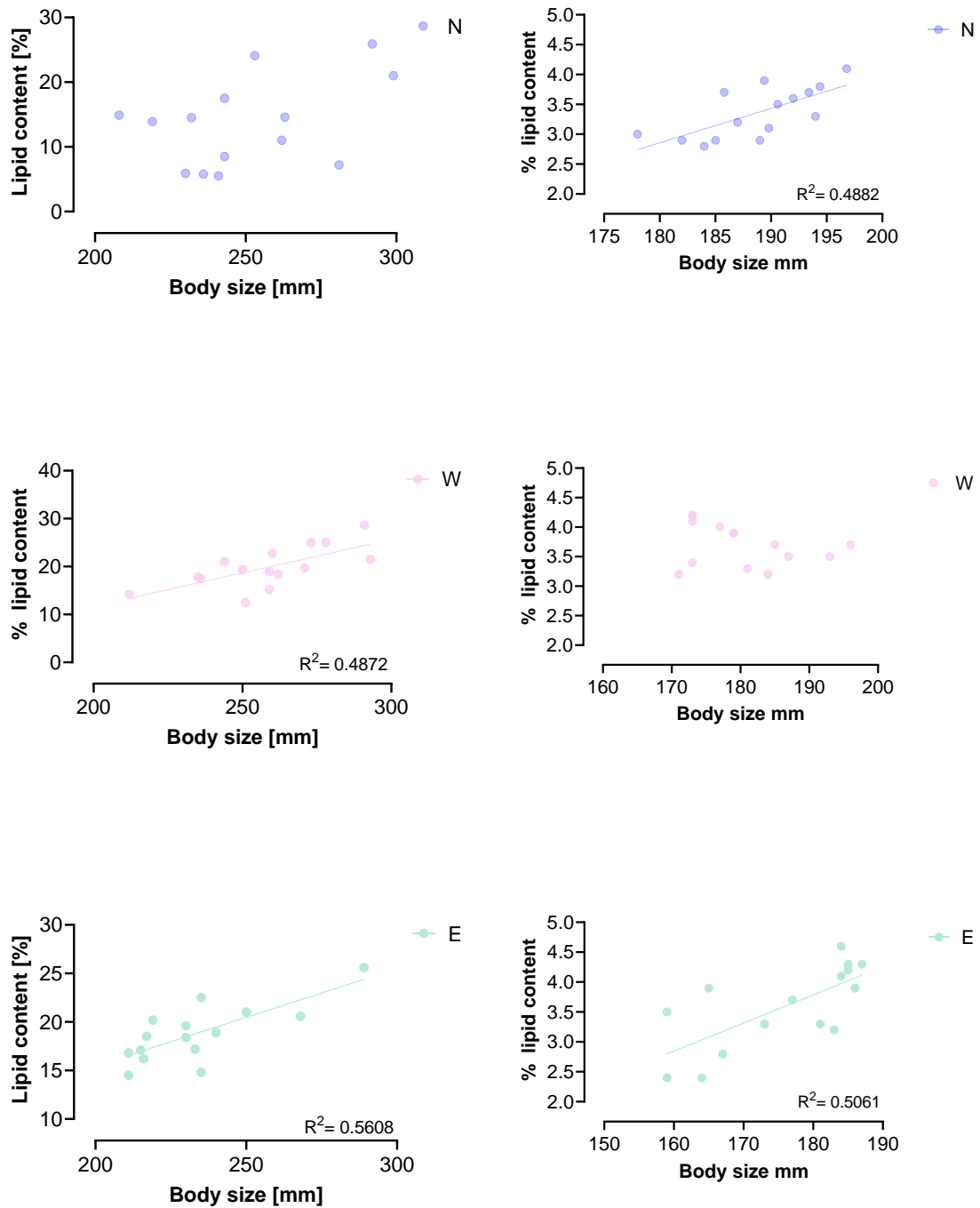


Figure 5.5 Linear regressions (including goodness of fit) between body size and lipid content in tissue (left) and whole-fish (right) for each location. Linear regression is only shown for statistically significant relationships between both factors (Pearson correlation; $p < 0.05$).

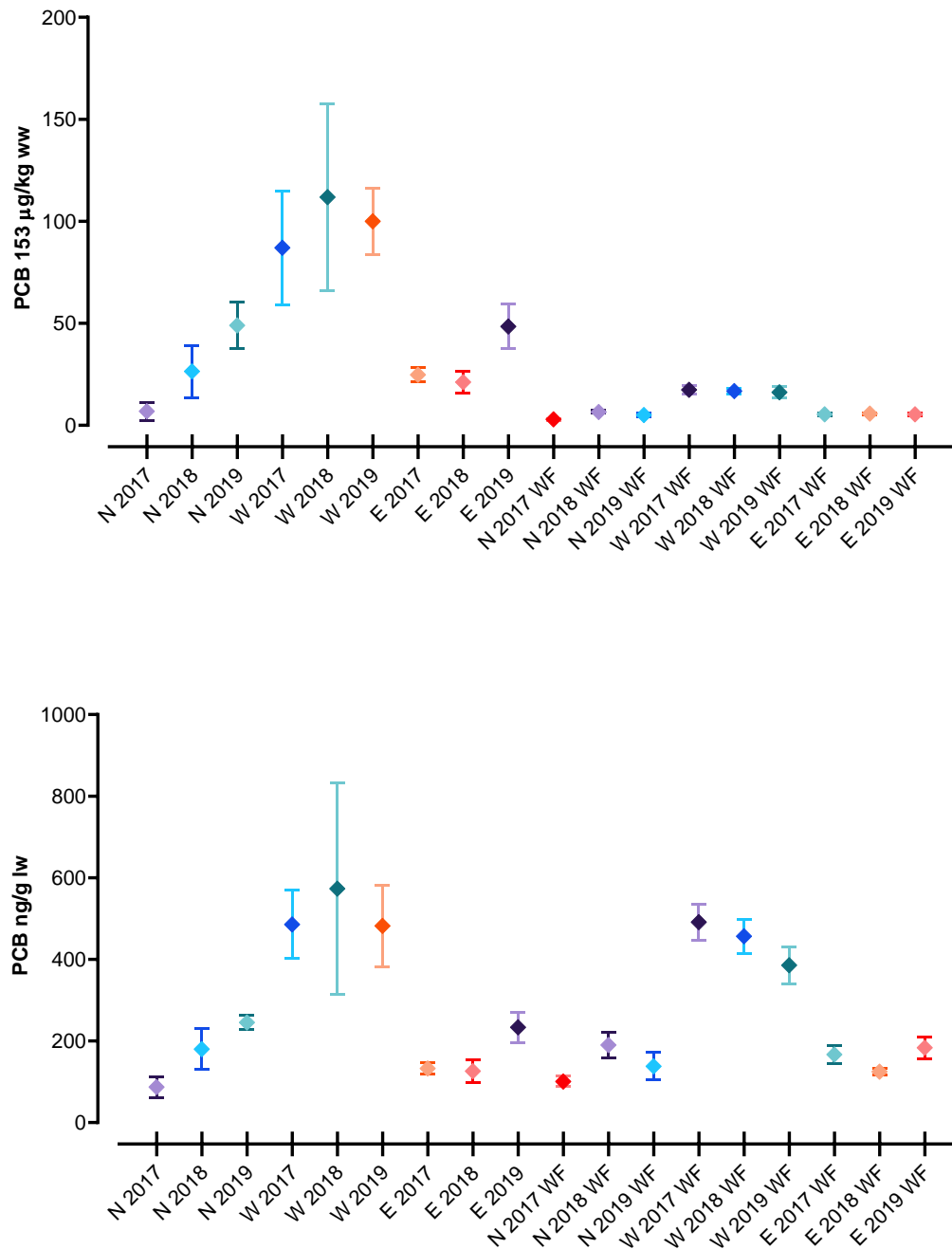


Figure 5.6 Concentrations of PCB 153 (Mean \pm STD) quantified in liver and whole-fish (WF) over all years and sample locations given in wet weight (ww, top) and lipid weight (lw, bottom).

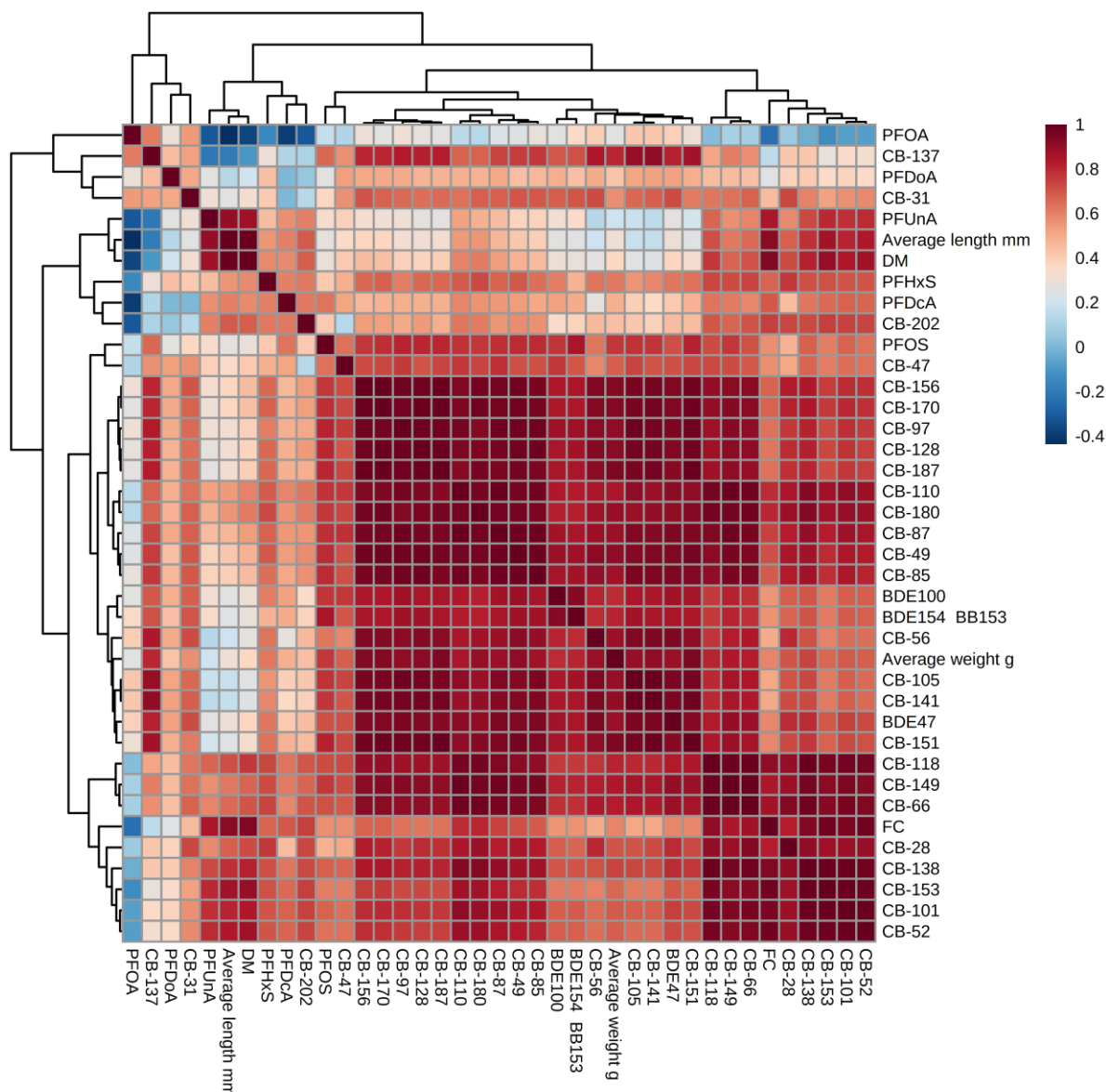


Figure 5.7 Pearson correlation matrix for chemicals screened within this present study. Grouping of PCB concentration detected in fish in years (2017, 2018, and 2019) and tissue matrix (liver and whole-fish) and running a Pearson correlation in MetaboAnalystR 3.0 to understand the relationship between co-variables. [DM= dry matter, FC= fat content]

5.2.1 Effect of variability

As there is a considerable margin in the estimated concentrations in liver, these concentrations can be either higher or lower than the actual concentrations. If the actual concentrations in the liver are close to a regulatory limit (BAC, EC, EAC, FEQG), the estimated concentrations can easily change from “below” to “above” the limit and vice versa. For managerial purposes, this is not desirable. To give an insight into this phenomenon, the measured contaminant concentrations in livers of the last decade, depicted in the report Biomonitoring part I, have been visually scored as on average below or above a regulatory limit. At a concentration in liver more than 2 times (in case of POPs) or 3 times (in case of metals) lower or higher than a particular regulatory limit, an estimated concentration is not likely to change this situation (in compliance or not). However, when the concentrations in the liver are close to a particular limit, an estimated concentration can easily affect whether or not it is in compliance with this limit. In table 7 below these two scenarios are shown with colour codes.

Table 7 Overview of possible effect of estimating liver concentrations.

Green; low risk, the concentrations in liver are more than 2 (in case of POPs) or 3 times (in case of metals) lower or higher than the regulatory limit. Using an estimated concentration is not likely to have an effect on compliance.

Red; high risk; the concentrations in liver are less than 2 (in case of POPs) or 3 times (in case of metals) lower or higher than the regulatory limit. Using an estimated concentration is likely to have an effect on compliance.

Nota bene The colour only indicates whether or not there is a risk on a different interpretation of the result (does the liver comply to the regulatory limit or not). It does not indicate if the levels are in compliance or not.

	Water		Cadmium		Kwik		Lood		PCB28		PCB52		PCB101		PCB118		PCB138		PCB153		PCB180		HCB		BDE-28		BDE-47		BDE-99		BDE-100		HBCDD	
			BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC	BAC	EC		
N			Red	Green	Green	Green	Red	Red	Green	Green	Green	Green	Red	Green	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	
Z			Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	
E			Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	
D			Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	
W			Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	
S			Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	

6 Conclusions

Overall, the results shown within the present study are in line with expectations and confirmed by observations in the literature, where tissue concentrations of chemicals in liver and fillet are higher than whole body burdens of contaminants for most of the compounds. Lead is an exception as it predominantly accumulates more in bones than in soft tissues⁴, therefore, the whole-fish concentrations tend to be higher. Hence, the organ/tissues selected within the present study represent the respective target tissue for bioaccumulation.

Generally, for organics e.g. PCB's, extrapolation of data is possible with tissue to whole body conversion factors. We observed significant correlation between body size and lipid content across the majority of locations and years. The ranges and variability between locations and years are comparable, therefore, reducing uncertainties for correlations. In contrast, metals have individual and more variable ratio's between whole body and liver/fillet and extrapolation need to be done with caution and higher variability factors need to be considered as well. However, for mercury, the ratio's are confirmed with literature values and they are comparable between species.

Overall, data presented within this study is the first step towards superseding the need for monitoring different matrices (e.g., whole-fish and liver). The predictive conversion factors can be applied to estimate the liver/fillet concentrations based on whole body burden of chemicals. However, how to address uncertainties into account as well as implication of such CFs needs to be considered. To date, no predictive approaches are applied within biota monitoring.

7 Future Recommendations

How to take into account uncertainty ?

The environmental quality assessment under the umbrella of OSPAR and WFD targets to protect the ecosystems via secondary poisoning, hence, the whole-fish concentrations are the most suitable matrix to assess the risk for ecosystem health. Human safety is the second target of WFD, implying that only consumable parts of fish need to be evaluated. We have shown that the intra-species differences for PCBs (calculated with the conversion factor) are within factor 2, for metals within factor 3, and for other chemicals within factor 3 of the actual measured concentration.

It must be noted that predictions are not a common approach within regulatory directives using biota monitoring and there are no harmonized approaches to take into account uncertainty factors. One approach to overcome these uncertainties is proposed by the World Health Organization (Figure 7.1). Such uncertainty factors can differ depending on the underlying data of predictions. The factor 3.16, for example, is been used for intraspecies differences in toxicokinetic (fate of the compound within the body of the animal) within the approach of WHO. If data is poorly presented the uncertainty factor could be more conservative. However, with complex modelling, particularly those which take into account the physiology and biology as well as physico-chemical properties of compounds, the uncertainty factors could be less conservative as the exposure scenarios are well predicted and can be extrapolated to other species or scenarios.

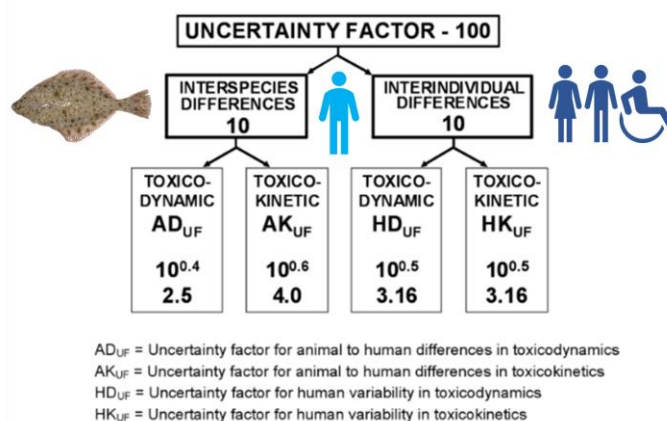


Figure 7.1 Uncertainty factor to harmonize the approaches to assess the risk from exposure to chemicals (Figure adjusted from²³)

How to implement the empirical approach in future studies?

As explained previously, to predict internal concentrations in fish species and extrapolate these to any exposure scenarios more complex models need to be considered. With the present empirical approach, only interpolations are possible when the sampling approach remains unchanged. A tiered approach, as shown in Figure 7.2, can be one option to implement such predictions within biota monitoring. After screening for chemicals in whole fish, the concentration ranges that allow

interpolations to tissue levels (liver or fillet) can be used as a replacement. However, if interpolations are not possible and the levels detected for the specific compound differ significantly (within the minimum and maximum level found in whole-fish) in subsequent years (following sampling efforts) the liver samples should be taken and analysed, as extrapolations are not possible with the current approach. However, the novel values can serve as a baseline for conversion factors that encompass wider concentration ranges, hence, the subsequent year the approach can start on tier 1. As mentioned prior, uncertainty factors need to be considered if such approaches are actively implemented within biota monitoring.

Tiered approach for application

Figure 7.2 Tiered approach for a possible application of conversion factors in biota monitoring

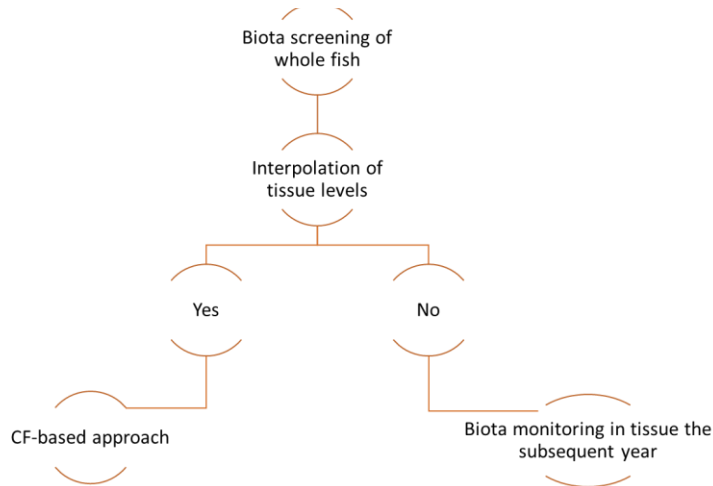


Figure 5.2 Tiered approach for a possible application of conversion factors in biota monitoring

Figure 7.2 Tiered approach for a possible application of conversion factors in biota monitoring

To decrease the uncertainty and to draw relationships based on predictive power, computer aided tools (toxicokinetic modelling) can be taken into consideration. These novel tools allow an indispensable approach to look beyond straight forward biomonitoring data and mathematical define uncertainties, using computing power and statistical approaches. However, a discussion within experts and researchers on how to approach uncertainties is highly recommended prior to implementations. Apart from this, the presented tiered approach can be applied for predictions of concentrations in liver based on levels detected in whole-fish with limited predictive power.

Figure 6.2 Tiered approach for a possible application of conversion factors in biota monitoring

8 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. This certificate is valid until 15 December 2021. The organisation has been certified since 27 February 2001. The certification was issued by DNV GL.

Furthermore, the chemical laboratory at IJmuiden has EN-ISO/IEC 17025:2017 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2021 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation. The chemical laboratory at IJmuiden has thus demonstrated its ability to provide valid results according a technically competent manner and to work according to the ISO 17025 standard. The scope (L097) of de accredited analytical methods can be found at the website of the Council for Accreditation (www.rva.nl).

On the basis of this accreditation, the quality characteristic Q is awarded to the results of those components which are incorporated in the scope, provided they comply with all quality requirements. The quality characteristic Q is stated in the tables with the results. If, the quality characteristic Q is not mentioned, the reason why is explained.

The quality of the test methods is ensured in various ways. The accuracy of the analysis is regularly assessed by participation in inter-laboratory performance studies including those organized by QUASIMEME. If no inter-laboratory study is available, a second-level control is performed. In addition, a first-level control is performed for each series of measurements.

In addition to the line controls the following general quality controls are carried out:

- Blank research.
- Recovery.
- Internal standard
- Injection standard.
- Sensitivity.

The above controls are described in Wageningen Marine Research working instruction ISW 2.10.2.105. If desired, information regarding the performance characteristics of the analytical methods is available at the chemical laboratory at IJmuiden.

If the quality cannot be guaranteed, appropriate measures are taken.

9 References

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10 Justification

Report C065/20

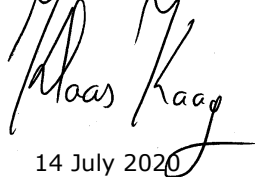
Project Number: 4316100131

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved: Dr.N.H.B.M. Kaag

Researcher

Signature:

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Date:

14 July 2020

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With knowledge, independent scientific research and advice, **Wageningen Marine Research** substantially contributes to more sustainable and more careful management, use and protection of natural riches in marine, coastal and freshwater areas.



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11 Appendix

The supplementary information consists of tables with the raw data.

Table 1 Sample analysis ID and the respective biometric information of all biota samples caught for this present project.

Sample Analysis ID	Sample matrix	Year	N	Average weight [g]	Average weight liver [g]	Average length [mm]	STD	CV [%]
N1	Filet	2017	5	152.2	2.8	223	12	5%
N2	Filet	2017	5	230.2	5.4	263	18	7%
N3	Filet	2017	5	247.6	4.1	261	41	16%
N4	Filet	2017	5	220.4	5.2	253	2	1%
N5	Filet	2017	5	159	3.4	231	17	7%
N6	Liver	2017	5	168.6	4	230	16	7%
N7	Liver	2017	5	188.4	4.2	243	22	9%
N8	Liver	2017	5	173.4	3.9	236	12	5%
N9	Liver	2017	5	195.4	4.5	241	9	4%
N10	Liver	2017	5	251.4	5.3	262	44	17%
N11	Whole-fish	2017	5	89.2		184	11	6%
N12	Whole-fish	2017	5	93.4		185	12	7%
N13	Whole-fish	2017	5	77.2		178	10	6%
N14	Whole-fish	2017	5	94.4		189	9	5%
N15	Whole-fish	2017	5	91.6		182	14	8%
N16	Filet	2018	5	151.6	1.6	233	26	11%
N17	Filet	2018	5	241	2.4	288	23	8%
N18	Filet	2018	5	219	2.2	273	22	8%
N19	Filet	2018	5	207.4	2.9	254	36	14%
N20	Filet	2018	5	197	2.7	253	40	16%
N21	Liver	2018	5	122	1.8	219	18	8%
N22	Liver	2018	5	170.4	2.4	243	27	11%
N23	Liver	2018	5	146.8	1.7	232	15	6%
N24	Liver	2018	5	250.4	2.3	281	39	14%
N25	Liver	2018	5	307.6	4.1	301	17	6%
N26	Whole-fish	2018	5	74.8		190.6	7	4%
N27	Whole-fish	2018	5	71.8		187	6	3%
N28	Whole-fish	2018	5	78.2		189.4	8	4%
N29	Whole-fish	2018	5	72.5		189.8	10	5%
N30	Whole-fish	2018	5	72.6		185.8	9	6%
N31	Filet	2019	5	290.6	4	306	27	9%

Sample Analysis ID	Sample matrix	Year	N	Average weight [g]	Average weight liver [g]	Average length [mm]	STD	CV [%]
N32	Filet	2019	5	251	3	286	22	8%
N33	Filet	2019	5	160.2	2	246	58	24%
N34	Filet	2019	5	190.2	3	250	55	22%
N35	Filet	2019	5	195.4	2.2	256	35	14%
N36	Liver	2019	5	291.4	4.7	292	36	12%
N37	Liver	2019	5	293.6	4.3	299	28	9%
N38	Liver	2019	5	197	2.6	253	45	18%
N39	Liver	2019	5	109	1.5	208	4	2%
N40	Liver	2019	5	208.2	2.3	263	43	16%
N41	Whole-fish	2019	5	88.6		193.4	11	6%
N42	Whole-fish	2019	5	100.4		196.8	9	5%
N43	Whole-fish	2019	5	93.2		312.2	267	85%
N44	Whole-fish	2019	5	89		194	3	1%
N45	Whole-fish	2019	5	88.6		194.4	8	4%
W1	Filet	2017	5	162	2.6	235	14	6%
W2	Filet	2017	5	135.4	2	232	20	9%
W3	Filet	2017	5	116.8	1.3	223	7	3%
W4	Filet	2017	5	139	1.6	232	12	5%
W5	Filet	2017	5	127.6	1.8	221	16	7%
W6	Liver	2017	5	185.8	3	251	40	16%
W7	Liver	2017	5	192.6	2.9	250	49	20%
W8	Liver	2017	5	186.4	3.2	244	47	19%
W9	Liver	2017	5	158.8	2.7	235	23	10%
W10	Liver	2017	5	154.4	2.4	236	36	15%
W11	Whole-fish	2017	5	73.8		193	10	5%
W12	Whole-fish	2017	5	67		181	14	8%
W13	Whole-fish	2017	5	89		196	3	2%
W14	Whole-fish	2017	5	78		185	9	5%
W15	Whole-fish	2017	5	69.8		187	7	4%
W16	Filet	2018	5	222.6	3.2	264	53	20%
W17	Filet	2018	5	208	2.7	260	48	18%
W18	Filet	2018	5	173.2	3.1	238	39	16%
W19	Filet	2018	5	177	3	249	38	15%
W20	Filet	2018	5	271.8	4.6	288	12	4%
W21	Liver	2018	5	224.2	3.8	259	44	17%
W22	Liver	2018	5	214.8	4.7	259	38	15%
W23	Liver	2018	5	236.8	4.4	278	7	3%
W24	Liver	2018	5	243.8	4.4	271	50	18%
W25	Liver	2018	5	217.8	3.2	260	50	19%

Sample Analysis ID	Sample matrix	Year	N	Average weight [g]	Average weight liver [g]	Average length [mm]	STD	CV [%]
W26	Whole-fish	2018	5	61.8		173	14	8%
W27	Whole-fish	2018	5	57.2		171	14	8%
W28	Whole-fish	2018	5	68.2		179	9	5%
W29	Whole-fish	2018	5	57.8		173	15	9%
W30	Whole-fish	2018	5	72.8		179	11	6%
W31	Filet	2019	5	290	4.9	300	37	12%
W32	Filet	2019	5	283.2	4.8	301	48	16%
W33	Filet	2019	5	179.2	3	242	38	16%
W34	Filet	2019	5	204.2	3.4	253	48	19%
W35	Filet	2019	5	168.2	2.3	244	24	10%
W36	Liver	2019	5	285.6	5.6	291	48	16%
W37	Liver	2019	5	249.4	5.2	273	42	15%
W38	Liver	2019	5	274.8	5.8	293	17	6%
W39	Liver	2019	5	103	1.5	212	8	4%
W40	Liver	2019	5	203.2	2.4	262	26	10%
W41	Whole-fish	2019	5	68.4		184	16	9%
W42	Whole-fish	2019	5	67		177	18	10%
W43	Whole-fish	2019	5	70.2		184	11	6%
W44	Whole-fish	2019	5	55.8		173	9	5%
W45	Whole-fish	2019	5	55.8		173	9	5%
W46	Filet	2017	5	103.6	1.1	215	7	3%
W47	Filet	2017	5	197.2	3.5	253	29	11%
W48	Filet	2017	5	114.8	1.6	220	18	8%
W49	Filet	2017	5	125.6	2.5	222	9	4%
W50	Filet	2017	5	132.2	2.4	225	6	3%
W51	Liver	2017	5	119.8	1.7	219	19	9%
W52	Liver	2017	5	114	1.6	217	2	1%
W53	Liver	2017	5	142.2	2.1	230	25	11%
W54	Liver	2017	5	114.8	1.5	233	18	8%
W55	Liver	2017	5	163.6	2.7	240	31	13%
W56	Whole-fish	2017	5	75.6		186	11	6%
W57	Whole-fish	2017	5	68		177	17	10%
W58	Whole-fish	2017	5	62		173	19	11%
W59	Whole-fish	2017	5	71.2		181	7	4%
W60	Whole-fish	2017	5	70.2		183	8	4%
W61	Filet	2018	5	212.2	2.9	263	41	16%
W62	Filet	2018	5	139.4	2.2	230	26	11%
W63	Filet	2018	5	335.6	7	296	58	20%
W64	Filet	2018	5	182.8	2.9	252	22	9%

Sample Analysis ID	Sample matrix	Year	N	Average weight [g]	Average weight liver [g]	Average length [mm]	STD	CV [%]
W65	Filet	2018	5	193	3.8	243	27	11%
W66	Liver	2018	5	119.6	2.1	216	10	5%
W67	Liver	2018	5	119	1.9	215	6	3%
W68	Liver	2018	5	145.8	2.5	230	33	14%
W69	Liver	2018	5	108.8	1.7	211	5	2%
W70	Liver	2018	5	111.4	1.9	211	4	2%
W71	Whole-fish	2018	5	72.6		185	14	8%
W72	Whole-fish	2018	5	68.8		184	12	7%
W73	Whole-fish	2018	5	72.6		185	6	3%
W74	Whole-fish	2018	5	74.6		187	9	5%
W75	Whole-fish	2018	5	76		184	14	8%
E71	Filet	2019	5	227.6	2.7	271	39	14%
E72	Filet	2019	5	248	3.2	279	17	6%
E73	Filet	2019	5	215.6	3.1	267	35	13%
E74	Filet	2019	5	195.8	2.2	259	33	13%
E75	Filet	2019	5	147.8	1.7	238	8	3%
E76	Liver	2019	5	219.6	3.2	268	31	12%
E77	Liver	2019	5	274.4	4.4	289	33	11%
E78	Liver	2019	5	157.6	2.3	235	41	17%
E79	Liver	2019	5	162.4	2.3	235	12	5%
E80	Liver	2019	5	191.4	2.1	250	41	16%
E81	Whole-fish	2019	5	51.2		165	10	6%
E82	Whole-fish	2019	5	51.2		167	10	6%
E83	Whole-fish	2019	5	43.4		159	9	6%
E84	Whole-fish	2019	5	52.2		164	12	7%
E85	Whole-fish	2019	5	47.4		159	6	4%

Table 2 Metal concentration in wet weight (mg/Kg) for each sample ID.

Sample Analysis ID	Total Hg	Cd	Cu	Pb	Zn
N1	0.08	0.026	9.2	0.012	33
N2	0.098	0.023	12	0.016	35
N3	0.058	0.036	13	0.011	36
N4	0.12	0.023	11	0.01	35
N5	0.1	0.024	11	0.01	33
N11	0.05	0.002	0.57	0.019	23
N12	0.06	0.002	0.61	0.017	21
N13	0.062	0.0031	0.59	0.045	22
N14	0.054	0.0027	0.61	0.027	23
N15	0.058	0.0023	0.61	0.044	22
N16	0.087	0.068	33	0.042	49
N17	0.12	0.15	22	0.031	46
N18	0.13	0.12	20	0.034	46
N19	0.14	0.079	21	0.035	44
N20	0.13	0.081	22	0.047	45
N26	0.072	0.005	0.79	0.064	23
N27	0.06	0.004	0.79	0.038	23
N28	0.076	0.004	0.78	0.029	22
N29	0.068	0.004	0.76	0.035	24
N30	0.069	0.005	0.8	0.038	25
N31	0.21	0.13	19.5	0.049	49
N32	0.2	0.11	18.2	0.036	48
N33	0.2	0.2	14.3	0.033	43
N34	0.17	0.096	21.5	0.032	48
N35	0.17	0.099	28.7	0.028	56
N41	0.068	0.0035	0.67	0.03	22
N42	0.063	0.0027	0.68	0.022	23
N43	0.073	0.004	0.64	0.034	23
N44	0.062	0.0032	0.68	0.034	28
N45	0.07	0.0026	0.65	0.034	32
W1	0.12	0.19	15	0.016	36
W2	0.11	0.25	20	0.027	44
W3	0.12	0.32	26	0.029	52
W4	0.09	0.24	16	0.047	36
W5	0.1	0.22	30	0.024	53
W11	0.062	0.013	1.2	0.048	22
W12	0.069	0.021	1.3	0.055	26
W13	0.065	0.014	1.3	0.046	23
W14	0.063	0.014	1.1	0.059	26
W15	0.065	0.013	1.1	0.043	25
W16	0.16	0.49	12	0.013	32
W17	0.14	0.41	17	0.016	39
W18	0.11	0.27	20	0.028	41
W19	0.12	0.3	17	0.023	37
W20	0.13	0.32	25	0.021	46
W26	0.065	0.016	0.98	0.043	24
W27	0.06	0.014	0.9	0.058	25
W28	0.066	0.011	0.77	0.041	26
W29	0.066	0.013	0.84	0.038	26
W30	0.062	0.012	0.87	0.029	26
W31	0.21	0.37	26.9	0.02	58
W32	0.22	0.35	27.8	0.02	58

W33	0.15	0.21	24.7	0.026	50
W34	0.21	0.28	27.7	0.023	54
W35	0.17	0.19	19.4	0.024	46
W41	0.053	0.012	0.75	0.097	25
W42	0.045	0.01	0.85	0.05	26
W43	0.045	0.0081	0.65	0.042	25
W44	0.05	0.0084	0.77	0.038	25
W45	0.05	0.0084	0.77	0.038	25
W46	0.15	0.27	37	0.033	74
W47	0.19	0.33	29	0.022	58
W48	0.09	0.14	25	0.03	59
W49	0.089	0.21	26	0.022	52
W50	0.085	0.19	27	0.023	62
W56	0.075	0.0082	1	0.032	22
W57	0.064	0.0096	1.1	0.032	22
W58	0.064	0.0097	1.1	0.034	21
W59	0.06	0.013	1.1	0.026	20
W60	0.068	0.011	1.1	0.03	21
W61	0.094	0.14	25	0.023	50
W62	0.084	0.23	25	0.022	47
W63	0.1	0.23	32	0.019	55
W64	0.099	0.19	23	0.018	52
W65	0.13	0.15	22	0.019	46
W71	0.081	0.017	1.5	0.041	23
W72	0.083	0.013	1.3	0.041	26
W73	0.083	0.017	1.4	0.035	21
W74	0.086	0.015	1.2	0.029	21
W75	0.086	0.015	1.4	0.025	20
E71	0.15	0.23	38.3	0.044	57
E72	0.22	0.33	31.8	0.037	58
E73	0.17	0.21	24.8	0.027	49
E74	0.15	0.48	26	0.044	55
E75	0.12	0.35	28.5	0.035	55
E81	0.038	0.0086	1	0.059	24
E82	0.041	0.013	0.98	0.074	27
E83	0.034	0.011	0.97	0.083	27
E84	0.041	0.012	0.85	0.11	30
E85	0.04	0.011	1	0.069	28

Table 3 BDE and HBCD concentrations for each sample ID given in wet weight. Below limit of quantification is indicated with '<'.

Sample Analysis ID	BDE100	BDE153	BDE154 + BB153	BDE183	BDE28	BDE47	BDE66	BDE85	BDE99	HBCD	HCB	HCBd	Heptachloor
N6	<0.1	0.1	0.4	<0.09	<0.07	0.2	<0.2	<0.1	<0.09	<1.2	0.4	<0.3	<0.3
N7	0.2	<0.08	0.4	<0.09	<0.07	0.4	<0.2	<0.1	<0.09	<1.2	0.5	<0.3	<0.3
N8	<0.1	<0.07	0.3	<0.08	<0.06	0.2	<0.2	<0.1	<0.07	<1.0	<0.08	<0.3	<0.3
N9	<0.09	<0.06	0.5	<0.07	<0.05	0.2	<0.1	<0.09	<0.06	<0.8	0.3	<0.3	<0.3
N10	0.2	<0.07	0.5	<0.08	<0.06	0.3	<0.2	<0.1	<0.07	<1.0	0.6	<0.3	<0.3
N11	<0.030	<0.020	<0.016	<0.021	<0.018	<0.084	<0.087	<0.033	<0.021		<0.12	nb	
N12	<0.031	<0.021	<0.016	<0.022	<0.019	0.095	<0.091	<0.034	<0.022		<0.12	nb	
N13	<0.031	<0.021	<0.016	<0.022	<0.019	0.093	<0.091	<0.034	<0.022		<0.12	nb	
N14	<0.030	<0.020	<0.016	<0.022	<0.019	<0.086	<0.089	<0.033	<0.021		<0.12	nb	
N15	<0.030	<0.020	<0.016	<0.021	<0.018	<0.084	<0.087	<0.033	<0.021		<0.10	nb	
N21	<0.1	<0.07	<0.08	<0.1	<0.2	0.4	<0.3	<0.1	<0.1	<2.1	1.8	<0.4	<1.4
N22	<0.1	<0.07	<0.07	<0.09	<0.2	1	<0.3	<0.1	0.2	<2.0	1.7	<0.4	<1.3
N23	<0.1	<0.07	<0.08	<0.1	<0.2	0.7	<0.4	<0.1	<0.1	<2.2	1.8	<0.4	<1.3
N24	<0.1	<0.08	<0.09	<0.1	<0.2	0.5	<0.4	<0.1	<0.1	<2.4	0.9	<0.4	<1.3
N25	<0.1	<0.08	<0.09	<0.1	<0.2	1	<0.4	<0.1	<0.1	<2.4	1.7	<0.4	<1.3
N26	0.1	0.06	0.06	<0.05	<0.02	0.3	<0.02	<0.03	0.06		<0.5	<0.02	
N27	0.08	0.06	0.06	<0.05	<0.02	0.2	<0.02	<0.03	0.06		<0.5	<0.02	
N28	0.09	0.06	0.06	<0.05	<0.02	0.3	<0.02	<0.03	0.06		<0.4	<0.02	
N29	0.09	0.06	0.06	<0.05	<0.02	0.3	<0.02	<0.03	0.06		<0.4	<0.02	
N30	0.1	0.06	0.06	<0.05	<0.02	0.3	<0.02	<0.03	0.06		<0.4	<0.02	
N36	0.6	<0.09	0.4	<0.3	<0.05	2.1	<0.05	<0.2	0.3	<0.6	<0.14	<0.64	<0.61
N37	0.6	<0.1	0.4	<0.4	<0.05	1.5	<0.05	<0.2	<0.2	<0.7	<0.16	<0.71	<0.67
N38	0.5	<0.1	0.2	<0.4	<0.06	1.2	<0.06	<0.2	0.3	<0.8	<0.15	<0.69	<0.65
N39	0.4	<0.1	0.2	<0.4	<0.06	1.1	<0.06	<0.2	0.2	<0.8	<0.15	<0.67	<0.64
N40	0.5	<0.1	0.2	<0.4	<0.05	1.4	<0.05	<0.2	0.2	<0.7	<0.15	<0.66	<0.63
N41	0.04	<0.04	<0.04	<0.05	<0.02	0.2	<0.02	<0.03	<0.03		0.3	<0.02	
N42	<0.03	<0.04	<0.04	<0.05	<0.02	0.1	<0.02	<0.03	<0.03		0.3	<0.02	
N43	0.03	<0.04	<0.04	<0.05	<0.02	0.1	<0.02	<0.03	<0.03		0.3	<0.02	
N44	0.04	<0.04	0.05	<0.05	<0.02	0.2	<0.02	<0.03	<0.03		0.3	<0.02	
N45	<0.03	<0.04	<0.04	<0.05	<0.02	0.2	<0.02	<0.03	<0.03		0.3	<0.02	

W6	0.3	0.3	0.5	<0.07	<0.05	1.2	<0.2	<0.1	<0.07	<0.9	0.5	<0.3	<0.3
W7	0.4	0.2	0.5	<0.09	0.1	1.7	<0.2	<0.1	<0.08	<1.1	1.1	<0.3	<0.3
W8	0.9	0.3	0.8	<0.08	0.3	3.2	<0.2	<0.1	0.1	<1.1	1.6	<0.3	<0.3
W9	0.5	0.2	0.5	<0.09	0.2	1.6	<0.2	<0.1	<0.09	<1.2	1.3	<0.3	<0.3
W10	0.3	0.2	0.3	<0.08	0.1	1.2	<0.2	<0.1	<0.08	<1.1	1.4	<0.3	<0.3
W11	0.05	<0.021	0.025	<0.022	<0.019	0.37	<0.090	<0.034	<0.021		<0.15	nb	
W12	0.052	<0.021	0.02	<0.022	<0.019	0.35	<0.090	<0.034	<0.021		<0.15	nb	
W13	0.13	0.021	0.079	<0.021	0.066	0.64	<0.088	<0.033	<0.021		<0.17	nb	
W14	0.057	<0.020	0.026	<0.022	0.021	0.37	<0.089	<0.033	<0.021		nb	nb	
W15	0.089	<0.020	0.038	<0.021	<0.018	0.49	<0.087	<0.033	<0.021		<0.15	nb	
W21	0.3	<0.06	<0.07	<0.08	<0.2	1.6	<0.3	<0.09	0.3	<1.8	<0.8	<0.4	<1.4
W22	0.1	<0.06	<0.06	<0.08	<0.2	1.6	<0.3	<0.09	0.2	<1.7	<0.8	<0.5	<1.7
W23	<0.1	<0.08	<0.08	<0.1	<0.2	1.1	<0.4	<0.1	0.2	<2.3	<1.0	<0.4	<1.5
W24	0.2	<0.07	<0.08	<0.1	<0.2	2.5	<0.3	<0.1	0.2	<2.1	<1.1	<0.5	<1.7
W25	0.2	<0.07	<0.08	<0.1	<0.2	1.7	<0.4	<0.1	0.2	<2.2	1	<0.4	<1.4
W26	0.1	0.05	0.07	<0.05	<0.02	0.4	<0.02	<0.03	0.05		<0.2	<0.02	
W27	0.1	0.06	0.08	<0.05	<0.02	0.4	<0.02	<0.03	0.04		<0.2	<0.01	
W28	0.1	0.06	0.09	<0.05	<0.03	0.5	<0.02	<0.03	0.06		<0.3	<0.02	
W29	0.1	0.07	0.09	<0.05	<0.02	0.6	<0.02	<0.03	0.07		<0.8	<0.04	
W30	0.1	0.06	0.08	<0.05	<0.02	0.4	<0.02	<0.03	0.05		<0.2	<0.02	
W36	0.4	0.1	0.3	<0.3	<0.05	1.1	<0.05	<0.2	<0.2	<0.7	<0.15	<0.68	<0.65
W37	0.7	<0.1	0.3	<0.4	<0.05	3.3	<0.05	<0.2	0.3	<0.7	<0.14	<0.61	<0.58
W38	0.4	0.2	0.4	<0.4	<0.06	1.4	<0.05	<0.2	0.2	<0.7	<0.14	<0.61	<0.58
W39	0.3	0.1	0.2	<0.3	<0.05	0.8	<0.05	<0.2	<0.2	<0.7	<0.15	<0.69	<0.66
W40	0.4	<0.1	0.3	<0.4	<0.05	1.3	<0.05	<0.2	<0.2	<0.7	<0.14	<0.65	<0.61
W41	0.06	<0.04	0.05	<0.05	<0.02	0.2	<0.02	<0.03	<0.03		0.09	<0.02	
W42	0.08	<0.04	0.07	<0.05	<0.02	0.3	<0.02	<0.03	<0.03		0.1	<0.02	
W43	0.1	<0.04	0.08	<0.05	<0.02	0.4	<0.02	<0.03	0.03		0.1	<0.02	
W44	0.09	<0.04	0.06	<0.05	<0.02	0.3	<0.02	<0.03	0.03		0.1	<0.02	
W45	0.09	<0.04	0.06	<0.05	<0.02	0.3	<0.02	<0.03	0.03		0.1	<0.02	
W51	0.1	0.2	0.5	<0.06	0.2	0.8	<0.1	<0.08	<0.05	<0.7	2.6	<0.4	<0.4
W52	<0.2	0.2	0.6	<0.1	0.1	0.4	<0.2	<0.2	<0.1	<1.5	2.7	<0.4	<0.4
W53	0.1	<0.06	0.4	<0.07	0.08	0.5	<0.1	<0.09	<0.06	<0.9	2.6	<0.3	<0.3

W54	0.1	0.2	0.5	<0.06	0.1	0.4	<0.1	<0.09	<0.06	<0.8	2.4	<0.3	<0.3
W55	0.1	0.1	0.9	<0.08	0.3	0.8	<0.2	<0.1	0.2	<1.0	1.9	<0.3	<0.3
W56	<0.032	<0.022	<0.017	<0.023	0.031	0.1	<0.094	<0.035	<0.022		0.48	nb	
W57	<0.033	<0.022	<0.018	<0.024	0.034	0.12	<0.10	<0.036	<0.023		0.45	nb	
W58	<0.032	<0.022	<0.017	<0.023	0.034	0.12	<0.094	<0.035	<0.022		0.39	nb	
W59	<0.034	<0.023	<0.018	<0.024	0.033	0.11	<0.10	<0.038	<0.024		0.36	nb	
W60	<0.034	<0.023	<0.018	<0.024	0.028	0.11	<0.10	<0.037	<0.023		0.41	nb	
W66	<0.1	<0.07	<0.08	<0.1	<0.2	0.5	<0.4	<0.1	0.2	<2.2	2	<0.4	<1.2
W67	<0.1	<0.08	<0.08	<0.1	<0.2	0.4	<0.4	<0.1	<0.1	<2.3	1.6	<0.4	<1.2
W68	<0.1	<0.08	<0.08	<0.1	<0.2	0.5	<0.4	<0.1	<0.1	<2.3	1.9	<0.3	<1.2
W69	<0.1	<0.08	<0.08	<0.1	<0.2	0.5	<0.4	<0.1	<0.1	<2.3	1.7	<0.4	<1.2
W70	<0.1	<0.07	<0.08	<0.1	<0.2	0.3	<0.4	<0.1	<0.1	<2.2	1.2	<0.3	<1.2
W71	0.04	0.06	0.06	<0.05	<0.03	0.2	<0.02	<0.03	0.05		<0.5	<0.03	
W72	0.06	0.06	0.06	<0.05	<0.02	0.1	<0.02	<0.03	0.05		<0.5	<0.02	
W73	0.04	0.05	0.06	<0.05	<0.02	0.1	<0.02	<0.03	0.05		<0.5	<0.02	
W74	0.04	0.05	0.06	<0.05	<0.02	0.1	<0.02	<0.03	0.04		<0.5	<0.02	
W75	0.05	0.05	0.05	<0.05	<0.02	0.1	<0.02	<0.03	0.04		<0.5	<0.01	
E76	0.3	<0.1	0.3	<0.4	<0.05	0.8	<0.05	<0.2	<0.2	<0.7	<0.14	<0.64	<0.61
E77	0.3	<0.09	0.3	<0.3	<0.05	0.9	<0.05	<0.2	<0.2	<0.6	<0.14	<0.63	<0.60
E78	0.2	0.1	0.2	<0.4	<0.05	0.6	<0.05	<0.2	<0.2	<0.7	<0.13	<0.58	<0.55
E79	0.3	<0.1	0.3	<0.3	<0.05	0.7	<0.05	<0.2	0.2	<0.7	<0.13	<0.58	<0.55
E80	0.2	<0.1	0.2	<0.3	<0.05	0.7	<0.05	<0.2	<0.2	<0.7	<0.14	<0.64	<0.61
E81	<0.03	<0.04	0.05	<0.05	<0.02	0.1	<0.02	<0.03	<0.03		0.5	<0.02	
E82	<0.03	<0.04	<0.04	<0.05	<0.02	0.1	<0.02	<0.03	<0.03		0.3	<0.02	
E83	<0.03	<0.04	<0.04	<0.05	<0.02	0.09	<0.02	<0.03	<0.03		0.3	<0.02	
E84	<0.03	<0.03	<0.04	<0.05	<0.02	0.09	<0.02	<0.03	<0.03		0.3	<0.02	
E85	<0.03	<0.04	<0.04	<0.05	<0.02	0.1	<0.02	<0.03	<0.03		0.4	<0.02	

Table 4 PFAS concentrations for each sample ID given in wet weight. Below limit of quantification is indicated with '<'.

Sample Analysis ID	PFBA	PFBS	PFDCa	PFDoA	PFDS	PFHpA	PFHpS	PFHxA	PFHxS	PFNA	PFOA	PFOS	PFPeA	PFTeA	PFTrA	PFUnA
N6	<0.6	<0.5	<0.2	<0.2	<0.2	<0.6	<0.6	<0.6	<0.2	<0.2	<0.6	3.2	<0.6	<0.6	<0.6	0.2
N7	<0.6	<0.6	<0.2	<0.2	<0.2	<0.6	<0.6	<0.6	<0.2	<0.2	<0.6	7.8	<0.6	<0.6	<0.6	0.2
N8	<0.9	<0.8	<0.3	<0.3	<0.3	<0.9	<0.9	<0.9	<0.3	<0.3	<0.9	2.7	<0.9	<0.9	<0.9	0.3
N9	<0.6	<0.6	<0.2	<0.2	<0.2	<0.6	<0.7	<0.6	<0.2	<0.2	<0.6	3.6	<0.6	<0.6	<0.6	0.2
N10	<0.5	<0.5	<0.2	<0.2	<0.2	<0.5	<0.5	<0.5	<0.2	<0.2	<0.5	3.5	<0.5	<0.5	<0.5	0.4
N11	nb	<0.3	<0.2	<0.2	<0.3	<0.3	<0.3	<0.8	<0.3	<0.2	0.3	<0.3	<0.8	<0.3	<0.2	0.2
N12	nb	<0.2	<0.1	<0.1	<0.2	<0.2	<0.2	<0.6	<0.2	<0.1	0.5	<0.2	<0.6	<0.2	<0.1	0.3
N13	nb	<0.3	<0.1	<0.1	<0.3	<0.3	<0.3	<0.7	<0.3	<0.1	0.8	<0.3	<0.7	<0.3	<0.1	<0.1
N14	nb	<0.2	<0.1	<0.1	<0.3	<0.3	<0.3	<0.7	<0.3	<0.1	0.5	<0.3	<0.7	<0.3	<0.1	<0.1
N15	nb	<0.3	<0.2	<0.2	<0.3	<0.3	<0.3	<0.8	<0.3	<0.2	0.5	<0.3	<0.8	<0.3	0.7	<0.2
N21	nb	<3.2	<1.7	<1.7	<0.3	<1.7	<1.5	<1.7	<1.5	<1.7	<1.7	22	<1.7	<3.4	<1.7	<1.7
N22	nb	<2.7	<1.5	<1.5	<0.3	<1.5	<1.3	<1.5	<1.3	<1.5	<1.5	24	<1.5	<2.9	<1.5	<1.5
N23	nb	<3.2	<1.7	<1.7	1.8	<1.7	<1.6	<1.7	<1.5	<1.7	<1.7	20	<1.7	<3.5	<1.7	<1.7
N24	nb	<2.6	<1.4	<1.4	0.3	<1.4	<1.3	<1.4	<1.3	<1.4	<1.4	17	<1.4	<2.8	<1.4	<1.4
N25	nb	<3.5	<1.9	<1.9	1	<1.9	<1.7	<1.9	<1.7	<1.9	<1.9	28	<1.9	<3.7	<1.9	<1.9
N26	nb	<0.5	<0.2	<0.6	<0.2	<0.6	<0.5	<0.6	<0.5	<0.2	<0.2	5.4	<0.6	<1.2	<1.2	<0.2
N27	nb	<0.5	1.7	<0.6	<0.2	<0.6	<0.5	<0.6	<0.5	<0.2	<0.2	7.1	<0.6	<1.1	<1.1	<0.2
N28	nb	<0.6	1.4	<0.7	<0.3	<0.7	<0.6	<0.7	<0.6	<0.3	<0.3	6.5	<0.7	<1.4	<1.4	<0.3
N29	nb	<0.6	<0.3	<0.8	<0.3	<0.8	<0.7	<0.8	<0.7	<0.3	<0.3	11	<0.8	<1.5	<1.5	<0.3
N30	nb	<0.6	<0.3	<0.7	<0.3	<0.7	<0.6	<0.7	<0.6	<0.3	<0.3	9.3	<0.7	<1.4	<1.4	<0.3
N36	<0.7	<0.1	1	0.6	<0.1	<0.1	<0.1	<0.1	<0.1	0.6	0.6	11	<0.1	<0.1	<0.1	0.5
N37	<0.6	<0.1	1.5	0.4	<0.1	<0.1	<0.1	<0.1	<0.1	1	1.6	15	<0.1	<0.1	<0.1	0.6
N38	<0.8	<0.1	3.7	0.5	<0.2	<0.2	<0.2	<0.2	<0.2	1	1.3	16	<0.2	<0.2	<0.2	1.1
N39	<0.9	<0.1	0.8	0.3	<0.2	<0.2	<0.2	<0.2	<0.2	1.2	1.4	29	<0.2	<0.2	<0.2	0.7
N40	<0.7	<0.1	1.3	0.3	<0.1	<0.1	<0.1	<0.1	<0.1	0.6	0.6	6.9	<0.1	<0.1	<0.1	0.4
N41	<0.7	<0.6	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.6	1	0.9	4.2	<0.7	<0.7	<0.7	<0.7
N42	<0.7	<0.6	<0.7	<0.7	<0.6	<0.7	<0.6	<0.7	<0.6	0.9	0.8	3.9	<0.7	<0.7	<0.7	<0.7
N43	<0.2	<0.2	0.4	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.4	0.6	5.5	<0.2	<0.2	<0.2	<0.2

N44	<0.2	<0.2	0.7	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.8	0.7	6.9	<0.2	<0.2	<0.2	<0.2
N45	<0.2	<0.2	0.6	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.4	0.4	4.2	<0.2	<0.2	<0.2	<0.2
W6	<1.0	<1.0	<0.4	1.7	<0.4	<1.0	<1.1	<1.0	2.9	<0.4	1.3	110	<1.0	<1.0	<1.0	6.2
W7	<1.1	<1.1	<0.4	3.3	<0.4	<1.1	<1.1	<1.1	6.4	<0.4	<1.1	120	<1.1	<1.1	<1.1	11
W8	<1.2	<1.2	<0.5	2.8	<0.5	<1.2	<1.3	<1.2	7	<0.5	14	120	<1.2	<1.2	<1.2	6.9
W9	<0.5	<0.5	<0.2	2.1	<0.2	<0.5	<0.5	<0.5	5.5	<0.2	3.5	91	<0.5	<0.5	<0.5	4.9
W10	<1.0	<1.0	<0.4	0.8	<0.4	<1.0	<1.0	<1.0	3.1	<0.4	<1.0	82	<1.0	<1.0	<1.0	4.1
W11	nb	<0.2	2.9	0.9	<0.3	<0.3	<0.3	<0.7	<0.3	0.2	1	17	<0.7	0.6	0.9	1.7
W12	nb	<0.2	1.4	2.6	<0.3	<0.3	<0.3	<0.7	<0.3	<0.1	0.7	24	<0.7	0.6	1	2.1
W13	nb	<0.2	3.3	2.2	<0.2	<0.2	<0.2	<0.6	<0.2	0.6	1.1	24	<0.6	1.3	1	2.7
W14	nb	<0.2	1.8	1.9	<0.2	<0.2	<0.2	<0.6	<0.2	1.2	1.7	16	<0.6	0.6	0.9	2.7
W15	nb	<0.2	3.8	0.8	<0.2	<0.2	<0.2	<0.6	<0.2	0.8	1.4	37	<0.6	0.6	1.1	1.9
W21	nb	<2.2	<1.2	<1.2	0.6	<1.2	<1.1	<1.2	<1.0	<1.2	<1.2	100	<1.2	<2.3	<1.2	<1.2
W22	nb	<2.5	<1.4	<1.4	1.6	<1.4	<1.2	<1.4	<1.2	<1.4	<1.4	140	<1.4	<2.8	<1.4	<1.4
W23	nb	<2.4	<1.3	<1.3	0.6	<1.3	<1.2	<1.3	<1.2	<1.3	<1.3	120	<1.3	<2.6	<1.3	<1.3
W24	nb	<2.1	<1.1	<1.1	0.4	<1.1	<1.0	<1.1	<1.0	<1.1	<1.1	120	<1.1	<2.2	<1.1	<1.1
W25	nb	<2.4	<1.3	<1.3	<0.2	<1.3	<1.2	<1.3	<1.2	<1.3	<1.3	110	<1.3	<2.6	<1.3	<1.3
W26	nb	<0.5	1.7	<0.5	<0.2	<0.5	<0.5	<0.5	1.8	<0.2	<0.2	42	<0.5	<1.1	<1.1	<0.2
W27	nb	<0.7	2.3	<0.9	<0.3	<0.9	<0.8	<0.9	1.6	<0.3	<0.3	40	<0.9	<1.7	<1.7	3.2
W28	nb	<0.6	1.3	<0.8	<0.3	<0.8	<0.7	5.4	1.4	<0.3	<0.3	48	<0.8	<1.5	<1.5	<0.3
W29	nb	<0.6	3.2	<0.7	<0.2	<0.7	<0.6	<0.7	1.8	<0.3	<0.3	41	<0.7	<1.3	<1.3	4.8
W30	nb	<0.8	3.6	<0.9	<0.3	<0.9	<0.8	<0.9	2.3	<0.4	<0.4	43	<0.9	<1.8	<1.8	4.4
W36	<0.8	<0.1	6.8	1.9	<0.2	<0.2	1.6	<0.2	4.2	1.8	1.1	110	<0.2	<0.2	2.3	5.4
W37	<0.8	<0.1	10	2.6	<0.1	<0.2	2.1	<0.2	7.3	2.3	1.1	130	<0.2	<0.2	3.1	6.8
W38	<0.7	<0.1	7	1.9	<0.1	<0.1	1.7	<0.1	4.7	1.6	0.7	170	<0.1	<0.1	1.7	4.9
W39	<0.7	<0.1	5.6	1.6	<0.1	<0.1	2.2	<0.1	4.8	1.7	0.7	160	<0.1	<0.1	2.4	5.2
W40	<0.5	<0.09	10	3.1	<0.1	<0.1	2.4	<0.1	7.8	2.1	2.2	130	<0.1	<0.1	3.7	5.9
W41	<0.2	<0.2	4.9	0.9	<0.2	<0.2	0.9	<0.2	2.7	2	1.2	64	<0.2	<0.2	<0.2	3.2
W42	<0.3	<0.3	6.1	1.2	<0.3	<0.3	1	<0.3	2.8	2.1	1.1	73	<0.3	<0.3	<0.3	4.5
W43	<0.2	<0.2	5.1	0.8	<0.2	<0.2	0.9	<0.2	2.2	1.8	0.5	67	<0.2	<0.2	<0.2	3.2
W44	<0.2	<0.2	4.4	1.2	<0.2	<0.2	0.6	<0.2	1.3	1.5	0.6	56	<0.2	<0.2	<0.2	3.2
W45	<0.2	<0.2	4.4	1.2	<0.2	<0.2	0.6	<0.2	1.3	1.5	0.6	56	<0.2	<0.2	<0.2	3.2
W51	<1.2	<1.2	<0.5	<0.5	<0.5	<1.2	<1.3	<1.2	<0.5	<0.5	<1.2	19	<1.2	<1.2	<1.2	1

W52	<0.8	<0.8	<0.3	<0.3	<0.3	<0.8	<0.8	<0.8	<0.3	<0.3	<0.8	21	<0.8	<0.8	<0.8	0.6
W53	<0.9	<0.9	<0.4	<0.4	<0.4	<0.9	<1.0	<0.9	<0.3	<0.4	<0.9	15	<0.9	<0.9	<0.9	0.2
W54	<1.6	<1.6	<0.6	<0.6	<0.6	<1.6	<1.7	<1.6	<0.6	<0.6	<1.6	19	<1.6	<1.6	<1.6	0.8
W55	<1.0	<1.0	<0.4	<0.4	<0.4	<1.0	<1.1	<1.0	<0.4	<0.4	<1.0	21	<1.0	<1.0	<1.0	1.9
W56	nb	<0.2	0.4	0.7	<0.2	<0.2	<0.2	<0.6	<0.2	1.6	0.5	5.5	<0.6	1.3	0.7	0.6
W57	nb	<0.2	<0.1	<0.1	<0.2	<0.2	<0.2	<0.6	<0.2	0.7	1.8	4.2	<0.6	0.8	0.4	0.5
W58	nb	<0.2	2.8	<0.1	<0.2	<0.2	<0.2	<0.6	<0.2	0.8	0.9	5.4	<0.6	0.9	0.4	1
W59	nb	<0.2	1.9	<0.1	<0.2	<0.3	<0.2	<0.7	<0.2	1.2	0.8	5	<0.7	0.8	<0.1	0.5
W60	nb	<0.2	1.2	<0.1	<0.2	<0.2	<0.2	<0.5	<0.2	1	0.9	3.9	<0.5	<0.2	0.9	0.5
W66	nb	<2.7	<1.5	<1.5	0.7	<1.5	<1.3	<1.5	<1.3	<1.5	<1.5	28	<1.5	<2.9	<1.5	<1.5
W67	nb	<2.0	<1.1	<1.1	4.4	<1.1	<1.0	<1.1	<1.0	<1.1	<1.1	30	<1.1	<2.2	<1.1	<1.1
W68	nb	<2.5	<1.4	<1.4	<0.2	<1.4	<1.2	<1.4	<1.2	<1.4	<1.4	19	<1.4	<2.7	<1.4	<1.4
W69	nb	<3.1	<1.7	<1.7	<0.3	<1.7	<1.5	<1.7	<1.5	<1.7	<1.7	31	<1.7	<3.3	<1.7	<1.7
W70	nb	<3.8	<2.1	<2.1	8.6	<2.1	<1.8	<2.1	<1.8	<2.1	<2.1	27	<2.1	<4.1	<2.1	<2.1
W71	nb	<0.7	1.4	<0.9	<0.3	<0.9	<0.8	<0.9	<0.8	<0.3	<0.3	7.8	<0.9	<1.7	<1.7	<0.3
W72	nb	<0.6	1.8	<0.8	<0.3	<0.8	<0.7	<0.8	<0.7	<0.3	<0.3	7.9	<0.8	<1.5	<1.5	<0.3
W73	nb	<0.6	0.9	<0.7	<0.3	<0.7	<0.6	<0.7	<0.6	<0.3	<0.3	4.9	<0.7	<1.4	<1.4	<0.3
W74	nb	<0.7	<0.3	<0.8	<0.3	<0.8	<0.7	<0.8	<0.7	<0.3	<0.3	8.3	<0.8	<1.6	<1.6	<0.3
W75	nb	<0.6	0.9	<0.7	<0.2	<0.7	<0.6	<0.7	<0.6	<0.3	<0.3	7.5	<0.7	<1.4	<1.4	<0.3
E76	<0.4	<0.07	1.5	0.6	<0.08	<0.08	<0.08	<0.08	<0.08	1.5	0.9	14	<0.08	<0.08	1.3	1.6
E77	<0.7	<0.1	2.4	0.5	<0.1	<0.1	<0.1	<0.1	<0.1	1.6	0.7	15	<0.1	<0.1	0.7	1.3
E78	<0.5	<0.09	1.4	0.2	<0.10	<0.1	<0.10	<0.1	<0.10	2.1	1.5	21	<0.1	<0.1	0.3	0.9
E79	<0.8	<0.1	1.8	0.3	<0.1	<0.1	<0.1	<0.1	<0.1	2.1	2.1	17	<0.1	<0.1	<0.1	0.7
E80	<0.6	<0.1	3.8	0.6	<0.1	<0.1	<0.1	<0.1	<0.1	1.8	1	27	<0.1	<0.1	0.9	1.6
E81	<0.2	<0.2	0.9	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	1.3	0.8	7.1	<0.2	<0.2	<0.2	<0.2
E82	<0.2	<0.1	0.8	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	1	0.6	3.9	<0.2	<0.2	<0.2	<0.2
E83	<0.2	<0.2	0.9	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.9	0.5	4.2	<0.2	<0.2	<0.2	<0.2
E84	<0.2	<0.1	0.9	<0.2	<0.2	<0.2	<0.1	<0.2	<0.1	1	0.6	4.7	<0.2	<0.2	<0.2	<0.2
E85	<0.1	<0.1	0.4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.8	0.6	3.8	<0.1	<0.1	<0.1	<0.1

Table 5 PCB (200-128+174) concentrations for each sample ID given in wet weight. Below limit of quantification is indicated with '<'.</p>
</div>
<div data-bbox="62 158 978 865" data-label="Table">
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Sample Analysis ID
 CB-206 | CB-202 | CB-194 | CB-187 | CB-180 | CB-170 | CB-156 | CB-153 | CB-151 | CB-149 | CB-141 | CB-138 | CB-137 | CB-128+174 |

</thead>
 N6 | <0.2 | <0.1 | <0.2 | 1.5 | 0.9 | 0.3 | <0.2 | 4.8 | 0.7 | 1.7 | <0.2 | 2.7 | <0.1 | nb || N7 | <0.2 | <0.1 | <0.2 | 3.4 | 1.9 | 0.7 | 0.4 | 8.1 | 1.1 | 2.9 | <0.2 | 4.7 | <0.1 | nb |
| N8 | <0.2 | <0.1 | <0.2 | 1.2 | 0.6 | <0.2 | <0.2 | 3.5 | 0.5 | 1.1 | <0.2 | 2.1 | <0.1 | nb |
| N9 | <0.2 | <0.1 | <0.2 | 1.4 | 0.7 | <0.2 | <0.2 | 3.9 | 0.6 | 1.4 | <0.2 | 2.3 | <0.1 | nb |
| N10 | <0.2 | 0.3 | <0.2 | 5.6 | 3 | 1 | 0.5 | 14 | 2.1 | 4.5 | <0.2 | 8.1 | <0.1 | nb |
| N11 | nb | 0.11 | nb | 0.77 | 0.44 | 0.16 | 0.064 | 2.24 | 0.37 | 0.7 | <0.043 | 1.15 | <0.029 | |
| N12 | nb | 0.15 | nb | 1.04 | 0.53 | 0.2 | 0.087 | 3.12 | 0.53 | 1.01 | 0.07 | 1.55 | <0.030 | |
| N13 | nb | 0.15 | nb | 0.96 | 0.58 | 0.24 | 0.084 | 3.01 | 0.44 | 0.9 | 0.1 | 1.64 | <0.030 | |
| N14 | nb | 0.16 | nb | 1.09 | 0.7 | 0.25 | 0.11 | 3.32 | 0.48 | 0.78 | 0.082 | 1.68 | <0.029 | |
| N15 | nb | 0.13 | nb | 0.87 | 0.49 | 0.18 | 0.077 | 2.92 | 0.46 | 0.85 | 0.068 | 1.49 | <0.028 | |
| N21 | <2.1 | <1.4 | <4.3 | 6.3 | 8.3 | 4.3 | 0.6 | 15 | 2.5 | 5.1 | <0.2 | 8.5 | <0.1 | nb |
| N22 | <2.0 | <1.3 | <3.9 | 9 | 11 | 4.9 | 1 | 27 | 4.7 | 10 | 0.7 | 14 | <0.1 | nb |
| N23 | <2.1 | <1.4 | <4.1 | 8.3 | 6.4 | 3.3 | 1.1 | 27 | 4 | 7.4 | 0.5 | 16 | <0.1 | nb |
| N24 | <2.0 | <1.3 | <4.0 | 4.1 | 2.9 | 1.3 | 0.3 | 16 | 2.4 | 5.1 | <0.2 | 8.1 | <0.1 | nb |
| N25 | <2.1 | <1.4 | <4.2 | 13 | 10 | 4.3 | 1.5 | 47 | 7 | 14 | 0.8 | 25 | <0.1 | nb |
| N26 | <0.2 | 0.1 | 0.3 | 2.3 | 2 | 0.9 | 0.3 | 6.7 | 1.1 | 2.2 | 0.3 | 4.1 | 0.06 | |
| N27 | <0.2 | 0.1 | 0.3 | 1.9 | 1.6 | 0.8 | 0.3 | 5.6 | 1 | 1.9 | 0.3 | 3.4 | 0.04 | |
| N28 | <0.2 | 0.1 | 0.3 | 2.1 | 1.7 | 0.8 | 0.3 | 6 | 0.9 | 2 | 0.2 | 3.6 | 0.04 | |
| N29 | <0.2 | 0.2 | 0.6 | 2.7 | 3.6 | 1.2 | 0.3 | 7.4 | 1.3 | 2 | 0.4 | 4.1 | 0.08 | |
| N30 | <0.2 | 0.2 | 0.5 | 2.6 | 2.6 | 1 | 0.3 | 7.1 | 1.1 | 2.1 | 0.3 | 4.1 | 0.06 | |
| N36 | <0.73 | <0.47 | <0.36 | 19.3 | 14.4 | 5.38 | 1.31 | 61.1 | 10.7 | 20.8 | 1.57 | 29.9 | <0.21 | NB |
| N37 | <0.81 | <0.52 | <0.39 | 19 | 12.5 | 4.89 | 1.14 | 53.6 | 9.02 | 16 | 0.99 | 27 | <0.23 | NB |
| N38 | <0.78 | <0.50 | <0.38 | 18.4 | 14.2 | 5.57 | 1.26 | 55.7 | 9 | 18 | 1.39 | 28.2 | <0.22 | NB |
| N39 | <0.76 | <0.49 | <0.37 | 11.3 | 8.44 | 2.9 | <0.43 | 34.8 | 5.82 | 12.1 | 0.78 | 17 | <0.21 | NB |
| N40 | <0.75 | <0.49 | <0.37 | 12.9 | 7.13 | 2.52 | <0.43 | 39.6 | nb | 13.9 | 0.52 | 19.7 | <0.21 | NB |
| N41 | <0.04 | nb | nb | 1.6 | 0.9 | 0.4 | 0.2 | 5 | 1 | 1.9 | 0.1 | 2.6 | <0.03 | |
| N42 | <0.04 | nb | nb | 1.4 | 0.6 | 0.3 | 0.1 | 4.3 | 0.8 | 1.7 | 0.09 | 2.2 | <0.03 | |
| N43 | <0.04 | nb | nb | 1.4 | 0.7 | 0.4 | 0.1 | 4.3 | 0.8 | 1.7 | 0.1 | 2.1 | <0.03 | |
| N44 | <0.04 | nb | nb | 2 | 1.3 | 0.6 | 0.2 | 6.4 | 1.1 | 2.4 | 0.2 | 3.1 | 0.03 | |
| N45 | <0.04 | nb | nb | 1.9 | 1.1 | 0.5 | 0.2 | 5.2 | 1 | 1.9 | 0.1 | 2.5 | 0.03 | |
| W6 | <0.2 | 1.3 | 2.6 | 21 | 26 | 8.3 | 3.2 | 55 | 10 | 19 | 3.7 | 29 | 0.4 | nb |
</tbody>
</table>
</div>
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<p>68 of 73 | Wageningen Marine Research report C065/20</p>
</div>

W7	<0.2	2.6	6.7	43	57	19	8.1	110	25	30	9.2	56	0.9	nb
W8	<0.2	1.8	5.7	37	50	17	5.8	120	25	44	9.5	58	1.2	nb
W9	<0.2	1.6	3.3	29	35	11	4.4	84	17	30	5.4	41	0.6	nb
W10	<0.2	1.7	3.5	27	32	10	4.1	66	14	22	4.4	33	0.5	nb
W11	nb	0.64	nb	5.09	5.77	1.99	0.7	14.7	3.6	5.95	1.13	6.94	0.13	
W12	nb	0.72	nb	5.86	7.53	2.62	0.86	16.5	4.08	6.04	1.38	7.95	0.17	
W13	nb	0.85	nb	5.92	6.76	2.44	0.88	19.1	4.38	7.87	1.28	9.1	0.16	
W14	nb	1.03	nb	6.27	6.42	2.6	1.07	19.8	4.28	7.87	1.22	9.25	0.21	
W15	nb	0.74	nb	5.43	6.28	2.25	0.81	17	3.98	6.67	1.23	8.94	0.16	
W21	<2.2	<1.5	<4.4	26	35	12	3.9	100	15	27	4.2	50	1	nb
W22	<2.7	1.8	<5.4	34	46	16	5.3	100	18	24	6.2	51	1.1	nb
W23	<2.3	<1.5	<4.6	27	32	10	4.5	69	20	20	4.1	28	0.8	nb
W24	<2.7	2.8	<5.4	45	56	18	6.3	190	30	52	10	82	1.7	nb
W25	<2.2	<1.5	<4.5	33	35	12	4.4	100	22	35	6.5	47	1.1	nb
W26	<0.2	0.2	0.6	4.9	5.5	2	0.7	16	3.7	6.6	1.3	7.8	0.2	
W27	<0.2	0.2	0.5	4.6	5.8	2	0.7	16	3.6	6.4	1.2	8	0.2	
W28	<0.2	0.3	0.5	5.4	5.8	2	0.8	19	4.2	8.1	1.4	10	0.2	
W29	<0.2	<0.03	0.3	5.1	5.7	2.3	1	17	4.1	8.2	1.5	10	0.3	
W30	<0.2	0.2	0.6	5	5.4	1.9	0.8	16	3.8	6.5	1.3	8.2	0.2	
W36	<0.77	<0.50	<0.38	46	51	15.9	5.7	96	28	25.2	5.62	38.3	0.88	NB
W37	<0.70	<0.45	<0.34	43	53.7	17.5	6.15	126	30.5	45.7	9.04	62.8	1.1	NB
W38	<0.70	<0.45	<0.34	33.9	37.9	12.4	3.82	92.9	19.5	29	4.88	43	0.54	NB
W39	<0.79	<0.51	<0.38	27.9	32.2	10.3	2.74	82.1	16.5	25.3	4.28	38	0.48	NB
W40	<0.74	<0.47	<0.36	38.3	39.8	12	4.25	103	23.5	29.1	5.46	42.7	0.71	NB
W41	0.08	nb	nb	3.2	2.9	1.1	0.4	12	2.8	5.2	0.7	5.2	0.1	
W42	0.1	nb	nb	4.1	4.4	1.7	0.6	15	3.6	6.3	0.9	6.8	0.1	
W43	0.1	nb	nb	5.2	5.5	2	0.7	18	4.3	7.8	1.2	8.4	0.2	
W44	0.1	nb	nb	5.4	5.9	2.1	0.8	18	4.3	7.5	1.2	8.2	0.2	
W45	0.1	nb	nb	5.4	5.9	2.1	0.8	18	4.3	7.5	1.2	8.2	0.2	
W51	<0.2	1.5	2.2	16	15	5.5	2.2	28	5.5	10	1.3	15	0.2	nb
W52	<0.2	1.1	1.7	14	13	5.1	1.9	27	5.1	8.2	1.1	15	0.2	nb
W53	<0.2	1.2	1.8	13	12	4.4	1.6	25	4.6	8.3	1	13	0.2	nb
W54	<0.2	1	1.6	12	11	4.2	1.7	19	4	6.8	0.8	11	<0.1	nb
W55	<0.2	0.9	1.6	13	12	4.5	1.8	25	4.5	10	1	15	0.1	nb

W56	nb	0.34	nb	2.23	1.68	0.76	0.28	4.99	1.03	1.95	0.19	2.63	0.039	
W57	nb	0.37	nb	2.57	2.06	0.92	0.33	6.51	1.25	2.52	0.27	3.5	0.044	
W58	nb	0.34	nb	2.22	1.72	0.75	0.29	5.89	1.16	2.41	0.26	3.12	0.041	
W59	nb	0.35	nb	2.29	1.92	0.85	0.3	5.69	1.14	2.27	0.26	3.07	0.042	
W60	nb	0.34	nb	2.38	1.83	0.83	0.3	5.76	1.05	2.21	0.24	3.03	0.042	
W66	<1.9	<1.3	<3.9	12	7	2.9	1	20	4	7.6	0.6	11	<0.1	nb
W67	<1.9	<1.3	<3.8	12	6.6	2.8	1.2	15	3.1	5.1	0.4	8	<0.1	nb
W68	<1.9	<1.2	<3.7	12	10	4.3	1.3	25	5.3	10	1	14	0.1	nb
W69	<1.9	<1.3	<3.8	13	11	5.4	1.5	28	5.9	11	1.1	15	<0.1	nb
W70	<1.8	<1.2	<3.6	9.5	7.2	3.6	1.1	18	3.7	6.7	0.6	9.3	<0.1	nb
W71	<0.2	0.2	0.3	2.5	1.9	0.9	0.3	5.4	1.1	2.3	0.3	3.1	0.03	
W72	<0.2	0.2	0.2	2.4	1.7	0.8	0.3	5.7	1.1	2.5	0.3	3.3	0.05	
W73	<0.2	0.2	0.3	2.2	1.9	0.8	0.3	5	1	2.2	0.2	2.9	0.04	
W74	<0.2	0.2	0.4	2.5	2.2	1	0.3	5.9	1.2	2.4	0.3	3.4	0.04	
W75	<0.2	0.1	0.3	2.1	1.8	0.8	0.3	4.8	1	2.1	0.3	2.8	0.05	
E76	<0.73	<0.47	<0.35	25.0	16.0	5.86	1.95	55.2	10.8	14.4	1.36	27.7	<0.20	NB
E77	<0.71	<0.46	<0.35	26.5	16.4	5.35	1.74	62.9	9.5	17.6	1.63	30.6	<0.20	NB
E78	<0.65	<0.42	<0.32	18.5	11.9	4.19	1.18	38.9	8.19	11.1	0.88	19.3	<0.18	NB
E79	<0.66	<0.42	<0.32	22.4	13.4	4.37	1.25	48.0	9.70	14.9	1.21	23.6	<0.19	NB
E80	<0.73	<0.47	<0.35	18.2	10.8	3.85	1.14	37.3	11.6	8.9	0.66	17.9	<0.21	NB
E81	0.06	nb	nb	2.1	1.3	0.6	0.2	5.6	1	2.1	0.2	3	0.04	
E82	0.06	nb	nb	2.3	1.5	0.7	0.2	5.9	1.1	2.4	0.2	3.1	0.04	
E83	0.06	nb	nb	1.8	1.1	0.4	0.2	4.5	0.8	1.7	0.1	2.3	<0.03	
E84	<0.04	nb	nb	1.9	1	0.4	0.2	4.9	0.9	1.9	0.1	2.5	0.03	
E85	0.07	nb	nb	2.3	1.6	0.7	0.2	6	1.1	2.5	0.2	3.1	0.04	

Table 6 PCB (128-28) concentrations for each sample ID given in wet weight. Below limit of d quantification is indicated with '<'.

Sample Analysis ID	CB-128	CB-118	CB-110	CB-105	CB-101	CB-97	CB-87	CB-85	CB-66	CB-56	CB-52	CB-49	CB-47	CB-31	CB-28
N6	0.4	2	1.2	0.4	1.7	0.3	0.3	0.2	0.6	<0.3	1.3	0.8	<2.4	<0.2	0.4
N7	0.8	3.3	2.1	0.6	2.8	0.6	0.5	0.4	1	0.5	1.7	1.1	<3.0	1.1	0.3
N8	0.3	1.6	0.9	<0.2	1.2	<0.2	<0.2	<0.2	0.6	<0.4	1.1	0.8	<3.2	1.3	0.4
N9	0.4	1.7	1	0.3	1.2	0.3	0.3	0.2	0.5	<0.4	1	0.8	<3.1	1	<0.2
N10	1	5.2	3.1	0.8	4.2	0.9	<0.08	1.2	1.4	0.6	2.6	1.8	<3.3	1.3	0.6
N11	0.17	0.8	0.51	0.11	0.72	<0.084	0.12	0.056	0.35	0.1	0.62	0.42	<0.39	0.21	0.28
N12	0.25	1.03	0.67	0.17	1.12	0.14	0.12	0.1	0.47	0.11	0.82	0.56	<0.44	0.28	0.33
N13	0.25	0.88	0.58	0.15	0.86	0.11	0.13	0.1	0.38	0.1	0.63	0.42	<0.30	0.22	0.28
N14	0.25	0.92	0.56	0.15	0.95	0.095	0.1	0.079	0.37	0.091	0.65	0.44	<0.29	0.24	0.29
N15	0.22	0.82	0.56	0.16	0.98	0.11	0.13	0.074	0.36	0.11	0.65	0.45	<0.36	0.24	0.28
N21	<4.3	6.5	4.4	1.2	6.7	1.2	1.1	0.7	2.5	0.9	4	3.1	<6.7	2.2	2.4
N22	<3.9	10	8.5	1.6	12	1.9	1.7	1	3.8	1.1	7.2	5.2	<5.4	2.8	3.2
N23	<4.1	8.7	5.4	1.4	7.8	1.3	1.2	0.6	4	1.1	6.3	4.8	<7.8	2.8	3.3
N24	<4.0	5	3.7	0.8	5.3	0.8	0.8	0.4	2.4	0.7	4.2	3.1	<6.3	1.5	1.9
N25	<4.2	13	8.4	2.3	13	1.9	1.8	1.4	5.5	1.3	8.7	6.2	<7.5	3.5	4.1
N26	0.6	2.4	1.6	1.6	2.4	0.4	0.4	0.3	1.3	0.3	1.9	1.4	<1.1	1	1.3
N27	0.6	2.1	1.5	0.5	2.2	0.4	0.4	0.3	1.2	0.3	1.8	1.4	<1.1	1	1.1
N28	0.6	2.3	1.6	0.4	2.2	0.4	0.4	0.3	1.3	0.3	1.9	1.5	<1.2	1.1	1.4
N29	0.6	2.3	1.4	0.5	2.3	0.4	0.4	0.3	1.2	0.3	1.8	1.4	2.3	0.9	1.2
N30	0.6	2.4	1.5	0.6	2.2	0.4	0.4	0.3	1.3	0.3	1.8	1.4	<1.6	1	1.3
N36	4.04	19.5	15.2	2.81	21.7	3.87	3.2	1.69	8.46	1.64	13.3	11.7	5.65	4.31	5.31
N37	3.83	18.3	12.3	2.6	17.5	2.76	2.5	1.42	7.28	1.34	10.9	9.6	5.12	3.4	4.35
N38	3.96	17.4	13.1	2.81	17.9	2.81	2.37	1.38	7.01	1.17	11	9.5	4.79	3.44	4.34
N39	2.59	11	8.6	1.63	11.8	2.03	1.59	0.88	4.66	0.9	7.49	5.92	3.36	2.48	2.95
N40	NB	12.8	10	1.7	13.9	2.31	2	0.75	5.55	1.05	9.33	7.34	4.05	2.66	3.43
N41	0.5	1.7	1.2	0.3	1.8	0.3	0.2	0.2	0.8	0.2	1.4	0.9	0.6	0.4	0.6
N42	0.3	1.6	1.2	0.3	1.8	0.3	0.3	0.2	0.9	0.2	1.7	1.1	0.7	0.6	0.7
N43	0.3	1.5	1.1	0.3	1.8	0.3	0.3	0.2	0.8	0.1	1.4	1	0.6	0.5	0.6
N44	0.5	2	1.6	0.3	2.3	0.4	0.3	0.2	1	0.2	1.6	1.1	0.7	0.5	0.6
N45	0.4	1.9	1.3	0.3	1.9	0.3	0.3	0.2	0.9	0.1	1.5	0.9	0.7	0.4	0.5

W6	4.2	14	12	2.9	18	3	2.4	1.8	4.3	1.1	8.3	5.5	<6.8	3.9	4.6
W7	7.2	32	23	6.3	42	5.6	5.2	3.2	7.7	1.9	21	11	<10	6.4	9
W8	7.7	26	24	5.5	38	5.8	5.9	3.8	8.7	2.1	20	13	<13	9.4	12
W9	5.9	21	18	4.1	28	4.7	4	3	7	1.7	16	11	<8.7	8.1	11
W10	4.9	17	14	3.4	23	3.8	3.3	2.5	5.8	1.2	14	9.5	<10	6.8	8.6
W11	1	3.48	3.73	0.74	5.6	0.9	0.87	0.5	1.45	0.49	2.78	1.94	1.43	1.06	1.99
W12	1.14	3.76	3.7	0.78	5.5	0.95	0.9	0.5	1.43	0.46	2.71	1.74	1.31	0.93	1.85
W13	1.34	4.3	4.52	0.96	6.71	1.21	1.07	0.65	1.56	0.55	3.05	2.15	1.59	0.96	1.93
W14	1.59	5.44	6.02	1.09	6.8	1.57	1.15	0.72	1.85	0.6	2.92	2.14	1.55	1.22	2.1
W15	1.16	3.97	4.16	0.84	6.07	1.11	0.96	0.6	1.45	0.46	2.89	1.89	1.42	0.82	1.69
W21	6.1	19	17	3.8	24	3.9	4	2.7	6.2	1.4	12	7.8	<11	2.4	4.5
W22	6.2	22	16	4.3	25	4	3.6	2.3	5.7	1.2	11	6.3	<10	1.7	4
W23	<4.6	19	11	2.7	18	3.4	2.7	1.4	4.3	1.1	14	6.4	<6.8	2.4	4.2
W24	9.4	35	27	6.9	48	6.7	6.3	5.1	10	1.5	18	12	<15	3.1	7.9
W25	5.2	23	22	4.6	34	5.5	5.1	2.7	6.9	1.5	16	9.3	<10	2.3	4.9
W26	1	4	4.5	0.8	7	1.2	1.1	0.7	1.3	0.3	3.1	1.9	<1.6	<0.5	0.9
W27	1	3.9	4.4	0.9	6.9	1.1	1.1	0.6	1.3	0.3	2.9	1.8	<1.5	<0.5	0.8
W28	1.2	4.6	5.4	1.1	7.8	1.5	1.3	0.7	1.5	0.4	3.5	2.2	1.8	<0.6	1
W29	1.4	5.3	7.4	2	7.4	1.8	1.5	0.8	1.8	0.5	3.5	2	<1.6	0.9	1.3
W30	1	4	4.3	1	6.3	1.2	1.1	0.6	1.2	0.3	3.1	1.7	<1.6	<0.4	0.9
W36	5.08	28.3	16.5	4.05	23.8	4.81	4.2	1.97	5.22	0.99	16.9	8.93	4.91	2.14	3.84
W37	7.22	31.4	34.3	6.75	43.5	7.49	7.59	3.47	8.88	1.73	21.1	12.2	7.65	2.31	4.8
W38	5.79	22.7	19	4.02	26.3	4.46	4.13	2.19	5.68	1.11	11.6	7.66	4.68	1.75	3.23
W39	5.53	18.2	15.9	3.24	23.3	3.76	3.27	2.19	4.23	0.8	8.98	6.07	3.63	1.31	2.59
W40	6.4	25.4	18.2	4.19	30.4	4.51	4.12	2.71	5.86	0.81	13.8	8.82	5.42	1.82	3.77
W41	0.8	2.6	3.1	0.5	5.3	0.8	0.7	0.4	0.9	0.2	2.1	1.4	0.9	0.2	0.5
W42	1.1	3.5	4.3	0.8	7	1.1	0.9	0.6	1.3	0.3	3.2	2	1.3	0.4	0.8
W43	1.3	4.2	5.2	0.9	8.1	1.4	1.1	0.7	1.4	0.3	3.8	2.3	1.7	0.4	0.9
W44	1.2	4.2	5.2	0.9	7.8	1.4	1.1	0.7	1.4	0.3	3.5	2	1.4	0.3	0.8
W45	1.2	4.2	5.2	0.9	7.8	1.4	1.1	0.7	1.4	0.3	3.5	2	1.4	0.3	0.8
W51	2.7	9.1	5.9	1.7	7.7	1.5	1.2	1	2.4	0.9	3.8	2.3	<4.8	1.6	1.4
W52	2.6	8.2	5.4	1.6	6.6	1.2	1	0.8	2	0.8	3.7	2.1	<4.7	1.6	1.2
W53	2.1	7.2	4.5	1.2	6.6	1.2	0.9	0.7	1.7	0.7	3.7	2.4	<3.5	1.8	1.8
W54	2	6.6	4.1	1.2	5.4	1.1	0.9	0.7	1.7	0.8	3	1.8	<4.7	1.6	1.1

W55	2.6	7.8	5.3	1.6	7.1	1.5	1.1	0.8	2.1	0.9	3.2	2.2	<8.5	1.2	0.9
W56	0.47	1.44	1.11	0.31	1.49	0.28	0.24	0.16	0.55	0.19	0.73	0.46	<0.36	0.22	0.33
W57	0.59	1.61	1.29	0.33	1.81	0.33	0.27	0.19	0.6	0.21	0.79	0.5	<0.42	0.22	0.36
W58	0.54	1.47	1.3	0.32	1.87	0.33	0.26	0.17	0.57	0.19	0.77	0.52	<0.49	0.21	0.33
W59	0.56	1.4	1.22	0.3	1.67	0.29	0.26	0.17	0.53	0.18	0.73	0.46	<0.40	0.19	0.3
W60	0.55	1.46	1.19	0.32	1.55	0.28	0.24	0.17	0.52	0.19	0.67	0.43	<0.40	0.18	0.28
W66	<3.9	6.5	4.6	1.2	6.1	1.1	1.1	0.6	2.4	0.9	3.2	<2.0	<4.1	0.8	1.3
W67	<3.8	6.5	3.9	1.1	5.3	1	1	0.6	2.4	0.8	3.3	2.2	<4.7	1	1.5
W68	<3.7	7	5.3	1.3	7.3	1.3	1.1	0.7	2.6	0.8	3.8	2.2	<3.9	1.1	1.5
W69	<3.8	7.7	5.4	1.4	7.1	1.3	1.2	0.7	2.6	0.8	3.4	2.3	<4.9	0.9	1.4
W70	<3.6	5.4	3.4	0.9	4.6	0.9	0.8	0.4	1.9	0.7	2.5	<1.6	<5.3	0.9	1.1
W71	0.5	1.7	1.2	0.5	1.7	0.3	0.3	0.2	0.6	0.2	<0.9	0.5	<0.5	<0.3	0.4
W72	0.5	1.7	1.4	0.4	1.8	0.4	0.3	0.2	0.6	0.2	<0.9	0.5	<0.5	<0.3	0.4
W73	0.4	1.5	1.2	0.3	1.6	0.3	0.3	0.2	0.6	0.2	<0.9	0.5	<0.5	<0.3	0.4
W74	0.5	1.6	1.3	0.3	1.7	0.3	0.3	0.2	0.6	0.2	<0.9	0.5	<0.5	<0.3	0.4
W75	0.5	1.4	1.2	0.3	1.5	0.3	0.3	0.2	0.5	0.2	<0.7	0.4	<0.5	<0.3	0.4
E76	5.02	14.2	7.79	2.40	11.5	1.58	1.69	1.29	3.97	<0.65	4.00	2.98	1.94	1.13	2.07
E77	3.82	15.7	9.44	2.69	15.4	2.23	2.25	1.37	4.81	0.69	5.18	3.71	2.19	1.45	2.53
E78	4.16	10.1	5.61	1.54	8.47	1.2	1.24	0.78	2.84	<0.59	2.95	2.16	1.43	0.9	1.54
E79	4.73	12.0	7.93	1.98	12.3	1.84	1.92	1.16	4.02	0.72	4.46	3.66	1.90	1.42	2.43
E80	NB	10.8	5.33	1.54	8.26	1.01	1.14	0.67	3.01	<0.66	3.5	1.96	<1.29	1.01	1.71
E81	0.5	1.5	1.1	0.3	1.5	0.3	0.2	0.2	0.6	0.1	0.5	0.5	0.2	0.2	0.3
E82	0.6	1.4	1.2	0.3	1.5	0.3	0.2	0.2	0.5	0.1	0.5	0.4	0.2	0.1	0.2
E83	0.4	1.1	0.9	0.2	1.2	0.2	0.2	0.1	0.4	<0.08	0.4	0.3	0.2	0.1	0.2
E84	0.4	1.2	1	0.2	1.4	0.2	0.2	0.2	0.4	<0.08	0.5	0.4	0.2	0.1	0.2
E85	0.6	1.5	1.3	0.3	1.7	0.3	0.3	0.2	0.6	0.1	0.7	0.5	0.3	0.2	0.3