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Biota monitoring under the Water Framework Directive: On tissue choice and fish species selection☆

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ABSTRACT

The study addresses the topic of suitable matrices for chemical analysis in fish monitoring and discusses the effects of data normalization in the context of the European Water Framework Directive (WFD). Differences between species are considered by comparing three frequently monitored species of different trophic levels, i.e., chub (*Squalius cephalus*, n = 28), (bream, *Abramis brama*, n = 11), and perch (*Perca fluviatilis*, n = 19) sampled in the German Danube. The WFD priority substances dioxins, furans and dioxin-like polychlorinated biphenyls (PCDD/F + dl-PCB), polybrominated diphenyl ethers (PBDE), α-hexabromocyclododecane (α-HBCDD), hexachlorobenzene (HCB), mercury (Hg), and perfluorooctane sulfonic acid (PFOS) as well as non-dioxin-like (ndl)-PCB were analyzed separately in fillet and carcass and whole body concentrations were calculated. Hg was analyzed in individual fish fillets and carcasses, all other substances were determined in pool samples, which were compiled on the basis of fish size (3 chub pools, 1 bream pool, 2 perch pools). The data were normalized to 5% lipid weight (or 26% dry mass in the case of Hg and PFOS) for comparison between matrices and species.

Hg concentrations were generally higher in fillet than in whole fish (mean whole fish-to-fillet ratio: 0.7) whereas all other substances were mostly higher in whole fish. In the case of lipophilic substances these differences leveled after lipid normalization.

Significant correlations ($p \leq .05$) were detected between Hg and fish weight and age. Hg concentrations varied least among younger fish. PCDD/F, dl-PCB, ndl-PCB, PBDE, α-HBCDD and HCB correlated significantly ($p \leq .05$) with lipid concentrations. Fillet-to-whole fish conversion equations and/or conversion factors were derived for all substances except α-HCBDD. Although more data also for individual fish would be desirable the results are nevertheless a step on the way to translate fillet concentrations of priority substances to whole fish concentrations.

1. Introduction

Biota monitoring has become a valuable instrument in environmental assessment complementing the analysis of water, suspended particulate matter and sediment especially in the case of those substances that tend to accumulate in organisms and are difficult to determine in other matrices. In the European Water Framework Directive (WFD) eleven substances and substance groups have been identified for which the assessment of compliance with environmental quality standards (EQSs) is required in biota. For nine of these the EQS refers to fish, i.e., dicofol, dioxins, furans and dioxin-like polychlorinated biphenyls (PCDD/F+dl-PCB), heptachlor and heptachlor epoxide, hexabromocyclododecane (HBCDD), hexachlorobenzene (HCB), hexachlorobutadiene (HBCDD), mercury (Hg), perfluorooctane sulfonic acid (PFOS), and polybrominated diphenyl ethers (PBDE) (EC, 2000, EC, 2013). The EQSs were derived for the protection goals 'human health' and 'secondary poisoning of wildlife' with the more sensitive protection

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goal being decisive for determining the EQS.

The biota monitoring community typically faces the questions of what fish species to choose, what size of fish to target, what matrix to analyze (e.g., fillet or whole fish), whether to pool samples or analyze individual fish, how to convert data from one matrix or species to another, and how to assess compliance with target values. Fish species and size play a crucial role in contamination especially when it comes to substances that bioaccumulate and biomagnify in the food web. Normally the contamination increases with trophic position and age of the fish (Driscoll et al., 2013; EC, 2014).

Decisions are mostly governed by the underlying question regarding the protection goals – does the program address primarily the human health aspect – which would favor the analysis of fillet of large (predatory) fish - or is its major focus on the protection of piscivorous wildlife and relatively small whole fish would be the appropriate matrix?

This in concert with the wide range of monitored fish species and the analysis of pool samples as well as individual fish has resulted in a wide variety of data sets that are difficult to compare.

Examples are, e.g., Germany, where some federal states have generated long time series of monitoring data analyzing fillets and/or livers of individual fish belonging to more than 30 species (e.g., FGG Elbe, 2016; Fliedner et al., 2016a; Guhl et al., 2014; ICPR, 2011). Additionally, the German Environmental Specimen Bank (ESB) has generated a broad data base for pool samples of bream fillets and livers (www.umweltprobenbank.de). Likewise, multiple data exist from fish monitoring in other countries, e.g., in Europe (compilations see EC, 2014; Fliedner et al., 2016b), the U.S., and Canada (Batt et al., 2017; Environment and Climate Change Canada, 2017; Lazorchak et al., 2003; Stahl et al., 2009, 2013, 2014; U.S. EPA, 2017; Wathen et al., 2015).

For ecological and economic reasons it would be desirable to address both protection goals, human health and the protection of piscivorous predators, in just one program by converting fillet data to whole fish or *vice versa* and translating data from one fish species to another and from young fish to old (or *vice versa*). Moreover, from the economic point of view pooling of samples would be preferable.

The EU Guidance document No 32 (EC, 2014) addresses these aspects in the context of EQS compliance monitoring and gives general recommendations. It states, for instance, that when monitoring fish fillets "… Conversion factors for fillet-to-whole fish contaminant levels should be used, when available, to give more accurate risk estimates for secondary poisoning. …. Thus, MS (Member States) that wish to consider this option should derive conversion factors for HCBD, dicofol, HBCDD, HCB, PFOS, and preferably mercury, before implementing such an approach". Alternatively, lipid-normalized concentrations in any matrix/tissue can be used, provided the contaminant concentrations correlate with the lipid content.

The present study addresses these issues by presenting data of a tailored monitoring study conducted in the Danube in 2015. The focus is on the aspect fillet *vs.* whole fish, younger *vs.* older fish, differences between fish species and effects of normalization.

The data are analyzed and discussed with respect to the following questions relevant for risk assessment and EQS compliance check:

- How do contaminant concentrations in fillet and whole fish relate to one another?
- What are the effects of data normalization to lipid (respectively dry mass in the case of Hg and PFOS)?
- Can data normalization overcome tissue and species specific differences in contamination thus superseding the need for monitoring different matrices (e.g., whole fish and fillet) and supporting the comparison between different monitoring programs?

2. Material & methods

2.1. Sampling

Chub (*Squalius cephalus*, n = 28), bream (*Abramis brama*, n = 11), and perch (*Perca fluviatilis*, n = 19) were sampled at Kelheim in the middle section of the German Danube. All three are frequent species in German freshwaters and are already included in national monitoring programs. The sampling site Kelheim (Danube km 2404) is located downstream of the confluence of Danube and Rhine-Main-Danube Canal and upstream of the barrage Bad Abbach (Fig. 1). It reflects the state of the shipped Middle Danube. Fish migration in this area is hampered by many barrages.

The sampling took place in September 2015 after the spawning season. It was performed on two consecutive days using gillnets. Until processing the fish were interim-stored in freezers up to 48 h. For every fish biometric data (length, weight, age, and sex) were recorded. Then one fillet was removed completely and separated from its skin while the second fillet remained on carcass. Fillet and carcass (including the second fillet and the skin of the removed fillet) were weight separately before being individually shock-frozen in liquid nitrogen. Next, the tissue was pre-crushed, cryo-milled and stored as homogenized powder at temperatures below −150 °C in an inert atmosphere to minimize chemical alterations (Rüdel et al., 2009, 2015; Rüdel and Weingärtner, 2008).

In the following, the term 'carcass' refers to the carcass plus the one remaining fillet.

2.2. Pool preparation

Hg was analyzed in fillet and carcass of individual fish while all other substances and substance groups were determined in pool samples of fillets, respectively carcasses. The pools were composed of fish of comparable size (Table S1, Supplementary material).

2.3. Chemical analysis

Fillet and carcass were analyzed for the WFD priority substances mercury (Hg), dioxins and furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCB), polybrominated diphenyl ethers (PBDE, sum of BDE-congeners −28, −47, −99, −100, −153, −154), hexabromocyclododecane (HBCDD), hexachlorobenzene (HCB), and perfluorooctane sulfonic acid (PFOS). Additionally, non-dioxin-like (ndl-) PCB (sum of congeners CB-28, -52, −101, −138, 153, −180) were analyzed.

The analytical methods applied are widely used methods that are confirmed by regular analysis of certified reference materials and validated regularly in inter-laboratory proficiency test.

Analysis of Hg was performed at Fraunhofer IME by a dedicated atomic absorption spectrometry (AAS) method applying Direct Mercury Analyzer (DMA) instruments (Rüdel et al., 2010). All other substances were analyzed by Eurofins GfA Lab Service GmbH, Hamburg. The methods have been described in more detail in Fliedner et al. (2016b). Briefly, PCDD/F, PCB, and HCB were determined by high resolution gas chromatography and high resolution mass spectrometry (HRGC/HRMS). Liquid chromatography and tandem mass spectrometry (LC-MS/MS) was used for the analysis of HBCDD and PFOS. PBDE were determined by means of gas chromatography and mass spectrometry (GC/MS). Identification of target compounds was based on the comparison of retention time

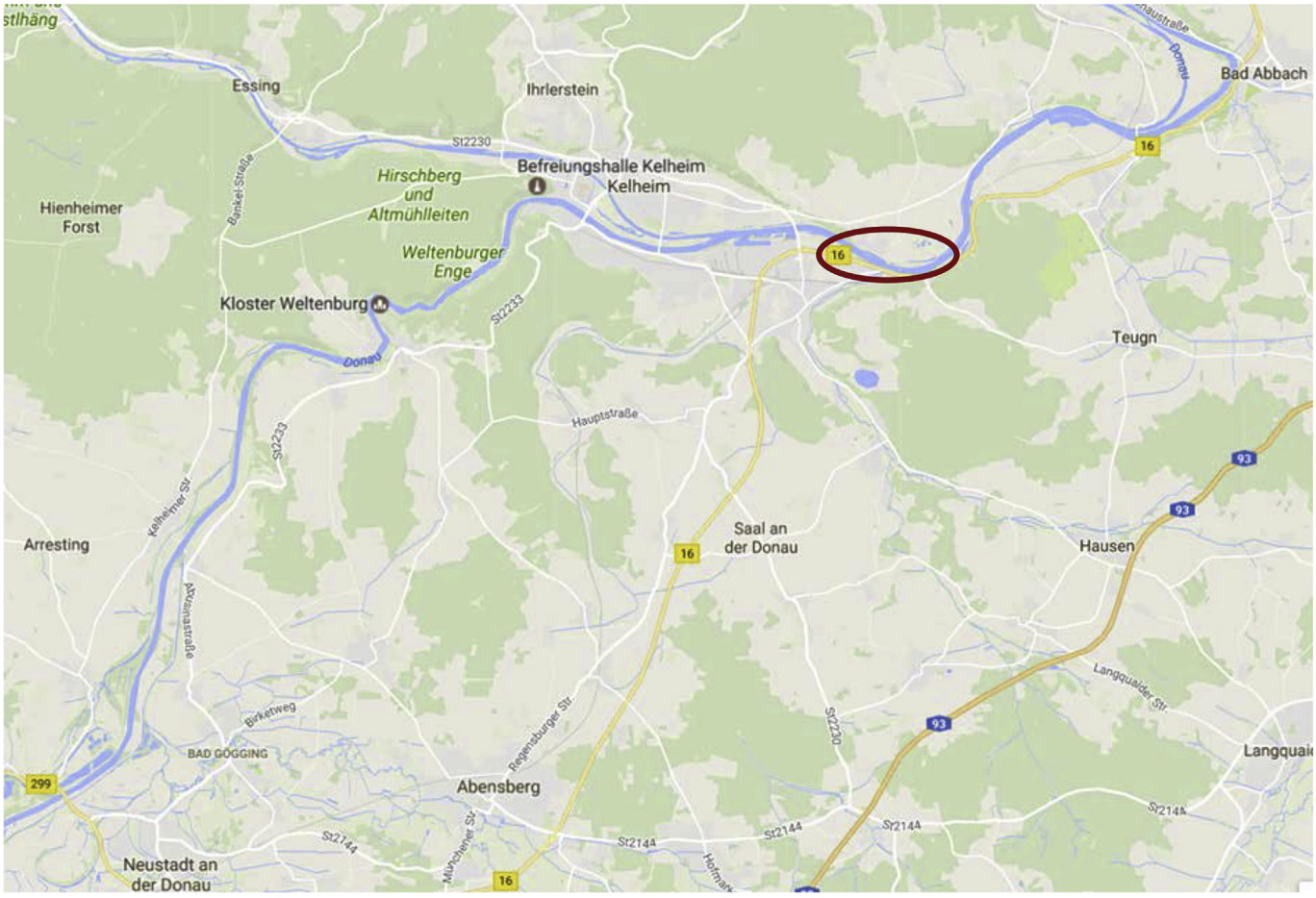

Fig. 1. Location of fish sampling site in the Danube (red circle). Source: Google maps (Kartendaten © 2017 GeoBasis-DE/BKG (© 2009), Google). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and relative isotope ratios between native and isotopic labelled internal standards. Quantification of target compounds was carried out by means of isotope dilution analysis with the use of internal and external standards. Method blanks including extraction, clean-up and measuring were monitored in parallel to each batch of samples. Furthermore, precision and accuracy were checked by analyzing in-house quality assurance pool samples, sample material of previous inter-laboratory proficiency studies or certified reference material along with each batch of samples.

The laboratories hold accreditations for the applied methods and all respective quality assurance/quality control (QA/QC) requirements were met. Both laboratories participate regularly and successfully in external proficiency tests and inter-laboratory tests, e.g., QUASIMEME.

Lipid determination was performed gravimetrically on pooled samples of fillets and carcasses according to the method described by Smedes (1999). Whole fish lipid contents were calculated based on lipid contents in fillets and carcasses taking into account the respective fractions of fillet and carcass in the fish and the share of the individual fish in the pool.

2.4. Calculation of whole fish contaminant concentrations

Whole fish concentrations were calculated according to Bevelhimer et al. (1997):

$$Conc\ (whole\ fish) = \frac{[(weight(whole\ fish) - weight(fillet)) \times Conc(carcass)] + [weight(fillet) \times Conc(fillet)]}{weight(whole\ fish)} \quad (1)$$

In the case of Hg, calculations were based on individual fish data, for all other substances calculations used the respective pool data (Table S1, Supplementary material).

2.5. Determination of the trophic levels

Trophic levels (TL) were determined based on the $^{15}N/^{14}N$ ratios of fish using soft bodies of zebra mussels (*Dreissena polymorpha*) sampled at Kelheim in 2014 as baseline organism (TL = 2). Cryo-milled tissue samples (pooled fish muscle samples) were freeze-dried prior to analysis. Stable isotope analysis (SIA) was performed by Agroisolab GmbH, Jülich, Germany. Tissue samples were

extracted with dichloromethane in a Soxhlet device for 6 h and dried overnight at 65 °C. Analysis was performed using a Carlo Erba NA1500 elemental analyzer combined with a Horizon continuous-flow isotope ratio mass spectrometer (CF-IRMS) from Nu-Instruments, Wrexham, UK. Leucine served as laboratory standard, calibrated against appropriate international standards (IAEA-N1, IAEA-N2 for $^{15}N/^{14}N$ and IAEA-CH6, IAEA-CH7 for $^{13}C/^{12}C$). SIA results are expressed in the δ unit notation (see, e.g., Post, 2002) as deviations from a standard (i.e., N_2 in air for $^{15}N/^{14}N$ and Pee Dee Belemnite for $^{13}C/^{12}C$):

$$\delta^{15}N = \left[\frac{R(sample)}{R\,(standard)} - 1\right] \times 10^3 \quad \text{with } R = {}^{15}N/{}^{14}N \tag{2}$$

Samples were analyzed as duplicates; reproducibility for all analysis was <0.4‰.

Trophic levels of the fish were calculated as follows (McCutchan et al., 2003; Post, 2002):

$$TL = \left(\frac{\delta 15N\,(fish)\,[‰] - \delta 15N\,(food\ source)[‰]}{3.4}\right) + 2 \tag{3}$$

$\delta^{15}N_{food\ source} = \delta^{15}N$ value of zebra mussels sampled at Kelheim/Danube in 2014.

2.6. Data normalization

Normalization followed the recommendations of the Guidance Document No. 32 (EC, 2014). Concentrations of lipophilic compounds were normalized to 5% lipid content to account for differences in lipid partitioning between fillet and carcass. Hg and PFOS concentrations were normalized to 26% dry mass (DM).

In the following the term 'measured' is used for the determined, non-normalized concentrations.

2.7. Data analysis

Regression and correlation analyses were performed using Microsoft EXCEL (Version 2010). Significance of correlations and regressions was determined using VassarStats (http://vassarstats.net/index.html). Pearson's correlation coefficients were calculated for individual fish data for Hg and biometrical parameters. All other correlations are based on pool data.

Fillet-to-whole fish conversion equations or -factors were derived according to the recommendations of the WFD Guidance Document (EC, 2014). The procedure outlined by Bevelhimer et al. (1997) includes a regression analysis followed by a test to determine whether the slope of the regression line differs from 1. If not so, the mean whole fish-to fillet ratio can be used as conversion factor. In the case the slope of a significant regression line is significantly different from 1 the respective equation of the regression line can serve as conversion equation. The difference of the regression slopes from slope = 1 was tested using an EXCEL-based tool designed by J. Wellmitz (German Environment Agency).

3. Results & discussion

3.1. Characterization of samples

The biometrical data of all fish and the preparation of pool samples are summarized in Table S1 (Supplementary material). Chub were between 5 and 11 years old and were divided into three pools according to their size (Table 1). Bream ranged between 4 and 12 years. Since the number of sampled bream was relatively small, only one pool was prepared including 8 fish of similar size. Perch were 4–8 years old and were divided into 2 pools whereby one male fish was excluded to create a solely female pool of similar sized fish.

For all three species the major fraction of body lipids was found in the carcasses. Lipid partitioning, however, varied between species, with most significant differences between fillet and carcass found in perch (Table 1).

The $\delta^{15}N$ values and calculated trophic levels were lowest for chub and highest for perch (Table 1). For chub and bream the calculated $\delta^{15}N$- based TLs were in accordance with the generic TL values based on diet studies (FishBase, Froese and Pauly, 2017). In the case of perch, however, the $\delta^{15}N$- based TLs were lower. One possible reason for this discrepancy may lie in the different approaches – stable isotope analysis with *Dreissena polymorpha* by definition as trophic level 2 on the one hand and diet studies on the other. Another explanation could be that perch in the Danube feed on a lower trophic level compared to the study sites from which the diet-based TL originates.

3.2. Hg/analysis of individual fish

Hg concentrations in chub ranged between 44.8 and 349 μg kg^{-1} wet weight (ww) in fillets and 29.0–242 μg kg^{-1} in whole fish. Levels in bream were similar, i.e., 28.1–372 μg kg^{-1} in fillets and between 19.6 and 240 μg kg^{-1} in whole fish. Higher concentrations were detected in perch (fillet: 131–509 μg kg^{-1}; whole fish: 93.1–348 μg kg^{-1}). The data are illustrated in Fig. 2 and summarized in Table S2 (Supplementary material). Due to its high biomagnification potential, measured Hg levels in perch (TL 3.7–3.8) are almost twice as high as in chub (TL 2.7–2.8) and bream (TL 3.1).

Hg accumulates in tissues rich in proteins carrying SH-groups like, e.g., muscle (Eisler, 2007). Accordingly, Hg concentrations are typically higher in fish fillets than in whole fish. The whole fish-to-fillet ratio based on measured Hg concentrations was around 0.6 in bream and around 0.7 in chub and perch (Table S2, Supplementary material).

Hg concentrations in fillet, carcass, and whole fish correlated significantly ($p \leq .05$) with fish length, fillet weight, carcass weight, whole fish weight, and age in chub ($n = 28$) and perch ($n = 19$) (Table S3, Supplementary material). For bream ($n = 11$), no respective correlations were found which is probably related to the relatively small number of fish. There were, however, slight but not significant relationships ($p < .1$) between Hg in bream fillet and whole fish, and fish length, and between Hg (bream fillet, carcass, whole fish) and age. Sex did not correlate with Hg in any species. In all three species a significant correlation (at least $p < .005$) was detected for Hg (whole fish, fillet, and carcass) and the dry mass fraction in carcass (in bream and perch also in fillet). In bream, the Hg whole fish-to-fillet ratio correlated negatively with fish length ($p = .026$) indicating that fish size had some influence on the partitioning of Hg in the body with relatively higher Hg fractions in fillets of bigger fish. No such correlation was detected in chub. However, the negative relationships ($p < .05$) between the Hg whole fish-to-fillet ratio in chub and the dry mass fractions in fillet and carcass also point to increased Hg fractions in fillets of larger fish. Like in bream, a slight (not significant) negative correlation with age was noticeable in chub. No significant correlations between the Hg whole fish-to-fillet ratio and biometric data were found in perch.

The data suggest that Hg levels vary less between younger fish (Fig. S1, Supplementary material). A possible explanation may be that exposure to environmental Hg not only increases with age but also varies more strongly between older individuals, e.g., through the uptake of differently contaminated food over a longer time. In the present study the youngest chub were already 5 years old and

Table 1
Characteristics of the pool samples prepared from chub (*Squalius cephalus*), bream (*Abramis brama*), and perch (*Perca fluviatilis*) sampled at Kelheim/Danube in 2015.

		chub 1	chub 2	chub 3	bream	perch 1	perch 2
N fish in pool sample		9	11	8	8	10	8
weight [g]		530 ± 149	991 ± 180	1695 ± 190	2240 ± 556	324.6 ± 29.0	756.6 ± 138
length [cm]		34.9 ± 2.7	42.5 ± 1.6	49.0 ± 1.2	56.1 ± 3.7	27.4 ± 0.91	35.7 ± 2.0
age [years]		5 ± 0	6.7 ± 0.6	9.5 ± 0.8	10.5 ± 1.2	4.2 ± 0.42	6.8 ± 0.7
lipid [%]	fillet	1.39	1.69	3.76	3.41	1.23	1.17
	carcass[a]	4.3	4.03	7.32	6.11	8.46	9.85
	whole fish	3.62	3.51	6.53	5.54	6.66	7.72
	WF-to-F ratio[b]	2.6	2.1	1.7	1.6	5.4	6.6
dry mass fraction [%]	fillet	19.8	19	21.1	20.8	20.1	20.8
	carcass[a]	25.2	25.4	28.7	29.1	31.8	34.2
	whole fish	23.8	23.8	26.9	27.3	28.7	30.8
δ^{13}Cfish [‰]		−28.6	−27.8	−26.2	−28.9	−28.6	−28.3
δ^{15}Nfish [‰]		13.6	13.3	13	14.5	16.5	16.8
trophic level[c]		2.8	2.7	2.7	3.1	3.7	3.8
generic TL value[d]		2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	3.1 ± 0.1	4.4 ± 0.0	4.4 ± 0.0

[a] Included in carcass is one fillet.
[b] Whole fish-to-fillet ratio.
[c] Based on a δ^{15}N value of 10.8 determined for zebra mussels (*Dreissena polymorpha*) sampled at Kelheim in 2014.
[d] Based on diet studies (Froese and Pauly, 2017).

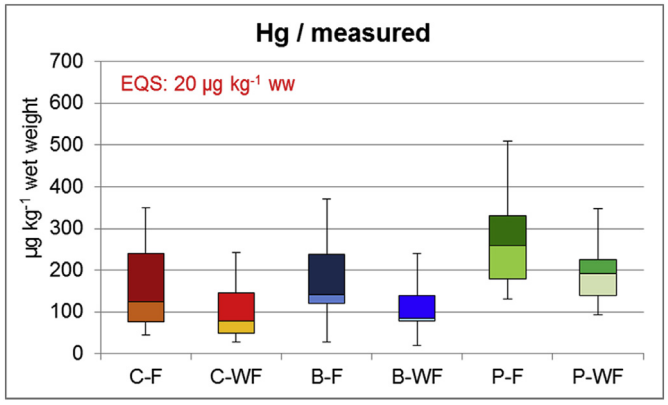

Fig. 2. Box-Plot diagram showing the upper and lower quantile and range of mercury concentrations in fillet (F) and whole fish (WF) of chub (*Squalius cephalus*, n = 28), bream (*Abramis brama*, n = 11), and perch (*Perca fluviatilis*, n = 19) sampled at Kelheim in the German Danube in 2015. C: chub, B: bream, P: perch; EQS: Environmental Quality Standard in fish.

30–40 cm in length. Nevertheless, their Hg levels varied clearly less than those of older fish. It is therefore assumed that the variability will further decrease in even younger fish. The German Working Group on Water Issues of the Federal States and the Federal Government LAWA (2016) recommends relatively small fish for biota monitoring under the WFD (e.g., chub of 23–30 cm length with an assumed age of 3–4 years). The recommendations of the LAWA are based on the assumption that less variability yields more robust data for trend monitoring. Our data support this recommendation. With respect to compliance monitoring this may also be relevant if the concentrations in fish are in the range of the EQS because exceedance or non-exceedance can easier be detected if the variability (i.e., the standard deviation of the mean concentration) is low. Moreover, if pooling of samples is intended, it would be advisable to focus on relatively young fish comparable to the age classes recommended by the LAWA (2016) because the associated information loss would be smallest.

3.3. PFOS/analyses of pool samples

Measured PFOS ranged between 1.92 and 12.3 µg kg^{-1} ww in pool samples of fillet and 5.85 and 36.0 µg kg^{-1} in whole fish. PFOS binds to proteins (Jones et al., 2003; Luebker et al., 2002) and is typically highest in liver, kidney and blood (Ahrens et al., 2009; Goeritz et al., 2013; Martin et al., 2003). Hence, the major fraction of PFOS is found in the carcasses of fish and not in fillets. In the present study the whole fish-to-fillet ratios based on measured PFOS concentrations ranged between 2.5 and 3.1 (Table S4, Supplementary material).

PFOS is known to enrich in the food web resulting in higher concentrations in predatory species like perch (e.g., Kannan et al., 2005; Martin et al., 2004a,b; UNEP, 2006). In the present study levels were lowest in chub, concentrations in bream and perch, however, were quite similar (Fig. 3, Table S4, Supplementary material).

Significant correlations were detected between PFOS in fillet, carcass and whole fish and δ^{15}N ($p < .05$) (Table S6, Supplementary material). However, no correlations were found between δ^{15}N and the PFOS whole fish-to-fillet ratios indicating that the partitioning of PFOS is not influenced by trophic level, fish species and size of fish (Table S7, Supplementary material). The finding that PFOS levels in fish are influenced by their trophic position (as measured by δ^{15}N) is in line with Babut et al. (2017) who identified δ^{15}N as an important variable influencing PFOS levels in fish from the Rhône.

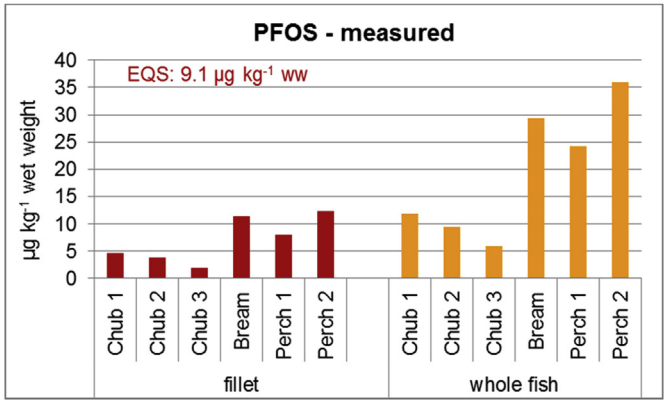

Fig. 3. PFOS concentrations (µg kg^{-1} wet weight) in fillet and whole fish sampled in the German Danube at Kelheim in 2015. Data refer to pool samples of chub (*Squalius cephalus*, chub 1: n = 9, chub 2: n = 11, chub 3: n = 8), bream (*Abramis brama*, n = 8), and perch (*Perca fluviatilis*, perch 1: n = 10, perch 2: n = 8). EQS: Environmental Quality Standard in fish.

However, in our study bream do not quite fit into this picture: despite a lower $\delta^{15}N$ value of 14.5 they had accumulated more PFOS than small perch (with $\delta^{15}N$ of 16.5). Possibly, differences in food contamination account for these findings.

3.4. Lipophilic substances/analyses of pool samples

The lipophilic substances PCDD/F + dl-PCB, ndl-PCB, PBDE, α-HBCDD and HCB accumulated mainly in the lipid-rich carcass. Accordingly, whole fish concentrations were higher compared to those in fillets (Fig. 4, Table S5, Supplementary material). Contaminant concentrations mostly increased with fish size/age; i.e., highest levels were detected in the pools with the largest fish (chub 3, respectively perch 2). The only exception was HCB which was equally high in both perch pools and also in small and medium-sized chub (Fig. 4E). The partitioning of PCDD/F+dl-PCB, ndl-PCB, PBDE, α-HBCDD, and HCB between tissues in the fish was linked to the lipid content as indicated by significant ($p < .05$) correlations between the whole fish-to-fillet ratios for lipids and the respective whole fish-to-fillet ratios for the substances (Table S7, Supplementary material).

PCDD/F + dl-PCB concentrations ranged between 0.55 and 4.81 ng kg^{-1} ww WHO(2005)-TEQ in pooled fillet tissue and between 1.74 and 11.4 ng kg^{-1} WHO(2005)-TEQ in whole fish pools (Fig. 4A). Levels of ndl-PCB were clearly higher (by factors around 12,800) with 8.23–68.7 μg kg^{-1} in fillets and 21.7–119 μg kg^{-1} in whole fish (Fig. 4B). Highest levels of both substances were found in fillets of large chub and, with respect to whole fish, in perch (Table S5, Supplementary material) and increased with fish age and size. The dominant ndl-PCB congeners in all samples were CB-153, CB-138 and CB-180. The whole fish-to-fillet ratios based on measured concentrations of PCDD/F+dl-PCB and ndl-PCB were in the range of 1.7–3.2 in bream and chub and 7.5–10 in perch (Table S5, Supplementary material). Correlation analyses for PCDD/F+dl-PCB and ndl-PCB in whole fish, fillets and carcasses revealed positive relations ($p < .05$) with the respective lipid contents and, except in fillets, also with the dry mass fractions (Table S6, Supplementary material). The whole-fish-to-fillet ratios for both, PCDD/F+dl-PCB and ndl-PCB correlated significantly with $\delta^{15}N$ ($p < .007$, respectively $p < .003$) suggesting that the trophic position of fish has some influence on the distribution of these substances in fish (Table S7, Supplementary material).

PBDE concentrations in fillet pools were in the range of 0.43–4.13 μg kg^{-1} ww. Levels in whole fish were clearly higher

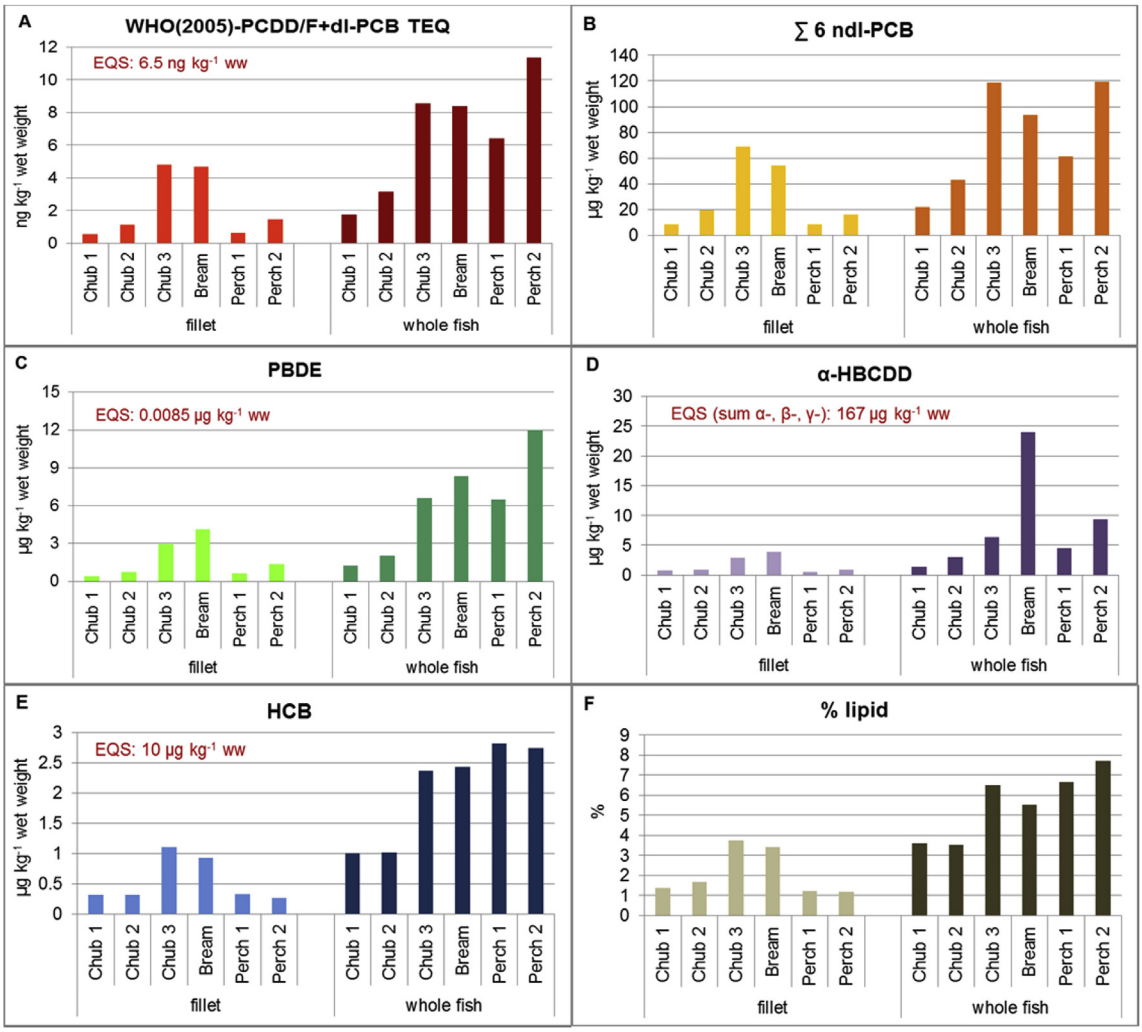

Fig. 4. Concentrations of lipophilic substances and %lipid in fillet and whole fish sampled at Kelheim/Danube in 2015. Data refer to pool samples of chub (*Squalius cephalus*, chub 1: n = 9, chub 2: n = 11, chub 3: n = 8), bream (*Abramis brama*, n = 8), and perch (*Perca fluviatilis*, perch 1: n = 10, perch 2: n = 8). Concentrations are given as ng kg^{-1} wet weight WHO(2005)-TEQ in the case of PCDD/F + dl-PCB and as μg kg^{-1} wet weight for all other substances. EQS: Environmental Quality Standard in fish. In the case of HBCDD the EQS of 167 μg kg^{-1} refers to the sum of the α-, β-, and γ-diastereomers of which, in freshwater fish, approximately 80% is α-HBCDD (Covaci et al., 2006; Rüdel et al., 2012).

ranging between 1.23 and 12.0 μg kg^{-1} ww (Fig. 4C, Table S5, Supplementary material). PBDE levels increased with fish age and size. The whole fish-to-fillet ratios ranged between 2.0 and 2.9 in bream and chub. Clearly higher ratios of 9.8 and 8.9 were observed for perch (Table S5, Supplementary material). These findings are in line with reported data from the Laurentian Great Lakes: For brown bullhead (*Ameiurus nebulosus*) and common carp (*Cyprinus carpio*) from Lake Ontario ∑PBDE whole fish-to-fillet ratios were in the range of 2.6–4.9 (Gandhi et al., 2017) which corresponds to the ratios for cyprinids in the present study. Furthermore, Su et al. (2017) found significant positive correlations between fish age and whole fish concentrations of PBDE in trout (*Salvelinus namaycush*) and walleye (*Sander vitreus*) from the Laurentian Great Lakes which supports the here observed increase in PBDE with fish age/size. The dominant congener was BDE-47 (on average 58% of the total PBDE mass), followed by BDE-100 in bream and chub (mean 19%) with one exception: in fillets of small chub (pool chub 1) BDE-209 was present at concentrations around 0.11 μg kg^{-1} constituting 20% of the total PBDE content in this sample (Fig. S2, Supplementary material). In the respective carcass sample, however, no corresponding high levels of BDE-209 were detected. So far we have no conclusive explanation for this finding, all the more as cyprinids have been found to effectively debrominate BDE-209 (Viganò et al., 2011). Moreover, Gandhi et al. (2017) found that while PBDE congener pattern may vary strongly among and within fish species, it hardly differs among fillet, whole fish (and eggs) within one fish. A measurement error can therefore not be excluded. In perch, the second most frequent congener was BDE-99 with around 22% (Fig. S2, Supplementary material). Species-specific PBDE metabolization has been described, e.g., by Roberts et al. (2011) who report that metabolization rates in carp were 10–100 times faster than in salmonid fish. Possibly BDE-99 was quickly metabolized in chub and bream whereas the process was slower in perch leading to the observed accumulation of BDE-99.

PBDE concentrations in whole fish, fillets and carcasses correlated significantly with lipid contents ($p < .05$). For carcasses and whole fish significant correlations were also detected between PBDE and dry mass ($p = .002$) whereas this was less obvious in fillets ($p = .060$). (Table S6, Supplementary material). Positive correlations were also found for δ^{15}N and PBDE in carcass ($p < .05$) and – although not significant – in whole fish ($p < .1$) suggesting that PBDE is biomagnified. Biomagnification of the BDE congeners –47 and –209 has previously been reported, e.g., by Law et al. (2006) for the food web of Lake Winnipeg (CA). Moreover, the distribution of PBDE in fish seems to be influenced by their trophic position as indicated by the observed significant correlation ($p = .004$) between the PBDE whole fish-to-fillet ratios and δ^{15}N (Table S7, Supplementary material).

Of the three WFD-relevant HBCDD diastereomers α-, β-, and γ-HBCDD, the present study focused on the analysis of α-HBCDD because of difficulties in chemical analysis during this measurement series. α-HBCDD is the predominant diastereomer in most fish samples constituting around 80% of the total HBCDD (e.g., Covaci et al., 2006; Rüdel et al., 2012). It is therefore assumed that the results for α-HBCDD will at least provide an estimate of the total HBCDD contamination of the fish.

Concentrations of α-HBCDD ranged between 0.54 and 3.91 μg kg^{-1} ww in fillet pools and between 1.37 and 24.0 μg kg^{-1} ww in whole fish with bream being the most contaminated species. The ratios between whole fish and fillets were in the range of 1.8–6.1 in chub and bream and 8.3–11.2 in perch (Table S5, Supplementary material). Correlation analysis revealed a significant correlation ($p = .003$) between the lipid content and α-HBCDD in fillet but not in carcass and whole fish (Table S6, Supplementary material). A significant correlation ($p = .002$) was also detected between the α-HBCDD whole fish-to-fillet ratio and δ^{15}N indicating that the partitioning of α-HBCDD is related to the trophic level (Table S7, Supplementary material).

HCB in fillet ranged between 0.26 and 1.10 μg kg^{-1}, with highest concentrations found in large chub (chub 3). In whole fish levels were between 1.01 and 2.82 μg kg^{-1} and highest concentrations were detected in perch. Whole fish-to-fillet ratios were in the range of 2.2–3.2 in bream and chub and 8.6–10.5 in perch and correlated significantly ($p = .003$) with δ^{15}N suggesting a trophic level dependent distribution of HCB in fish (Tables S5 and S7, Supplementary material). HCB correlated significantly with the lipid fraction in all three matrices ($p < .005$) and levels in whole fish and carcass correlated also with the dry mass fraction ($p < .005$, Table S6, Supplementary material). Furthermore, δ^{15}N correlated with HCB in carcass ($p < .05$) and, although not significantly, in whole fish ($p < .1$) indicating that HCB is enriched in the food web. Trophic magnification is well known for HCB (Houde et al., 2008; Moermond and Verbruggen, 2013).

3.5. Effects of data normalization

Following the recommendations of the WFD Guidance Document No 32 on Biotamonitoring (EC, 2014) the concentrations of Hg and PFOS were normalized to 26% dry mass and those of the lipophilic substances to 5% lipid content to account for differences between species and analyzed matrices.

Normalization of the Hg and PFOS data to 26% DM had only minor effects. In the case of Hg, fillet concentrations increased by around 30% (mean of all 58 fish) after normalization. The effects on whole fish were less pronounced with slight decreases in bream and perch (mean: –2% and –12%, respectively) and slight increases in chub (mean + 6%) (Table S2, Fig. S3A, Supplementary material). Based on normalized concentrations the whole fish-to-fillet ratio for Hg was around 0.5 in all three species (*vs.* 0.6–0.7 based on non-normalized concentrations).

Similarly, normalization led to around 28% higher PFOS concentrations in fillet while levels in whole fish decreased slightly in bream, perch and large chub and increased slightly in smaller and medium-sized chub (Table S4, Fig. S3B, Supplementary material). As a result, the whole fish-to-fillet ratios decreased from 2.5 to 3.0 (based on measured concentrations) to 2.0–2.4 after normalization.

In the case of lipophilic substances, normalization to 5% lipid typically led to higher concentrations in fillets and lower levels in whole fish (Table S5, Supplementary material). Substance concentrations were linked to lipid contents and accordingly, normalization to lipid adjusted the substance concentrations in fillet and whole fish (Amrhein et al., 1999; Gewurtz et al., 2011; Jürgens et al., 2013). As a result, the whole fish-to-fillet ratios decreased, from 1.7 to 11.2 for measured concentrations to 0.7–3.8 based on lipid normalized concentrations (Table S5, Supplementary material). Effects were strongest in perch where differences in lipid partitioning between fillet and carcass were most pronounced (Table 1, Fig. 5C). For both, small (perch 1) and large perch (perch 2) normalization reduced the whole fish-to-fillet ratios by more than 80%. In bream, the ratios decreased by about 39% after normalization. In chub, normalization effects on whole fish-to-fillet ratios were most pronounced in small chub (ratio decrease by around 62%) followed by medium size chub (ratio decrease by 52%) and large chub (ratio decrease by around 42%) (Fig. 5A and B).

The positive effects that lipid normalization has on aligning monitoring data of lipophilic substances in fillet and whole fish are emphasized in the WFD Guidance Document (EC, 2014) - provided that substance concentrations correlate with lipid levels (Hebert and Keenleyside, 1995). In the present study significant

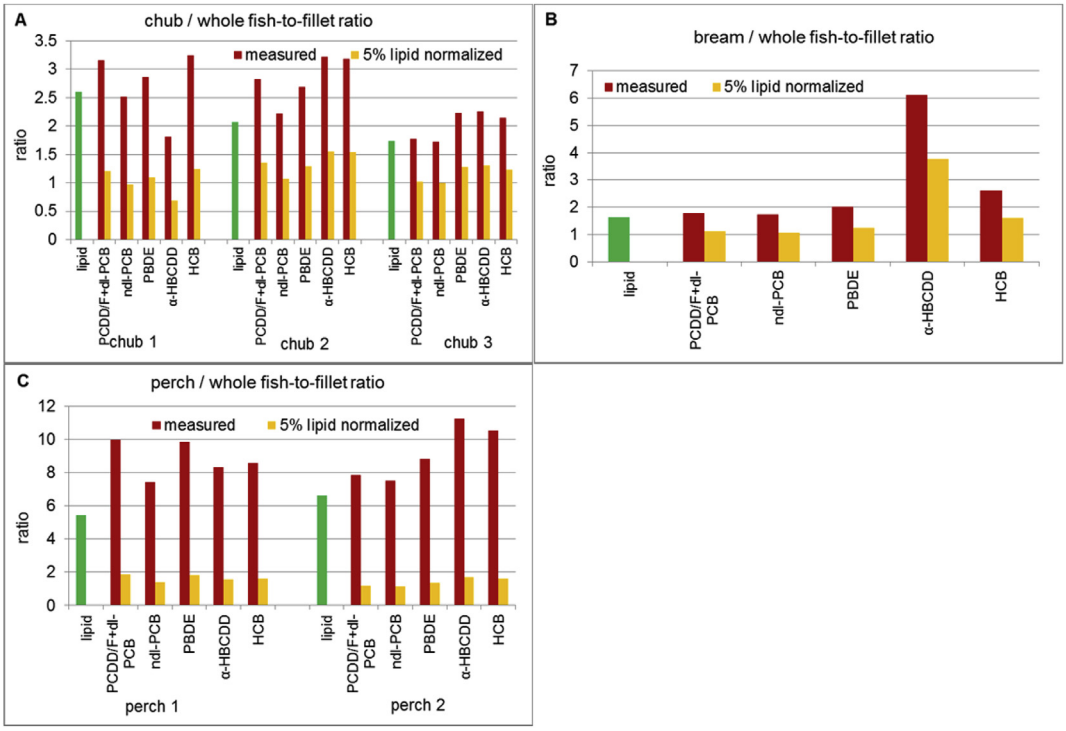

Fig. 5. Whole fish-to fillet ratios of measured and 5% lipid normalized concentrations of PCDD/F+dl-PCB, ndl-PCB, PBDE, α-HBCDD and HCB in chub (*Squalius cephalus*, chub 1: n = 9; chub 2: n = 11, chub 3: n = 8), bream (*Abramis brama*, n = 8), and perch (*Perca fluviatilis*, perch 1: n = 10; perch 2: n = 8) sampled 2015 at Kelheim/Danube.

correlations between lipid and substance concentrations were detected for all lipophilic substances and matrices (Table S6, Supplementary material).

3.6. Conversion of fillet concentrations to whole fish

The results of the regression analysis are summarized in Table 2.

In the case of Hg the analysis is based on logarithmized individual fish data. Concentrations in whole fish and fillets correlated significantly for all three species ($p < .0001$). For chub the slope of the regression line did not differ significantly from 1 and the fillet-to-whole-fish ratio can be used as conversion factor (Table 2). Fig. 6 shows the regression analysis based on the combined data of the individuals of all three species (n = 58). Again the slope does not differ significantly from 1 indicating that the whole fish-to-fillet ratio can be applied as conversion factor also when facing samples with more than one species.

Normalizing the data to 26% dry mass improved the correlation only marginally but the slope of the resulting regression line was significantly different from 1. This means that translating fillet to whole fish concentration using 26% DM normalized data should resort to the respective conversion equation (Table 2, CE 5).

Peterson et al. (2005) analyzed Hg in fillet and whole fish of 13 piscivorous and non-piscivorous fish species from 65 sites in western USA. Applying their fillet-to-whole fish equation our data (all individuals, n = 58) resulted in around 50% lower whole fish concentrations. These discrepancies may be explained by differences in the underlying data base, as Peterson et al. (2005) based their conversion equation on considerably more species from diverse sites whereas our study relied on three species from one sampling location.

Based on perch data from 6 Swedish lakes Fauxneld et al. (2015) derived a fillet-to-whole fish equation for Hg that led to only slightly higher whole fish concentrations (on average 7.6%) when applied on our perch data. These small differences are probably related to habitats and feeding habits.

All other substances covered in the present study were analyzed in pool samples limiting the number of data for regression analyses to n = 6.

For PFOS a highly significant relationship ($p < .0001$) was detected between fillet and whole fish concentrations (Fig. 7) indicating that the distribution of PFOS between tissues is independent of fish species, size and age. This has already been suggested by the missing correlation between the PFOS whole fish-to-fillet ratio and $\delta^{15}N$ (Table S7, Supplementary material). As with Hg, normalization to 26% dry mass improved the correlation slightly. The slopes of both regression lines differed significantly from 1 indicating that the conversion equations should be used for translating fillet concentrations to whole fish.

Regression analyses of the lipophilic substances were less conclusive yielding no significant relations between fillet and whole fish concentrations when based on measured data (Fig. S4, Supplementary material). Normalization to 5% lipid improved the fitting for all substances and resulted in significant correlations for PCDD/F+dl-PCB ($p = .0024$), ndl-PCB ($p = .0004$), PBDE ($p = .0009$), and HCB ($p = .0471$) (Table 2, Fig. S4, Supplementary material). The slopes of all regression lines did not differ significantly from 1 suggesting that the respective whole fish-to-fillet ratios can serve as conversion factors for translating lipid-normalized fillet to lipid-normalized whole fish concentrations. There was also a significant correlation ($p = .0034$) between α-HBCDD in fillet and whole fish. The regression, however, was biased by the extremely high α-HBCDD concentration in the bream whole fish sample (Fig. 4D) which led to erroneous results.

Comparative data from other studies are scarce. For PCB, Amrhein et al. (1999) derived mean whole fish-to-fillet ratios of 1.70 ± 0.80 and 1.47 ± 0.61 based on measured concentrations in coho salmon (*Oncorhynchus kisutch*) and rainbow trout

Table 2
Results of regression analyses using fillet *vs.* whole fish concentrations of the priority substances Hg, PFOS, PCDD/F+dl-PCB, ndl-PCB, PBDE, α-HBCDD and HCB determined in chub (*Squalius cephalus*), bream (*Abramis brama*), and perch (*Perca fluviatilis*) sampled at Kelheim/Danube in 2015. Concentration refer to μg kg^{-1} wet weight (in the case of PCDD/F+dl-PCB to ng kg^{-1}[1] wet weight WHO(2005)-TEQ). White fields: based on measured concentrations, grey shading: based on normalized data.

Substance	Data base		Fillet-to-whole-fish conversion equation (CE)	r^2	Pearson's correlation Coefficient (α = 0.05)	Sign. difference from slope = 1	Mean Conversion factor
Hg measured	Individual fish	chub n = 28	CE 1: $\ln(Conc_{whole\ fish}) = 0.9819 \times \ln(Conc_{fillet}) - 0.3419$	0.988	p <0.0001	no	0.7
	Individual fish	bream n = 11	CE 2: $\ln(Conc_{whole\ fish}) = 0.9301 \times \ln(Conc_{fillet}) - 0.1175$	0.998	p <0.0001	yes	
	Individual fish	perch n = 19	CE 3: $\ln(Conc_{whole\ fish}) = 0.8954 \times \ln(Conc_{fillet}) + 0.2404$	0.991	p <0.0001	yes	
	Individual fish	all species n = 58	CE 4: $\ln(Conc_{whole\ fish}) = 0.9954 \times \ln(Conc_{fillet}) - 0.38$	0.985	p <0.0001	no	0.7
Hg 26% DM norm	Individual fish	all species n = 58	CE 5: $\ln(Conc_{whole\ fish}) = 0.9283 \times \ln(Conc_{fillet}) - 0.2979$	0.987	p <0.0001	yes	
PFOS measured	Pool	n = 6	CE 6: $Conc._{whole\ fish} = 2.8459 \times Conc._{fillet} - 0.4636$	0.983	p <0.0001	yes	
PFOS 26% DM norm	Pool	n = 6	CE 7: $Conc._{whole\ fish} = 1.9266 \times Conc._{fillet} + 1.017$	0.997	p <0.0001	yes	
PCDD/F+dl-PCB 5% lipid norm	Pool	n = 6	CE 8: $Conc._{whole\ fish} = 0.8711 \times Conc._{fillet} + 1.5602$	0.888	p = 0.0024	no	1.3
ndl-PCB 5% lipid norm	Pool	n = 6	CE 9: $Conc._{whole\ fish} = 0.9452 \times Conc._{fillet} + 8.1822$	0.9527	p = 0.0004	no	1.1
PBDE 5% lipid norm	Pool	n = 6	CE 10: $Conc._{whole\ fish} = 1.2425 \times Conc._{fillet} + 0.3656$	0.9331	p = 0.0009	no	1.4
HCB 5% lipid norm	Pool	n = 6	CE 11: $Conc._{whole\ fish} = 1.2497 \times Conc._{fillet} + 0.2625$	0.5443	p = 0.0471	no	1.5

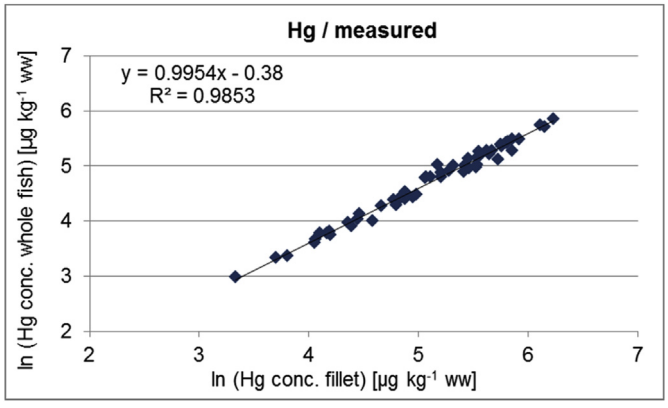

Fig. 6. Ln-transformed Hg concentrations (μg kg^{-1} wet weight) in fillet *vs.* whole fish for chub (*Squalius cephalus*, n = 28), bream (*Abramis brama*, n = 11), and perch (*Perca fluviatilis*, n = 19) sampled 2015 at Kelheim/Danube.

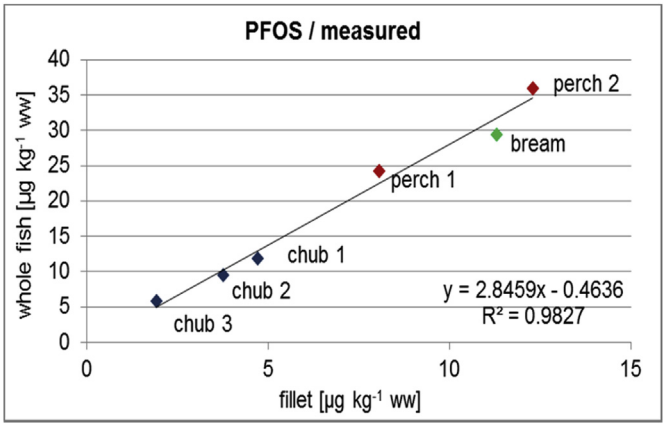

Fig. 7. PFOS concentrations (μg kg^{-1} wet weight) in fillet *vs.* whole fish for pool samples of chub (*Squalius cephalus*, chub 1: n = 9; chub 2: n = 11, chub 3: n = 8), bream (*Abramis brama*, n = 8), and perch (*Perca fluviatilis*, perch 1: n = 10; perch 2: n = 8) sampled 2015 at Kelheim/Danube.

(*Oncorhynchus mykiss*), respectively. Lipid normalization reduced the ratios to 0.98 ± 0.31 (coho salmon) and 0.85 ± 0.23 (rainbow trout). With respect to measured concentrations we found similar low whole-fish-to-fillet ratios for ndl-PCB only in bream and large chub (both 1.7). Following lipid normalization, however, the whole fish-to-fillet ratios in chub, bream and big perch were in the range of 0.97–1.14 (Table S5, Supplementary material) and are thus in good agreement with the salmon and trout data. This underlines

the lipid dependency of PCB accumulation and the need of lipid normalization when calculating concentrations in whole fish respectively fillet.

Stone (2006) analyzed total PBDE and total PCB in whole body and fillets of chinook salmon (*Oncorhynchus tshawytscha*) and found average whole fish-to-fillet ratios of 1.5 for PBDE and PCB based on measured concentrations. Normalization to lipid adjusted the data yielding whole fish-to-fillet ratios of 1. These ratios are clearly lower than what we detected for PBDE and ndl-PCB. Batt et al. (2017) used whole fish-to-fillet conversion factors of 1.83 and 1.50 for PCB and PBDE, respectively, which they derived from published data (i.e., Environment Canada, 2013; Stone, 2006; US Department of Energy, 1997; USGS, 2014). The PCB value matches best to our bream and chub ratios (i.e., 1.7–2.5) whereas the PBDE value was lower than the ratios we detected (i.e., 2.0–9.8).

For none of the other substances studied here published conversion factors or -equations for freshwater fish were available.

To put the results into context and show the applicability of the data conversion, the conversion equations and -factors were applied to fillet data compiled in two monitoring programs that ran in parallel to the present study, one by the German Environmental Specimen Bank (ESB), the other by the Bavarian Environment Agency (LfU). A short description of the programs and the results are given in the Supplementary material Part 2.

4. Compliance with reference values

The EU Guidance Document on biota monitoring (EC, 2014) recommends to determine EQS compliance with data not only normalized to lipid or dry mass but also adjusted to a common trophic level (i.e., TL 4 in the case of freshwater fish) to compensate for species specific differences in contamination. Trophic level adjustment, however, requires substance specific trophic magnification factors (TMFs) which in many cases are not available. Since TMFs may vary depending, e.g., on water body, food web characteristics, and geographic location, it is not always helpful to resort to published values even if these are available (Fliedner et al., 2016a). Ideally, TMFs are generated for the respective water body under investigation. This, however, is labor-intensive and expensive and seems impractical considering thousands of sites which have to be monitored EU-wide. Therefore, as long as relevant TMFs for the respective substances and water bodies are not available it seems reasonable to assess compliance using measured or lipid/dry mass normalized data.

In the present study we used *whole* fish concentrations (measured and lipid normalized) to assess compliance with the biota EQSs (EC, 2013) and with wildlife reference values from Canada (CCME, 2017; Environment Canada, 2013, 2016, 2017) and the U.S. (Lazorchak et al., 2003). The results are summarized in Table S8 (Supplementary material).

All fish samples exceeded the threshold values for Hg. The same was true for PFOS with only one exception (chub 3). In contrast, there was 100% compliance with the available threshold values for HBCDD, HCB, and ndl-PCB. In the case of PCDD/F+dl-PCB, large perch (perch 2), large chub (chub 3), and bream exceeded the EU EQS. All samples met the Canadian wildlife value for PBDE but exceeded the respective EU-EQS. This is because of the extremely low EU-EQS of 0.0085 $\mu g\ kg^{-1}$ PBDE (based on the protection goal human health). The respective EU value derived for secondary poisoning of piscivorous top predators is 44 $\mu g\ kg^{-1}$ ww which corresponds well to the Canadian Wildlife values and is met by all samples.

5. Conclusions

The results of the present study demonstrate the differences in contaminant concentrations between fish species, fish sizes, and fillet and whole fish.

Based on the present data the following conclusions can be drawn:

- More than one species of different trophic levels should be monitored at least at sites with priority substance concentrations in the range of the respective EQSs (as recommended by EC, 2014; LAWA, 2016).
- Basing risk assessment and EQS compliance of lipophilic substances solely on wet weight concentrations in fillets would significantly underestimate the risk for piscivorous predators. This is true also for PFOS.
- For Hg, risk assessment and EQS compliance based on fillet concentrations would overestimate the risk to piscivorous wildlife.
- Normalization to 5% lipid can partly overcome discrepancies between substance concentrations in fillet and whole fish. Note that this is only an option when substance concentrations correlate significantly with lipid contents.
- Normalization to 26% dry mass for non-lipophilic substances (e.g., Hg and PFOS) is only partly effective in adjusting fillet and whole fish data.
- Fish size/age must be considered when monitoring Hg contamination. Focus should be put on young fish (as recommended by EC, 2014; LAWA, 2016) to minimize variation among individuals.
- PFOS distribution between tissues is independent of fish species, trophic position and size/age at least in the fish species studied here.
- Measured and 26% normalized fillet data of Hg and PFOS can be converted to whole fish data *vice versa* using the respective conversion equations or -factors.
- Conversion of fillet concentrations to whole fish or *vice versa* is possible for 5% lipid normalized data of PCDD/F+dl-PCB, ndl-PCB, PBDE, and HCB using the respective conversion equations or -factors.

The results of the present study demonstrate that analyzing only fillet or only whole fish and only one species entails the risk of underestimating the potential hazard to human consumers or piscivorous wildlife or of overestimating the risk and triggering costly but unnecessary measures to achieve a good chemical status under the WFD.

With respect to the conversion of substance concentrations from one matrix to the other it has to be kept in mind that the underlying data base in the present study was relatively small for all substances but Hg. Nevertheless, the regression equations and -factors can help to at least get an estimate of the whole fish concentrations when only fillet is analyzed (or the other way around). In many cases this will be sufficient for checking EQS compliance (i.e., the identification of a clear exceedance or clear non-exceedance of the EQS). Further studies using individual fish are needed to derive broadly applicable conversion equations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.12.052.

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