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Food web on ice: a pragmatic approach to investigate the trophic magnification of chemicals of concern

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Abstract

Background: The trophic magnification factor (TMF) is a metric that describes the average trophic magnification of a chemical through a food web. TMFs may be used for the risk assessment of chemicals, although TMFs for single compounds can vary considerably between studies despite thorough guidance available in the literature to eliminate potential sources of error. The practical realization of a TMF investigation is quite complex and often only a few chemicals can be investigated due to low sample masses. This study evaluated whether a pragmatic approach involving the large-scale cryogenic sample preparation practices of the German Environmental Specimen Bank (ESB) is feasible. This approach could provide sufficient sample masses for a reduced set of samples allowing screenings for a broad spectrum of substances and by that enabling a systematic comparison of derived TMFs. Furthermore, it was assessed whether plausible TMFs can be derived with the 'Food web on ice' approach via a comparison with literature TMF values.

Results: This investigation at Lake Templin near Potsdam is the first TMF study for a German freshwater ecosystem and aimed to derive TMFs that are appropriate for regulatory purposes. A set of 15 composite biota samples was obtained and analyzed for an extended set of benchmark chemicals such as persistent organic pollutants, mercury and perfluoroalkyl substances. TMFs were calculated for all substances that were present in > 80% of the biota samples. For example, in the case of polychlorinated biphenyls, TMFs from 1.7 to 2.5 were determined and comparisons to literature TMFs determined in other freshwater ecosystems showed similarities. We showed that 32 out of 35 compounds analyzed had TMFs significantly above 1. In the remaining three cases, the correlations were not statistically significant.

Conclusions: The derived food web samples allow for an on-demand analysis and are ready-to-use for additional investigations. Since substances with non-lipophilic accumulation properties were also included in the list of analyzed substances, we conclude that the 'Food web on ice' provides samples which could be used to characterize the trophic magnification potential of substances with unknown bioaccumulation properties in the future which in return could be compared directly to the benchmarking patterns provided here.

Keywords: Bioaccumulation, Biomagnification, TMF, Aquatic food web, Freshwater, Fish, Mussels, Plankton, Stable isotope analysis, Trophic positions

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Background

With the Green Deal, the new chemicals strategy for sustainability and its Zero Pollution Action Plan, the European Union (EU) is setting ambitious incentives for a chemicals market that is safe for human health and the

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environment [1–3]. It has also been recognized that authorities should take greater account of data on the fate and behavior of substances in the environment when assessing the risks of chemicals. So far, bioaccumulation assessment is mainly based on laboratory data for substances, e.g., the determination of the 1-octanol/water partition coefficient [4], the bioconcentration potential of substances in fish [5], the bioaccumulation of sediment-associated chemicals in endobenthic oligochaetes worms [6], and the bioaccumulation of chemicals in soil oligochaetes [7]. The use of field data in regulatory bioaccumulation assessment has been discussed [8–10] but they are still underused. This concerns the systematic investigation of ecosystems for existing chemicals and the quantification of their accumulation in food webs. Trophic magnification factors (TMFs), which describe the average accumulation of a substance through a food web of interest, have been suggested to support the risk assessment of marketed chemicals which are already present in the environment and are subject of regulatory concern [11, 12]. So far, regulatory guidelines for TMF studies are missing, they are not standardized and cost-intensive compared to laboratory studies. With the here presented project 'Food web on ice', we propose a cost-efficient way to use archived samples of a single sampling campaign in a way that allows the derivation of TMFs for a range of current and future relevant chemicals. The investigation of accumulative reference chemicals, which are well studied and undisputed in regard to their persistent and bioaccumulative properties, allows a reliable check of the plausibility of the 'Food web on ice' approach. Furthermore, data on the accumulation of new potentially problematic substances that are less well studied can be compared to the accumulation of these reference chemicals in the same system.

The TMF is sometimes referred to as the 'conclusive evidence of the bioaccumulative nature of the compound' as it integrates bioaccumulation processes over an entire food web and under realistic environmental conditions [9]. The use of TMFs is also considered in the context of the European Water Framework Directive (WFD) for the derivation of Environmental Quality Standards (EQSs) and the normalization of concentrations in different fish species to one common trophic level for EQS compliance checking [13, 14]. The TMF approach has a long history: first basic investigations in regard to general food web transfer date back to the 1970s, when the accumulation of DDT along an aquatic food chain was observed [15] and the transfer of polychlorinated biphenyls (PCBs) was evaluated in artificial food chains under laboratory conditions [16, 17]. In TMF studies, a regression of concentration data determined in biota samples against the species' trophic positions, which are based on stable

isotope data for nitrogen, is performed [11]. A generally approved standardized approach for TMF studies is still lacking, although TMFs for several hundreds of chemicals have been derived in the past 20 years. Suggestions for a standardized study framework are available and were considered in this work [12, 18].

The performance of a TMF study requires a thorough preparation that involves a variety of crucial considerations. The most important aspect is the selection of the sampling site, followed by the identification of the food web and planning of the sampling and its time frame. Following the advice provided in review papers on the performance of a TMF study [11, 12], we designed a customized sampling campaign for an investigation in a German freshwater ecosystem. The following aspects regarding the selection of the sampling site have been considered in the planning of the campaign:

i. An important criterion for the reliable quantification of the accumulation of substances in food webs is that the ecosystem is in a state of equilibrium. TMF studies in very different regions have shown [19–21] that this is often not the case in rivers and streams [11]. Therefore, we were aiming for a lake ecosystem to investigate the TMF concept during this project.

ii. In order to allow for the reliable quantification of the target substances in all food web samples, and not only fish, the ecosystem must be exposed to a sufficiently high level of pollutants. Therefore, ecosystems with a slightly elevated anthropogenic input are preferred to assure sufficient detection limits for the desired compounds throughout the food web. As mentioned above, the pollutant input should be as consistent as possible to provide a steady-state like scenario. The input should not be too high; otherwise, toxic side effects could alter the organisms' physiology and with that the observed accumulation.

iii. The nutrient load of the ecosystem is of crucial importance, especially with regard to the available sample material of low trophic levels, e.g., plankton. A eutrophic freshwater ecosystem was selected to provide at least 1 kg (wet weight) of each of the different sample materials for subsequent homogenization, chemical analysis for benchmark chemicals, and archiving of sufficient sample material to derive additional TMFs for chemicals of emerging concern.

We aimed for representative species present in the selected lake that have a known food web connection. This is easier to observe empirically in the case of

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preferably pelagic species, but due to the expected coupling of carbon sources, a purely pelagic food web [13] cannot be expected in relative small, shallow lakes as the selected one.

For the food web selection, the following aspects were considered:

i. The selected food web items should be a representative, central part of the entire food web. This means that the sampled species should have a high abundance and express a dietary connection to provide a basis for a comprehensive evaluation of a substances' dietary transfer in the food web.

ii. Ideally, TMFs are derived along a distinct food web with one common source of nutrients at the base of the food web. Nutrient sources include primary producers in the water phase (pelagic source) and/or organic particles such as e.g., detritus in the sediment or periphyton (benthic source). Depending on the ecosystem, terrestrial input can contribute to the benthic carbon load and also present an independent carbon source [22, 23]. In general, mixing of nutrient sources is commonly observed in freshwater environments [24, 25]. In this study, it was attempted to focus on samples which are known to typically depend on pelagic carbon sources. Doing so ensured a maximum dietary connection of the biota samples taken from the ecosystem.

iii. Before the sampling campaign is conducted, information about the species' diversity, abundance, and feeding habits in the ecosystem should be available. In this study, it was collated with the help of local experts. For the selected lake, data from previous investigations were available and local scientists supported the current work.

iv. To fulfill the steady-state requirement, no migratory species should be included in the sample list as their chemical concentrations and stable isotope ratios may reflect habitats beyond the observed ecosystem. Local experts gave advice on this topic for the sampling campaign.

v. The selected species should not be endangered to obtain the necessary sampling permits. In the selected lake, sufficient non-endangered species were found for the current study.

In order to evaluate the applicability, benefits, and pitfalls of the TMF concept for regulatory assessment and monitoring issues, field samples were collected from the selected lake ecosystem in 2018. All samples were frozen immediately after sampling, cryo-milled, homogenized, and stored under cryogenic conditions according to the

protocols developed for the German Environmental Specimen Bank (ESB) [26] to generate samples for a 'Food web on ice'. This set comprising a plankton fraction, mussel and fish samples offers the possibility to analyze the trophic transfer of several chemicals, but also to investigate general aspects of biomagnification within the same set of samples without the need for further sampling.

In this proof-of-concept study, TMFs of POPs and other substances with well-known biomagnification properties (e.g., PCBs, mercury/methyl-mercury) were derived for the sampled food web and compared with TMFs from other ecosystems.

The following questions were aimed to be answered with this study:

i. Is the 'Food web on ice' sampling approach using large sample amounts feasible?

ii. Can the obtained food web be sufficiently characterized to allow the investigation of the accumulation behavior of chemicals with diverse properties?

iii. Can TMFs be derived with the 'Food web on ice' approach which are plausible and comparable to literature-reported TMFs from other freshwater ecosystems?

Material and methods
Sampling
Ecosystem description
The selected water body was Lake Templin near Potsdam, Germany. This shallow freshwater lake has a surface area of approx. 6 km^2 and a maximum depth of 12 m [27]. It is categorized as a shallow lake with a short water retention time (3–30 days), typical for Central European lowlands [28]. It receives its water from the River Havel which flows through Lake Templin. Before it enters the lake, the Havel flows past the city of Berlin and collects the outflow of various canals that cross the city. Among these is the Teltow Canal which receives the outflow of the waste water treatment plant (WWTP) Ruhleben, the largest WWTP of Berlin, during the summer. In the winter, the Havel receives the WWTP outflow directly some kilometers upstream. Additional input from leisure activities in Lake Großer Wannsee (Berlin) and Lake Templin itself also contribute to the chemical load. According to measurements of the Brandenburg State Agency (Landesamt für Umwelt Brandenburg, LfU), Lake Templin is a eutrophic water body.

Sampling of plankton fractions
Two different techniques for sampling of plankton fractions were applied in the southern part of Lake Templin. The respective sampling locations are shown in

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Additional file 1: A, Figure A1. The first method used a stacked cascade net at two different dates (June and July 2018) that allowed fractionation of the filtered sample. Two water pumps delivering a total flow of 6.2 m^3 h^{-1} transported the lake water through the threefold cascade net with mesh sizes of 250, 100, and 40 µm, respectively. The nets were regularly rinsed with lake water to prevent clogging. The sampled material was stored in cooled brown glass flasks until freezing in liquid nitrogen. The second method was applied to gain a higher plankton biomass for TMF determinations. An approx. 5-m-long coned net with 200 µm mesh width was used, which was supplied with lake water via a pump (Aquahandy, LINN Gerätebau) with approx. 50 m^3 h^{-1} pump flow. Sampling was conducted on 19th September 2018 over a period of about 6 h. In intervals of 30–60 min the net was cleaned from larger debris using a colander and the collected material was carefully compressed in the collection net to remove excess water. The moist plankton sample was directly transferred into liquid nitrogen, then freeze-dried due to the high water content of the collected material and subsequently stored in the gas phase above liquid nitrogen.

Mussel sampling

The freshwater mussel *Dreissena polymorpha* was sampled at four different spots in the southern part of Lake Templin (cf. Additional file 1: A, Figure A1) by staff of the Institute of Inland Fisheries (IfB; Potsdam-Sacrow) on 17–18th September 2018. Preferably submerged mussels growing on larger stones were sampled. However, since the water level was low due to a drought period in summer of 2018, this source of mussels was not available in large quantities. Therefore, sampled material was increased by collecting *D. polymorpha* from the upper layers of sediment-bound colonies using a dip net. The collected mussels were transferred to the near-by IfB facility. Mussels were sorted and dead individuals were removed from the bulk. The remaining mussels were maintained in fresh, aerated tap water in glass aquaria for at least 24 h to clear their guts from any potentially contaminated particles. Afterwards, the mussels were removed from the water, frozen in liquid nitrogen and transported to the Fraunhofer IME site and stored under cryogenic conditions in the ESB archive. The frozen mussels were sorted into two size classes (< 2 cm and > 2 cm), and after thawing, soft tissues were removed from the shells. Tissues were refrozen and stored in the gas phase above liquid nitrogen. The cryogenically cold mussels were allowed to thaw until the soft tissue could be removed from the shell [29]. Tissue samples were stored in the gas phase above liquid nitrogen (< − 150 °C) until

being processed by cryo-milling following ESB procedures [30].

Fish sampling

Different fish species (cf. Table 1) were sampled in the period of 17th–18th September 2018 by the IfB staff in the southern part of Lake Templin (cf. Additional file 1: A, Figure A1). Two different fishing methods were applied, including electrofishing (FEG 5000, electrode diameter approx. 45 cm, EFKO), and overnight deployment of gillnets with different mesh sizes. Collected fish were sorted by size and species, stored on ice and transported to the ESB laboratory. Biometric data of each fish was recorded (size, weight, sex). Fish smaller than 20 cm were treated as one composite sample per species. Fish larger than 20 cm were dissected under a clean bench. One skinless fillet, the gastro-intestinal tract (GIT), and approx. 20 scales were removed, the remaining carcass was cut into smaller pieces (approx. 6 cm per dimension). Commercially available pollack fillet (deep frozen) was thawed and treated as the Lake Templin fish catch after sampling and during transport. Finally, in the laboratory it was exposed during fish dissection and preparation, and used as a field blank. All tissue samples were shock frozen in liquid nitrogen and cryogenically stored until cryo-milling was performed as described above. Scales were carefully cleaned with distilled water, dried overnight on paper towels and stored in paper bags at room temperature. The age determination by examination of the scales was performed by IfB staff.

Sample preparation

Fish fillet, fish carcass, and mussel tissue samples were cryo-milled following procedures of the German ESB program [31]. In brief, all samples were manually crushed under liquid nitrogen cooling and milled with either a planetary mill (Pulverisette 5, Fritsch) or a large-scale cryomill (KHD Palla VM-KT), depending on the sample mass. The plankton fraction was freeze-dried to remove excess water and the resulting dry sample mass thoroughly mixed. Composite samples of the larger fish were generated, sorted by species. In a first step, fillet and carcass homogenates were re-combined considering the weight proportion of both compartments. Some portion of each fillet homogenate was kept in storage as backup in case fillet-specific questions may arise in the future. Individual fish (> 20 cm) were characterized for Hg content, C and N stable isotope pattern, age and size. Individuals of one species were combined in one pooled homogenate sample if differences between the determined characteristics were small. Of each homogenate, at least 80 subsamples of each > 3 g fresh material were generated and have been stored in the gas phase above liquid nitrogen.

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Table 1 Food web samples from Lake Templin (Potsdam, Germany) and their respective characteristics: age, lipid content, stable isotope values of whole fish in case of fish, and calculated trophic positions (TP) using a $\delta^{15}N$ enrichment of 2.3‰ per TP

Sample	Scientific name	Detailed description	Age (y)	Lipid content (%)±SD	Water content (%)	Protein content (mg/g)±SD	$\delta^{13}C$ (‰)±SD	$\delta^{15}N$ (‰)±SD	Calculated TP
Plankton fraction (200 µm)	–	–	–	0.52	90	14.3±1.0	−34.4±0.2	14.7±0.1	1.3
Dreissena, small	*Dreissena polymorpha*	Soft tissue (incl. breathing water) of mussels < 2 cm shell length	≤ 2.5	0.5±<0.1	97.0/95.9*	22.1±0.9	−31.3±0.1	16.4±0.1	2.1
Dreissena, large	*Dreissena polymorpha*	Soft tissue (incl. breathing water) of mussels > 2 cm shell length	≥ 2.5	0.3±<0.1	98.9/97.1*	15.9±0.4	−32.0±0.1	16.1±0.1	1.9
White bream	*Blicca bjoerkna*	Pool of 3 individual fish, GIT removed (length 28–30 cm)	5+–6+	7.4±0.1	71.2	49.2±0.8	−29.2±0.9[§]	18.6±0.2[§]	3.0
Roach	*Rutilus*	Pool of 3 individual fish, GIT removed (length 35–36 cm)	9+–10+	6.2±<0.1	70.5	53.1±2.7	−29.0±0.4[§]	18.9±0.4[§]	3.1
Roach, small	*Rutilus*	Pool of 17 individual whole fish (length: 9–11 cm)	< 1	4.6±0.1	79.5	56.2±11.3	−29.1±0.1	19.2±0.1	3.3
Bleak	*Alburnus*	Pool of 15 individual whole fish (length: 10–13 cm)	< 1	13.0±0.1	72.1	53.3±2.7	−28.7±0.1	19.3±0.1	3.3
Perch, small	*Perca fluviatilis*	Pool of 20 individual whole fish (length: 7–10 cm)	< 1	3.4±<0.1	77.4	15.3±1.7	−28.0±0.1	20.0±0.1	3.6
Pike	*Esox lucius*	Pool of 2 individual fish, GIT removed (length 36–42 cm)	2+	2.8±0.1	78.2	40.0±2.1	−28.0±0.8[§]	20.6±0.8[§]	3.9
Asp	*Aspius*	Pool of 2 individual fish, GIT removed (length 60–62 cm)	5+–7+	11.4±0.7	69.3	53.0±3.6	−27.8±0.1[§]	21.2±0.7[§]	4.2

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Table 1 (continued)

Sample	Scientific name	Detailed description	Age (y)	Lipid content (%) ± SD	Water content (%)	Protein content (mg/g) ± SD	δ^{13}C (‰) ± SD	δ^{15}N (‰) ± SD	Calculated TP
Perch 1	*Perca fluviatilis*	Individual fish, GIT removed (length 41 cm)	10+	5.4 ± 0.1	70.1	47.0 ± 0.7	− 27.0 ± 0.1	21.1 ± 0.1	4.1
Perch	*Perca fluviatilis*	Pool of 2 individual fish, GIT removed (length 28–32 cm)	6+	5.9 ± 0.1	73.0	44.2 ± 4.3	− 26.7 ± 0.4[§]	21.3 ± 0.5[§]	4.2
Pike-perch A	*Sander lucio-perca*	Pool of 3 individual fish, GIT removed (length 21–31 cm)	1+	2.4 ± 0.1	78.1	41.1 ± 1.6	− 27.6 ± 0.1[§]	21.5 ± 0.1[§]	4.3
Pike 1	*Esox lucius*	Individual fish, GIT removed (length 68 cm)	5+	3.0 ± < 0.1	75.9	44.2 ± 1.5	− 26.8 ± 0.1	21.6 ± 0.1	4.3
Pike-perch	*Sander lucio-perca*	Pool of 3 individual fish, GIT removed (length 24–26 cm)	1+	2.0 ± < 0.1	78.6	41.0 ± 1.3	− 26.3 ± 0.1[§]	22.4 ± 0.1[§]	4.7

[*] Water content in mussels with breathing water/water content in mussels without breathing water. Breathing water content is based on a mean value *of Dreissena polymorpha* data from the ESB database and refers to water content that is not part of the tissue, but surrounding water between shell and tissue

[§] Calculated values are the weighed means based on mass proportion in composite sample of the individual fish measurements

In case of the plankton fractions, subsamples of freeze-dried material were prepared. Freeze-drying of samples was performed using a lyophilization unit (Alpha 1–2 LDplus, Christ). Samples were dried for 7 days, weighed, dried for one additional day, and weighed again. Samples were considered dry if there was no loss in weight between both weighings.

Analyses

Stable isotope analyses (SIA) of ^{15}N/^{14}N and ^{12}C/^{13}C ratios of the freeze-dried food web samples were performed by agroisolab GmbH (Jülich, Germany) after lipid extraction [32, 33]. Results are given in per mill δ^{15}N and δ^{13}C, respectively. Total mercury content (total Hg) was analyzed following ESB protocols, using freeze-dried sample material on a DMA-80 (Direct Mercury Analyzer, MLS GmbH) [34, 35]. Freeze-dried food web subsamples were analyzed for methylmercury (MeHg) levels via SID-GC/ICP-MS (stable isotope dilution-gas chromatography coupled to inductively coupled plasma-mass spectrometry) as described elsewhere [35]. Analyses for PCBs and polybrominated diphenyl ester (PBDE) congeners, as

well as for dioxins, furans and selected organochlorine pesticides were conducted by Eurofins GfA Lab Service GmbH (Hamburg, Germany) following established and accredited protocols [36, 37] (cf. Table 2 for congener details). Lipid determinations were performed according to the protocol by Smedes [38], which is commonly used in bioaccumulation studies [5]. Protein content was determined using the Pierce BCA Protein Assay (Thermo Fisher) after extraction with the T-PER Protein Extraction Reagent (Thermo Fisher). As reference, the pollack fillet sample was measured in all analyses.

QC/QA of analyses

An overview of the quality control/quality assurance (QC/QA) measures applied during the different analyses of the food web sample set is presented in Additional file 1: B. The laboratories which conducted the analyses hold DIN EN ISO/IEC 17025 accreditations for most of the analytical parameters (stable isotope analysis, Hg, MeHg, PCBs, PBDEs, organochlorine pesticides). All samples were analyzed in duplicates. Limits

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of quantification (LOQs)/limits of detection (LODs) are provided in Additional file 1: B.

Calculations

TMF calculation

TMFs were calculated for non- or slowly metabolized substances. It is known that metabolism rates have a central impact on the accumulation of substances and that metabolism capacities can be species-specific [39, 40]. Only target substances that were detected with concentrations above the limit of quantification (>LOQ) in ≥80% of the food web samples were evaluated further. In case that a substance could not be quantified in a sample at concentrations >LOQ, it was substituted by a default concentration of ½ LOQ value. The natural logarithm of the biota concentrations, which had been normalized beforehand was calculated (i.e., lipid normalization for lipophilic substances, dry weight normalization for Hg/MeHg and per- and polyfluoroalkyl substances (PFAS)). Additionally, PFAS concentrations were protein-normalized. A basic trophic position (TP) calculation as described by Post [33] was applied and is displayed in Eq. 1:

$$\text{TP} = \lambda + \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{baseline})}{2.3}. \tag{1}$$

An increment of ^{15}N enrichment per trophic level $\Delta^{15}\text{N} = 2.3$ ‰ [41] was applied. Usually, an increase of 3.4 ‰ δ15N per trophic level is suggested as increment [32, 45]. A comparison of the trophic positions (TPs) calculated for samples from Lake Templin using this mean enrichment increment with empirical trophic levels listed in the online database 'fishbase.org' [42] shows that the calculated TPs tend to be lower than would be expected. However, the calculated values fit well to the empirical data when an increment of 2.3‰, derived by McCutchan et al. [40] for the aquatic environment, was used (cf. Additional file 1: C). The mean $\delta^{15}N$ value of both *D. polymorpha* samples was used to define the baseline. As primary consumers, *D. polymorpha* have a trophic position (λ) of 2. In case of fish, whole fish $\delta^{15}N$ values were used as basis for the TP calculation. A linear regression of the logarithmized concentration data against trophic positions was performed using the 'Fit linear regression model' (fitlm) function in Matlab (Matlab R2017b, MathWorks®). In order to calculate a substance's TMF, the antilog of the regression slope was formed:

$$\text{TMF} = e^{\text{slope}}. \tag{2}$$

The 95% confidence of a TMF was calculated based on the 95% confidence interval (CI) of the slope, utilizing Eq. 2.

Results and discussion

Food web

The characteristics-based sorting of the obtained fish, mussel and plankton fractions resulted in 15 different food web samples for further analyses: One plankton fraction of >200 μm, two different mussel samples (composite samples of small and large individuals) and 12 fish samples. Only the 200-μm plankton fraction could be considered for analyses, since it was the only plankton fraction delivering a sufficient yield of biomass in the sampling campaign.
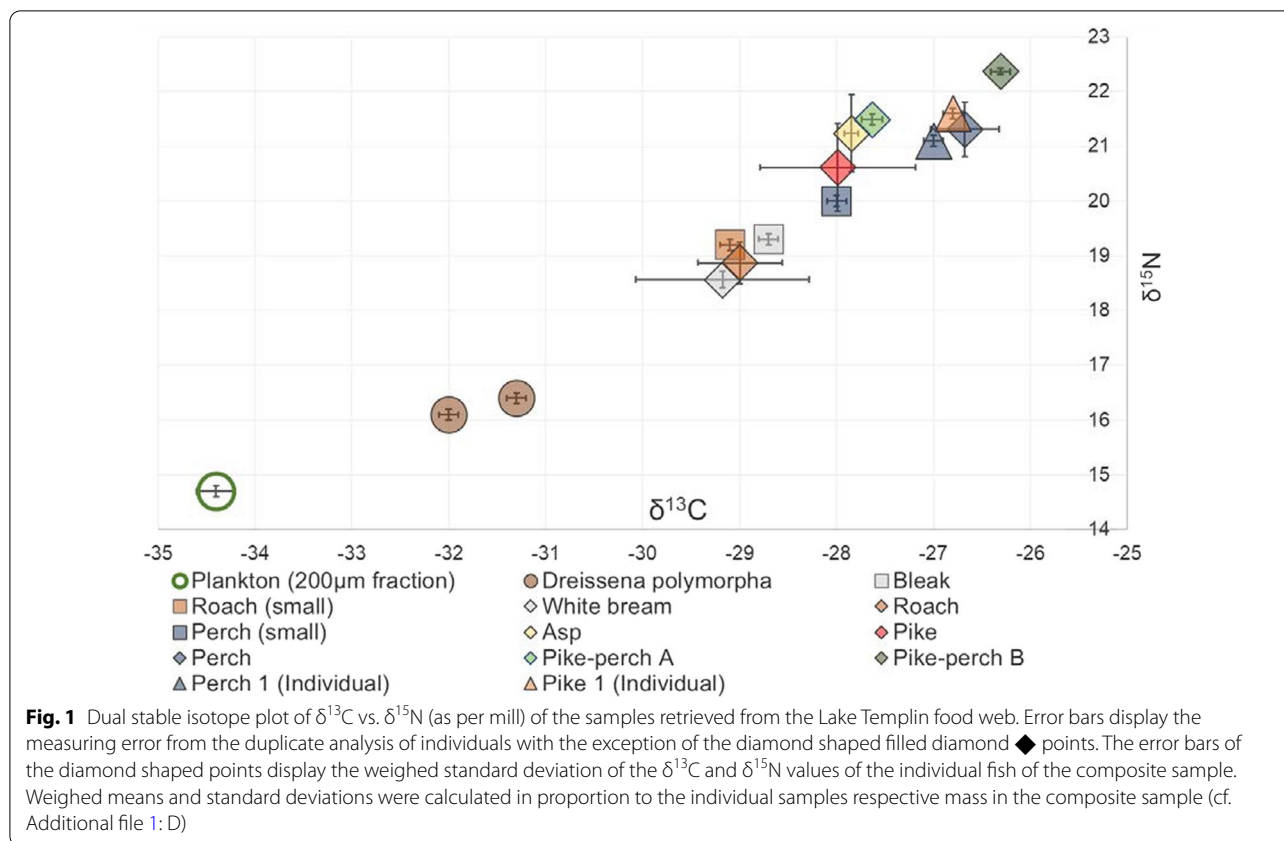
Stable isotope analysis (SIA)

The dual-isotope plot of the $\delta^{13}C$ vs. $\delta^{15}N$ ratios of the analyzed samples (Fig. 1) reveals a wide range for both ratios in the Lake Templin food web. One roach and one white bream were excluded from the respective composite samples, due to their divergent isotope ratios compared to the rest of the species sampled in this campaign. The largest pike and perch were both treated as individual samples. Although their isotope ratios are comparable to those detected in the other samples of the same species, their age, length, weight, and total Hg content were considerably different. For pike-perch, two independent composite samples (A, B) were generated, based on observed differences in their stable isotope patterns.

Stable isotope ratios of the fish pools for larger individual fish are displayed as weighed mean values (cf. Additional file 1: D). These were calculated based on the mass proportions of each fish in the composite sample. All food web samples showed a correlation between the enrichment of ^{15}N and the respective $\delta^{13}C$ ratio (cf. Figure 1). The linearity between $\delta^{13}C$ and $\delta^{15}N$ ratios indicates a coupling of at least two carbon sources, since the $\delta^{13}C$ ratio of the food web samples increases more than would be expected by a single carbon source. An increase of 0.4–1‰ $\delta^{13}C$ per trophic position can be assumed [41, 42]. However, the increase in our sample set is larger. It can be assumed that increasing amounts of at least one other carbon source are incorporated with increasing trophic level. More detailed analyses such as analyses for additional stable isotope patterns and additional sampling such as for submerged and emergent macrophytes, benthic algae or periphyton, different sediment types and organic matter of terrestrial origin would have been necessary to resolve this in more detail and with sufficient accuracy.

Biota concentrations of selected target substances

The substance class that showed the highest biota concentrations in the food web were the PCBs with the PCB congeners 153 and 138 making up most of the detected

Fig. 1 Dual stable isotope plot of $\delta^{13}C$ vs. $\delta^{15}N$ (as per mill) of the samples retrieved from the Lake Templin food web. Error bars display the measuring error from the duplicate analysis of individuals with the exception of the diamond shaped filled diamond ◆ points. The error bars of the diamond shaped points display the weighed standard deviation of the $\delta^{13}C$ and $\delta^{15}N$ values of the individual fish of the composite sample. Weighed means and standard deviations were calculated in proportion to the individual samples respective mass in the composite sample (cf. Additional file 1: D)

PCB burdens, (up to 268 ng g^{-1} ww and 187 ng g^{-1} ww were found in the individual perch sample and in *A. aspius*, respectively). The next highest concentrations were detected in fish samples for the p,p'-DDT metabolites p,p'-DDD and p,p'-DDE with concentrations of up to 203 and 173 ng g^{-1} ww, respectively. Mercury levels in fish samples were consistently higher than the WFD EQS of 20 ng g^{-1} ww with concentrations of up to 206 ng g^{-1} ww as observed for perch 1. The ratio of MeHg to total Hg levels measured for the plankton fraction was low (13.6% MeHg in relation to total Hg). However, for whole fish and mussel samples this ratio was high (approx. 0.83 MeHg/total Hg in samples of larger fish to about 1 in, e.g., samples of pooled, small fish). Perfluorooctanesulfonic acid (PFOS) was detected with concentrations of up to 46.6 ng g^{-1} in fish samples (asp). PBDEs were present in lower concentrations with PBDE congener BDE 47 exhibiting the highest concentrations in fish tissues (2.9 ng g^{-1}, perch 1). However, this congener could not be detected in mussel tissues. BDE 100, which exhibited the second highest levels, was detected in all tissue samples. The highest concentrations were detected in tissues of the individual perch sample with 0.43 ng g^{-1} ww. Concentrations of up to 0.020 ng g^{-1} ww were measured for 2,3,7,8-tetra CDF. Other PCDD/F such as 2,3,4,7,8-Penta

CDF, 1,2,3,7,8-penta CDF and 2,3,7,8-tetra CDD were detected in all tissue samples but mussels with maximum levels of 0.0046, 0.0033, and 0.0003 ng g^{-1} ww, respectively. A detailed overview of the detected tissue concentrations is presented in Additional file 1: E.

TMFs of chemicals in the Lake Templin food web and comparison with literature TMFs

In total, we evaluated 35 substances belonging to seven different substance classes in this study. Three substances showed no significant correlation between the concentration in the samples and their trophic positions. For eleven TMF datasets, the mussel concentrations were below the limit of quantifications and were substituted with ½ LOQ concentrations for the TMF evaluations. These TMFs are based on a more imbalanced food web with only the plankton fraction representing lower trophic positions.

PCBs

The most prominent substance class found in the various samples are PCBs, which have long been known to be persistent, bioaccumulative and to exhibit toxic effects [43]. In the Lake Templin food web, 15 PCBs were identified in all samples, 2 additional PCBs (congeners 28 and 52) were detected in all samples but not

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in the two mussel samples. The regression of the lipid-normalized and logarithmized concentrations against the trophic level of each sample revealed that all PCBs except PCB 81 express a significant accumulati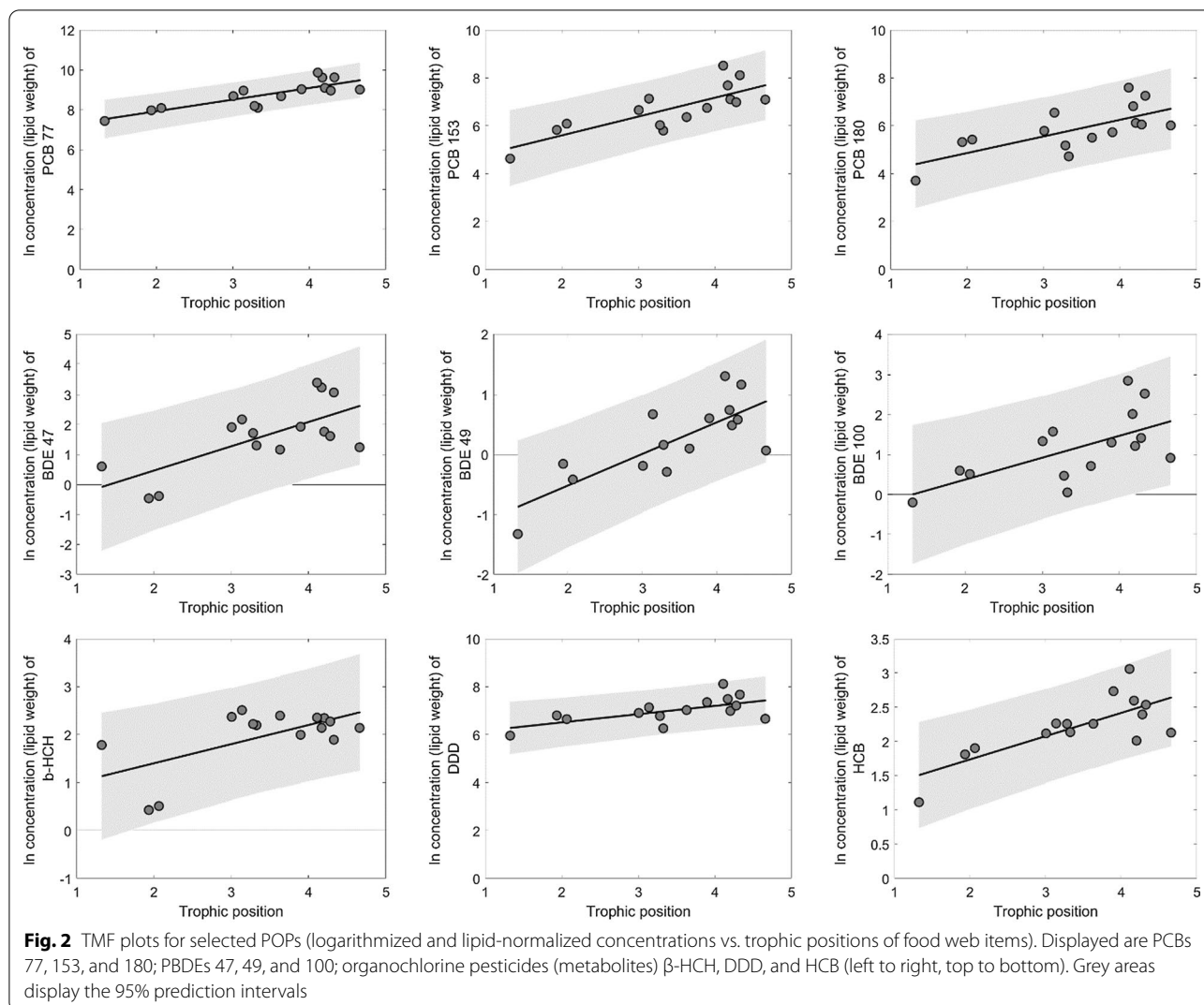on in the food web ($p < 0.05$) and the derived TMFs range between 1.7 and 2.5 (see Table 2). Typical plots for PCBs 77, 153, and 180 are presented in Fig. 2.

PCBs have been analyzed in several other TMFs studies; however, not every PCB congener has been analyzed comparably often. For example, 28 studies in freshwater ecosystems report TMFs for PCB 153,

Table 2 TMFs calculated for different substances identified in the sampled food web of Lake Templin (Potsdam, Germany)

Class	Substance/congener	TMF	95% CI	*p*-value	R^2
Whole food web data available					
Lipid-normalized data TMFs					
PCB	77	**1.8**	1.4–2.2	0.0001	0.706
	81	1.6	0.7–3.4	0.2311	0.108
	101	**2.0**	1.4–2.9	0.0007	0.603
	105	**2.1**	1.5–2.9	0.0002	0.671
	114	**2.2**	1.6–3.1	0.0003	0.647
	118	**2.1**	1.5–3.0	0.0003	0.643
	123	**2.5**	1.7–3.7	0.0002	0.657
	126	**2.3**	1.6–3.2	0.0002	0.666
	138	**2.1**	1.5–3.0	0.0004	0.634
	153	**2.2**	1.5–3.2	0.0005	0.624
	156	**2.1**	1.4–3.1	0.0017	0.543
	157	**2.1**	1.4–3.1	0.0018	0.540
	167	**2.2**	1.3–3.6	0.0049	0.469
	180	**2.0**	1.3–3.1	0.0036	0.491
	189	**1.9**	1.2–3.2	0.0134	0.386
PBDE	49	**1.7**	1.3–2.2	0.0006	0.606
	100	**1.7**	1.2–2.6	0.0110	0.403
Organochlorine pesticides	p,p'-DDD	**1.4**	1.1–1.8	0.0117	0.398
	p,p'-DDE	**1.9**	1.4–2.6	0.0007	0.603
PCDD/F	2,3,7,8-TetraCDF	**1.6**	1.3–2.0	0.0001	0.686
Dry weight-normalized TMFs					
Mercury compounds	Total Hg	**2.2**	1.6–3.0	0.0001	0.690
	MeHg	**2.9**	2.0–4.3	< 0.0001	0.737
PFAS	PFOS (linear)	**3.0**	1.8–4.8	0.0003	0.643
	PFDoA	**1.9**	1.3–2.9	0.0037	0.489
No concentration > LOQ in mussels detected					
Lipid-normalized data TMFs					
PCB	28	**1.7**	1.3–2.2	0.0005	0.624
	52	**2.0**	1.5–2.6	0.0001	0.687
PBDE	28	1.5	1.0–2.3	0.0657	0.237
	47	**2.3**	1.4–3.7	0.0035	0.494
	153	**1.7**	1.2–2.4	0.0044	0.477
	154	**1.8**	1.2–2.8	0.0124	0.400
Organochlorine pesticides	HCB	**1.4**	1.2–1.7	0.0012	0.564
	β-HCH	**1.5**	1.1–2.0	0.0142	0.381
PCDD/F	1,2,3,7,8-PentaCDF	**1.5**	1.0–2.1	0.0339	0.302
	2,3,4,7,8-PentaCDF	**1.5**	1.0–2.1	0.0394	0.287
	2,3,7,8-TetraCDD	1.1	0.7–1.8	0.5419	0.029

95% CI is the 95% confidence interval for the calculated TMF. Bold values: Significant correlation between TP and logarithm of normalized concentrations ($p < 0.05$).
Mussel samples < LOQ were substituted with ½ LOQ value for the TMF calculation

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Fig. 2 TMF plots for selected POPs (logarithmized and lipid-normalized concentrations vs. trophic positions of food web items). Displayed are PCBs 77, 153, and 180; PBDEs 47, 49, and 100; organochlorine pesticides (metabolites) β-HCH, DDD, and HCB (left to right, top to bottom). Grey areas display the 95% prediction intervals

whereas for PCB 118 only 5 TMFs were found. The following comparison was based on ≥ 20 reported TMFs in the literature. For PCB congeners 52, 101 138, 153, and 180 sufficient individual TMFs calculated from freshwater ecosystems are available, (cf. Additional file 1: G). The mean TMFs for these PCBs range from 2.0 to 3.9 in the respective ecosystems. Although the available data are incomplete, PCBs with a higher degree of chlorination display a higher variability in the TMF values. TMFs of PCBs derived for the Lake Templin food web range from 1.8 (PCB 77) to 2.5 (PCB 123). Literature values are in a comparable range (cf. Additional file 1: G). From the comparison with the literature data it can be concluded that the Lake Templin TMFs describe the accumulation potential for PCBs realistically.

PBDEs

The second largest class of compounds analyzed in this study are PBDEs. Only two congeners were found in all food web samples above LOQ, BDE 49 and BDE 100. A TMF of 1.7 was determined for both congeners. In both cases a significant linear relationship ($p < 0.05$) was determined for the regressions. Four additional congeners, BDEs 28, 47, 153, and 154 were found in all but the two mussel samples. The determined TMFs range from 1.5 to 2.3 and apart from BDE 28, all regressions show a significant linear relationship ($p < 0.05$). TMF plots for BDEs 47, 49, and 100 are presented in Fig. 2.

Just like PCBs, PBDEs are also a group of substances that have been included in several TMF investigations, providing a good data basis for comparison. Again, not all congeners have been analyzed in similar frequency. BDE 49 is the only congener investigated in the Lake Templin food web for which no freshwater TMF could be found in

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the literature. For BDEs 28, 153, 154, and 100 three to five TMFs were found and nine TMFs were found for BDE 47. Consistently, the highest TMFs for PBDEs were found in the Lake Ontario food web with values ranging from 5.1 to 9.5 [44]. Similarly high TMFs were derived for PBDE 47 with a value of 5.7 in the Lake Mjøsa food web [18, 45], and a TMF of 5.2 for the Lake Winnipeg food web As shown in Additional file 1: G, TMFs from other studies are in a range comparable to those determined at Lake Templin, with mean values ranging from 1.4 to 2.4 [46, 47].

Organochlorine pesticides

Although the samples were analyzed for a spectrum of organochlorine pesticides and some of their metabolites, only a limited number of compounds were found. In this evaluation we focused on four substances that are persistent in the environment and not readily metabolized in biota. Representative TMF plots are displayed in Fig. 2. The legacy insecticide DDT, which was applied in Germany until the mid-1980s, is one of the targeted persistent and lipophilic substances in this study [48]. In its commercial formulation, DDT was a mixture of the desired p,p'-DDT and impurities such as o,p-DDT, and p,p-DDD, whereas the latter and p,p-DDE are also formed as metabolites in the environment [49]. Due to the fact that they are less readily metabolized than their parent substance p,p-DDT, they are usually found in higher concentrations in aquatic animals [48]. In this study, we did not evaluate the accumulation of p,p-DDT along the Lake Templin food web, to exclude the influence of different biotransformation activities on the observed accumulation. Therefore, only the relatively more stable metabolites p,p-DDD and p,p-DDE were evaluated. Both were detected in all food web samples and the regressions were significant ($p < 0.05$). The TMFs ± 95% CI for p,p-DDD and p,p-DDE were 1.4 (1.1–1.8; Fig. 2, Table 2) and 1.9 (1.4–2.6), respectively. DDT and its metabolites have been analyzed in different freshwater ecosystems and respective TMFs have been derived. 9 TMFs for DDD and 34 TMFs for DDE were found in the literature. A comparison of the literature data with the results from this study is shown in Additional file 1: G. The by far highest TMFs with values of up to 6 and 9 for DDE and DDD, respectively, were determined by Govaerts et al. [50]. DDD TMFs have been derived mainly from riverine ecosystems in Africa (7 out of 13 TMFs), while the remaining ones were obtained in sub-alpine or high-altitude lakes [51–53], which are all rather different to Lake Templin. The Lake Templin TMF for DDD ranges at the lower end of TMFs found in the literature, being lower than the mean TMF minus SD of the literature data (cf. Additional file 1: G). Similar observations were made for

DDE, when only TMFs excluding tropical regions were included in the comparison.

Another organochlorine pesticide with lipophilic characteristics is gamma-hexachlorocyclohexane (γ-HCH), also known as lindane. Apart from γ-HCH, its α and β isomers, which originate from impurities or environmental degradation processes, are also commonly found in the environment [54]. In Lake Templin the highest residues are found for these isomers in the order β-HCH > γ-HCH > α-HCH (cf. Additional file 1: E). Since several different degradation pathways for all isomers have been identified in the past that are strongly dependent on biotic (e.g., microbial community composition) and abiotic (e.g., oxygen availability, sunlight exposure) factors, it is not easy to determine the most persistent HCH isomer [54–56]. But due to its high concentrations, for Lake Templin it seems that β-HCH is the most stable one; accordingly, it was selected for this study. In all samples except mussels, this isomer could be detected, and the lipid-normalized and logarithmized concentrations of the tissues showed a significant linear correlation ($p < 0.05$) with the, respectively, determined TPs. A TMF of 1.5 (1.1–2.0) was obtained, indicating an enrichment of β-HCH along the food web (Fig. 2). Only a few TMFs were found for β-HCH. Ecosystems that have been analyzed are river basins in South Africa and a lake (system) south of Beijing in North-East China [57, 58]. With a minimum TMF of 1.3 and a maximum TMF of 1.6 a narrow range of TMF values was observed. Based on the currently available data, the Lake Templin TMF for β-HCH seems to be in the range of expected TMF values.

The final organochlorine pesticide evaluated in the Lake Templin food web was HCB. The TMF of 1.4 (1.2–1.7) shows its accumulation through the food web (Fig. 2). In total, 23 TMFs for HCB that were derived from data of different freshwater ecosystems were found in the literature. A mean TMF of 2.8 ± 1.6 can be calculated from the dataset, which is twice as high as the TMF of 1.4 calculated from the Lake Templin data. The TMFs of HCB have a wide range, but no dependence on the ecosystem can be identified. Overall, literature TMFs are > 1 with one exception, confirming the high bioaccumulation potential of HCB in different ecosystem types.
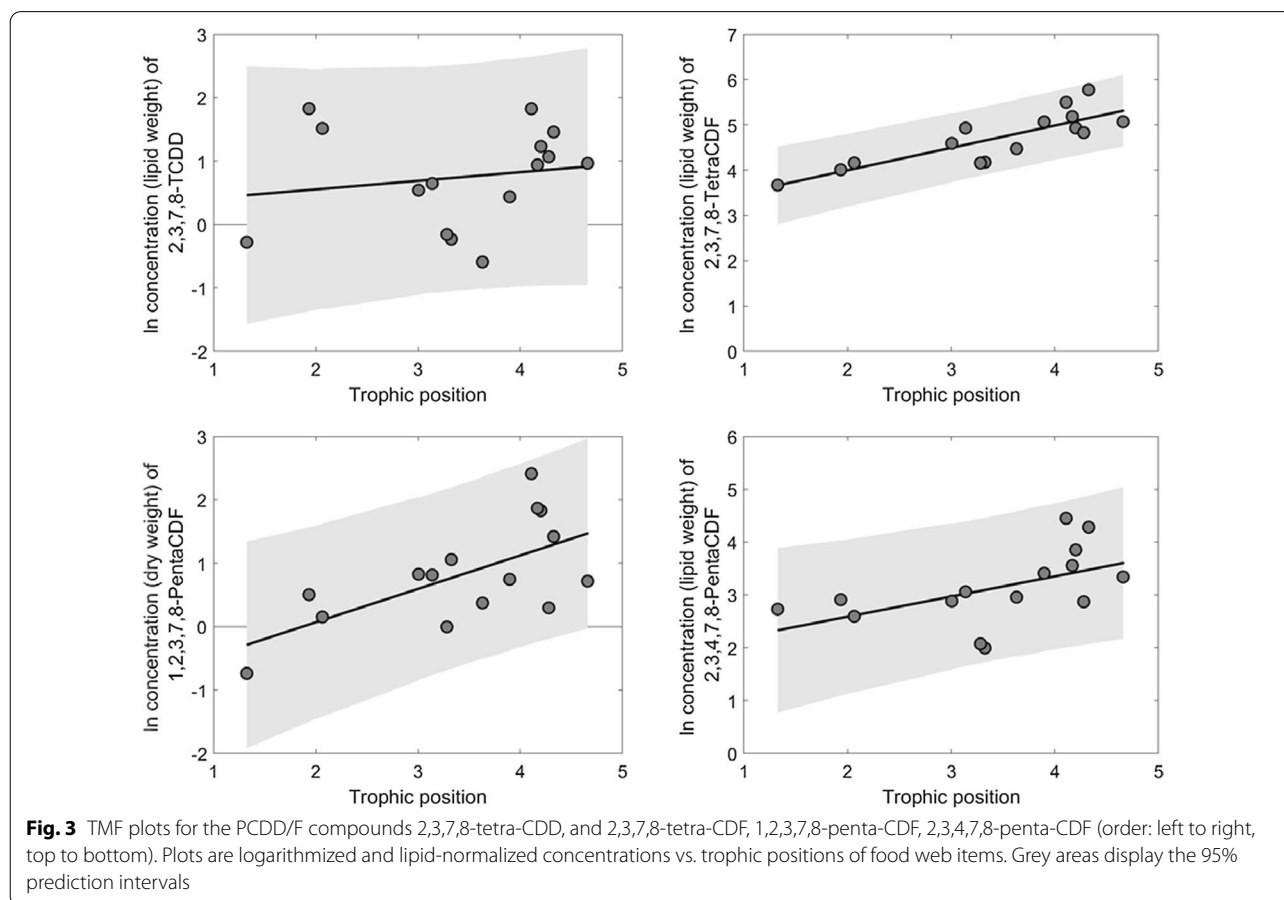
Polychlorinated dibenzodioxins/furans (PCDD/Fs)

Another class of lipophilic substances analyzed are PCDD/Fs. Only 2,3,7,8-tetra-CDF was detected in all food web samples and showed a significant correlation ($p = 0.0001$) between trophic position and lipid-normalized tissue concentration resulting in a TMF of 1.6 (1.3–.0). Additionally, two furans (1,2,3,7,8-penta-CDF and 2,3,4,7,8-penta-CDF) and one dioxin (2,4,7,8-tetra-CDD) were detected in all samples but mussels. For both

furans a significant ($p < 0.05$) linear relationship is present, whereas this is not the case for 2,3,7,8-tetra CDD. For both penta-CDFs TMFs of 1.5 (1.0–2.1) based on lipid-normalized tissue concentrations were derived (Table 2). Plots of the relation between tissue concentration and TP for all four PCDD/F substances are displayed in Fig. 3. No freshwater TMFs are available so far for single PCDD/Fs. One study was found that describes the transfer of the PCDD/F group in a riverine freshwater ecosystem near an e-waste dismantling area in China, which showed a dilution trend in the food web [59]. However, this ecosystem is not representative due to its heavy pollution and results should thus be interpreted with caution. It cannot be excluded that substance concentrations in heavily polluted ecosystems reach toxic levels which may alter the metabolic processes in the exposed organisms. Observed effects are therefore hardly transferrable to other ecosystems. It can furthermore not be excluded that the detection of accumulating substances with low concentrations was masked by compounds with a limited accumulation potential, but higher tissue concentrations when congener groups are being analyzed. Other studies also detected trophic dilution for PCDD/F-mixtures in food webs of marine environments [60, 61], but some furan classes apparently show an accumulation potential [62]. The data presented by Naito et al. further implies that accumulation is mainly related to 2,3,7,8-substituted PCDD/Fs. Clear trophic dilution can be observed for the sum of PCDD/F when these congeners are eliminated from the calculation. For some ecosystems BMFs or biota-sediment accumulation factors (BSAFs) were derived which show that accumulation of PCDD/Fs depends on the investigated pairs of prey and predator [63, 64]. In case of, e.g., 2,3,7,8-tetra-CDD it was shown that species-specific metabolism does occur [65] and thus its TMF in this study may only be used for the plausibility check under reservations. To our best knowledge, the data from Lake Templin demonstrate for the first time freshwater food web TMFs for single PCDD/Fs and can confirm the accumulation properties of some 2,3,7,8-substituted PCDFs (Table 2, Fig. 3).
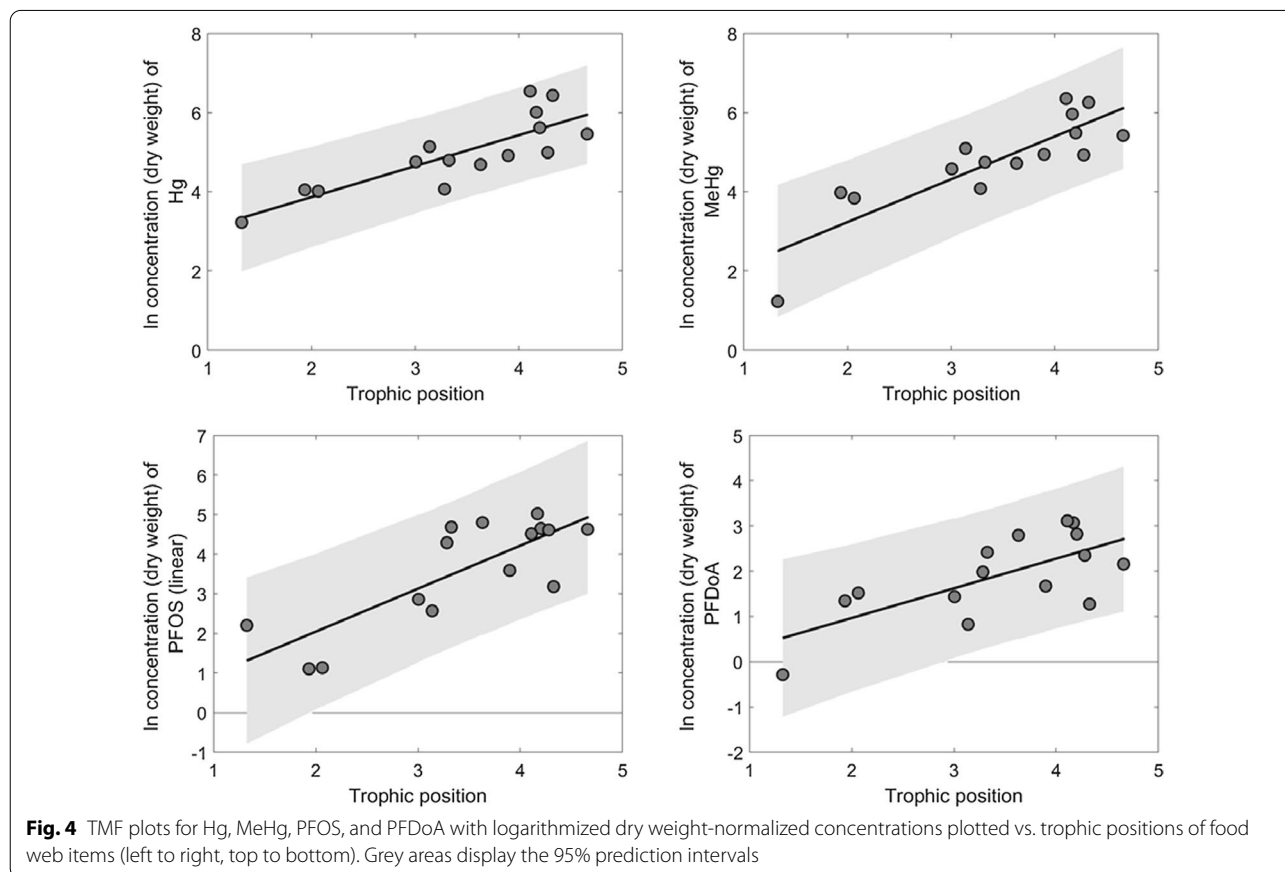
PFAS

The Lake Templin food web samples were also analyzed for PFAS. These synthetic substances have been applied as coating for different products (e.g., textiles, paper) to

Fig. 3 TMF plots for the PCDD/F compounds 2,3,7,8-tetra-CDD, and 2,3,7,8-tetra-CDF, 1,2,3,7,8-penta-CDF, 2,3,4,7,8-penta-CDF (order: left to right, top to bottom). Plots are logarithmized and lipid-normalized concentrations vs. trophic positions of food web items. Grey areas display the 95% prediction intervals

provide water or fat-repellant properties. PFOS has been detected in numerous environmental matrices. It is not readily biodegradable and furthermore bioaccumulative, toxic, and is subject to long range transport. Accordingly, it was declared a POP in 2009 [43]. In contrast to many of the aforementioned substances, PFOS does not express a lipophilic behavior. Instead, an affinity towards proteins, especially water-soluble proteins such as serum proteins (e.g., serum albumin) has been described [66]. Previous studies have suggested a normalization of PFOS tissue concentrations to protein concentrations [67–69], which has so far rarely been performed in TMF studies [68]. A screening for different PFAS compounds showed that linear PFOS was detectable in all samples of the Lake Templin food web. A dry weight-based TMF for linear PFOS of 3.0 (1.8–4.8) was obtained. Another PFAS that was detected in all samples is perfluorododecanoic acid (PFDoA) with a TMF based on dry weight-normalized data of 1.9 (1.3–2.9). The corresponding plots displaying the relation between dry weight-normalized, logarithmized concentrations and trophic level are given in Fig. 4. A comparison of the different normalization procedures is shown in Additional file 1: F. The screening revealed that also other PFAS can be detected in some of

the fish samples. With a lower level of quantification in the analytical method, TMFs for more PFAS may be calculated for the Lake Templin food web.

The TMF for PFOS in the food web of Lake Templin is high in comparison to other TMFs for PFOS in freshwater ecosystems described in the literature. A total of 32 different TMFs for PFOS or PFOS-isomers are available, which are summarized in Additional file 1: G. Since there are different isomers (i.e., linear or branched form) for PFOS, a subset of TMFs for 'linear PFOS' was created from the available literature data set. This group includes only five studies with an average TMF of 2.9 ± 1.6 (wet weight), originating from three river and one lake ecosystem [20, 70, 71]. The TMF of 5.8 (2.7–12.1) derived for Lake Templin, which was calculated after protein normalization, is significantly higher. Its recalculation based on wet weight data yields a similar TMF of 5.3 (2.6–10.9). Both values are much higher than the average reported TMF for linear PFOS from other freshwater ecosystems. And even when other PFOS isomers were included in the literature comparison, both, the protein- and wet weight-normalized TMFs from Lake Templin remained comparatively higher. There are data available that suggest the involvement of precursors of PFAAs via transformation

Fig. 4 TMF plots for Hg, MeHg, PFOS, and PFDoA with logarithmized dry weight-normalized concentrations plotted vs. trophic positions of food web items (left to right, top to bottom). Grey areas display the 95% prediction intervals

in the observed accumulation of, e.g., PFOA and PFOS [71]. More detailed analyses may be needed to further elucidate this possible input of PFAS into the Lake Templin food web.

Another PFAS that was analyzed in the Lake Templin food web was perfluorododecanoic acid (PFDoA), which was detected in all food web samples. The wet weight-normalized TMF was 3.5, and the protein-normalized TMF was 3.7. Compared to literature data, this TMF is also comparatively high as seen for PFOS. A literature TMF of 3.9 was derived in a lake ecosystem in China, the lowest TMF with a value 1, was derived from a food web in Lake Ontario. In comparison, river ecosystems in France display intermediate PFDoA TMFs of around 1.8–2.7 (cf. Additional file 1: G).

Hg and MeHg
The accumulation potential of mercury (Hg) and its main organic species, the methylmercury cation (MeHg), have long been documented and its emission and impact on human health and the environment is regulated in the Minamata Convention on Mercury [72]. The transfer of Hg and MeHg through food webs has been observed and studied in the field [73]. Hg and MeHg are not preferentially stored in the lipid fraction of tissues; accordingly, it is not reasonable to normalize their tissue concentrations to the lipid content. Instead, either dry weight-, wet weight-, or protein-normalized concentrations are recommended [13]. In Fig. 4, TMF plots based on dry weight-normalized concentrations for Hg and MeHg are presented. The determined TMF for Hg of 2.2 (1.6–3.0) is based on a clear and highly significant linear regression ($p \leq 0.0001$) for dry weight-normalized concentrations. In case of MeHg, the determined TMF is slightly higher with a value of 2.9 (2.0–4.3) for dry weight-normalized concentrations, also displaying a highly significant linear correlation between trophic positions and tissue concentrations ($p < 0.0001$). Additional file 1: F shows the calculated TMFs based on wet weight- and protein-normalized concentrations. Especially the latter one is not commonly performed and our values may be used for future comparisons. The determined TMF values based on wet weight- and protein-normalized concentrations are similar and, in all cases, higher than the respective dry weight-based TMF. A potential explanation for the differences of fresh weight- and dry weight-based TMFs is the relatively high water content of the plankton fraction and the mussel samples compared to the fish tissue.

The fate and behavior of mercury in the environment has been studied for many decades and recent investigations and reviews also cover TMFs [21, 73]. In both studies, TMFs obtained in different ecosystems are evaluated, with Lavoie et al.[73] compiling a global synthesis

and Jardine et al. [21] focusing their analyses on stream ecosystems. The meta-study by Lavoie et al. [73] classified TMFs into different subgroups for freshwater and marine ecosystems, e.g., latitude, species composition, or ecosystem type. Around 100 different studies on mercury and methylmercury involving freshwater ecosystems were included in the meta-analysis. Most studies were conducted in lake ecosystems of temperate regions and analyzed food webs consisting of fish and other species. As seen in previous comparisons, the range of calculated TMFs can differ considerably between ecosystems, which was also observed for Hg/MeHg, showing TMFs with large standard deviations, sometimes even larger than the mean TMF [73]. Both studies share a general trend that MeHg seems to have a higher accumulation potential compared to Hg, irrespective of the studied ecosystem. This observation is in agreement with the results from Lake Templin. The mean wet weight TMFs in Lavoie et al.'s [73] review are close to the wet weight TMFs calculated in the Lake Templin food web, but a direct comparison is not informative due to the large standard deviation in the review's dataset. Due to the fact that Lake Templin is a lake ecosystem which shares some characteristics with a riverine ecosystem, comparing the obtained results with both, riverine and lake ecosystems seems advisable. The review by Lavoie et al. [73] shows that the mean TMFs of Hg calculated from lake data tend to be higher than TMFs for riverine ecosystems. In case of MeHg it is the other way around as confirmed by Jardine et al. [21]. 21 streams were investigated and dry weight-normalized mean TMFs of 1.9 and 3.5 for Hg and MeHg were observed, respectively, under the assumption of a trophic enrichment increment ($\Delta^{15}N$) of 2 ‰ [21]. The Lake Templin TMFs for Hg and MeHg (see Table 2) are well comparable to the mean TMFs from the 21 riverine systems evaluated by Jardine et al. [21] in their study.

Study feasibility
In this study, we demonstrated that the 'Food web on ice' sampling approach in general is feasible. For the selected 15 biota samples sufficient material could be collected to provide sample material for a large number of different analyses. The obtained food web items could be sufficiently characterized regarding trophic positions so that the investigation of the bioaccumulation behavior of chemicals with different properties was possible. Nevertheless, the experiences from the practical work could help to improve future investigations with similar aims.

In the literature, there is comprehensive expert advice available regarding the planning and realization of TMF studies [11–13]. In this study, however, several challenges of the conceptual design of the TMF study became apparent. For instance, plankton abundance underlies a

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temporal fluctuation in its composition (especially phytoplankton) and therefore the primary energy source and its associated chemical levels may vary over the course of the year [74]. In addition, the anthropogenic input can often derive from a point source (e.g., waste water treatment plant) or can exhibit temporal shifts (e.g., sunscreen is used in summer, and de-icing agents in winter), a circumstance that can violate the steady-state requirement. However, with its specific characteristics, Lake Templin fulfills most of the requirements for a suitable aquatic ecosystem for TMF studies described in the introduction. Its stream-lake characteristics could be discussed as one potential drawback as it may not fully meet the steady-state requirement. Data regarding the water exchange rate display a considerable span (3–30 days). Accordingly, a representative water sampling and analysis campaign would be necessary to characterize this water body in more detail. In general, more information about the study site Lake Templin and its native species might help in the ascertainment of the observed effects. Most prominent might be a detangling of the carbon sources that build the basis of the food web. Analyses of additional input sources that may play important roles, such as sediments, benthic algae, or terrestrial input (e.g., leaves) for additional stable isotopes (e.g., δ^{34}S, δ^{18}O) could be helpful. As often in field work, sampling site selection called for some tradeoffs. Sufficient anthropogenic input to allow for detection in organisms of lower trophic positions, eutrophication to provide sufficient organisms of lower trophic levels, and accessible locations for sampling had to be present. The anthropogenic input should not be too high, otherwise toxic side effects may alter the results. We managed to derive a considerable number of TMFs for legacy benchmark pollutants and gained a sufficient number of samples for further analyses. For future studies of this type, a higher number of individual fish of each species might be advisable to provide a larger statistical basis.

A commonly addressed source of uncertainty in the calculation of TMFs is the enrichment increment that describes the ^{15}N increase defining one trophic position, since it may vary depending on, e.g., an organism's physiology [11]. Commonly, an increment of 3.4‰ is assumed to represent one trophic position for aquatic organisms of freshwater ecosystems. In our study, we applied an increment of 2.3‰ to obtain realistic trophic positions, which directly affects the TMF calculation. This resulted in TMFs that are lower than the ones calculated with trophic positions obtained with an increment of 3.4‰ would be. There are indications in the literature that show that an application of lower increments may be appropriate in stream ecosystems [21, 75]. Since Lake Templin is flown through by River Havel it cannot be excluded that

the sampled food web may display characteristics similar to those of stream ecosystems. The proper determination of trophic positions still needs some refinement to facilitate a better comparison of TMFs from different ecosystems.

The large span in the δ^{13}C values of the Lake Templin food web samples indicates that a broad food basis is consumed. Additional samples of, e.g., sediment or benthic invertebrates may have been useful to retrace the nutrient flow through the food web more effectively. Additional samples from the lower trophic level part of the food web may have also been helpful to provide a more balanced sampling design, as the current TMFs are more representative of a chemical transfer through fish food webs. The cascade net sampling for the plankton unfortunately did not provide enough sampling mass for the targeted fraction sizes. To this end, a revised and upscaled sampling method would have been needed.

Plausibility check

The analysis of the bioaccumulation behavior of numerous lipophilic compounds with known trophic magnification potential in this study at Lake Templin revealed that plausible TMFs could be derived with the 'Food web on ice' approach.

In total, TMFs were derived for 35 substances for the food web of Lake Templin, which belong to six different substance classes. The plausibility of the project design can be checked by comparing these values with the data of other TMF studies in freshwater ecosystems. The set of analyzed well-known legacy pollutants includes lipophilic, proteinophilic, and overall accumulating substances, covering different accumulation types. Furthermore, all selected substances are not- or only slowly metabolized by aquatic organisms, which should minimize the effect of species-specific differences in the metabolic activity between species from the Lake Templin food web. A general effect of metabolic rates on TMFs was demonstrated in the past based on a global meta-analysis [76]. Since the metabolic rate of a substance is not easy to predict in silico [39] and can vary even among different fish species [40] we wanted to exclude this effect for our plausibility check by focusing on substances with limited metabolism. The TMFs determined for the Lake Templin food web are in agreement with the data from the other TMF studies. The freshwater TMFs for PCDD/Fs which were derived for the first time, are the basis for future comparisons with upcoming TMFs for these compounds from other ecosystems. The plausibility check is required to evaluate the results with regard to the two most important applicability domains the TMF has been suggested for: The regulatory bioaccumulation assessment in chemical legislation and the normalization of

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biota concentrations in context of environmental legislation, e.g., the EU WFD.

Evaluation under regulatory aspects

In the regulatory assessment of the bioaccumulation potential of a substance of concern, a threshold-based approach is commonly applied [77]. The crucial bioaccumulation endpoint in the regulatory chemical risk assessment of industrial chemicals, pesticides, biocides, medicinal products, and other substances is the bioconcentration factor (BCF) which expresses the retention of a test substance in fish accumulated from the surrounding medium [78–80]. The standard procedure to determine the BCF for regulatory applications is the flow-through bioconcentration fish test according to the OECD 305 test guideline (OECD 2012). For BMF studies this study guideline recommends the use of reference substances that are known to accumulate, are of known bioconcentration potential, and undergo low metabolism in fish for a plausibility check to demonstrate the appropriateness of the test conditions. Comparable plausibility criteria for TMF studies are have been proposed at a smaller scale, e.g., suggesting single PCB or PBDE congeners [45]. Many of the Stockholm Convention POPs that were analyzed in the Lake Templin TMF study fulfill the requirements of suitable benchmarking substances as recommended in the OECD TG 305 [5]. For our Lake Templin samples, we successfully demonstrated that a range of substances listed under the Stockholm Convention bioaccumulate across the food web. The TMF values > 1 for the investigated compounds with known bioaccumulation potential and low metabolism indicate the integrity of the study design and that the 'Food web on ice' approach is fit for purpose.

In cases where the TMF only slightly exceeds the threshold value of 1, such as e.g., some PBDEs in this study, additional criteria should be considered: The *p*-value for the linear regression between the logarithm of biota concentrations and trophic positions of food web items, which is given together with the 95% confidence interval (CI). In the Lake Templin data only two of the reported TMFs are below the threshold value of 1 in the lower 95% CI, furthermore the p-value for the linear regression of these two substances is > 0.05, indicating that these TMFs are of limited reliability. In future, more advanced statistical approaches that have been suggested to enhance the reliability of TMF values, e.g., Starrfelt et al. [81], may be applied.

Evaluation under aspects of the WFD

In context of the European Water Framework Directive, TMFs are recommended for normalization processes in compliance monitoring against an environmental quality standard (EQS) and in trend monitoring. This requires a numerical TMF value rather than an answer to the question, whether the TMF is above or below 1. A recent analysis illustrated challenges associated with the normalization approach which may result in an overestimation of TMF corrected biota concentrations [82].

Conclusion and outlook

Although the study displayed some drawbacks in regard to the question, whether the 'Food web on ice' enables a sufficient characterization of the samples, the study design and the plausibility check against literature data confirms that this approach to TMF investigations can be a valuable tool for future TMF investigations.

In regulatory routines TMFs are useful to refine the data from lab-based bioaccumulation assessment and to support monitoring strategies. A plausibility check for legacy pollutants regulated under the Stockholm Convention has shown that the sampling design applied at Lake Templin leads to TMF values that are in good agreement with accumulation data from other freshwater food web studies.

This offers the possibility to use the remaining sample material archived in the German Environmental Specimen Bank for TMF investigations of chemicals of emerging concern and other contaminants where TMF values are missing.

The strength of the 'Food web on ice' approach is that with time additional TMFs can be added in a cost-effective manner and the new data can be compared to data generated before for well-known legacy pollutants in the same samples. While deriving safe TMF values for less studied substances is still a challenge, it may be helpful to compare incoming data with accepted benchmarking TMF values of legacy pollutants studied in the same ecosystem, each of which accumulates to a different extent in food webs. Novel analysis techniques such as non-target screening and multi-target analysis have the potential to optimize the use of the samples and generate a significant amount of new TMF data. This may also allow for the rapid screening of the bioaccumulation potential of substances which have not been prioritized for field-based bioaccumulation assessment so far. The application of the 'Food web on ice' approach in other ecosystems may allow advanced statistical methods to determine the underlying factors causing different TMF levels and contribute to a better understanding of the accumulation of substances in food webs. Finally, the 'Food web on ice' approach can contribute to the standardization of TMF studies and stimulate the regulatory use of TMF values, as it drastically reduces costs and new values can be derived quickly and at any time from the archived samples.

In case of strongly metabolizable substances, the ability of the respective analyzed species to biotransform these substances probably needs to be considered. How this can be achieved in a standardized way is subject to additional research. More refined mathematical methods could help to receive a more precise picture of the likelihood to derive a TMF > 1. In addition, a more profound knowledge on the main driving forces behind the variations TMFs can express in different freshwater ecosystems could help to further increase the precision of the decision-making in the assessment of chemical substances. In order to provide a more holistic plausibility check, an evaluation of substances with known biodilution tendencies is advisable. For metals such as lead, vanadium and thallium and the metalloid arsenic, biodilution was observed in the field and hence, these elements may be a good starting point [83, 84]. However, biodilution of chemicals is not consistently described in the literature, which calls for the analysis of a larger group of targets to enable a detailed evaluation.

As a next step it is planned to use the Lake Templin samples for the analysis of non-regulated compounds, e.g., plasticizers, biocides, pharmaceuticals and plant protection products and also to apply Non-Target Screening techniques. Substances that are less resistant to degradation in the environment and are more prone to metabolism in biota than the persistent legacy pollutants already analyzed in this study are also to be investigated. Investigations will reveal to what extent their concentration decreases with increasing trophic position in the food web and how the trophic dilution of substances in the ecosystem can be mapped with the 'Food web on ice' approach. The 'Food web on ice' offers beneficial prospects for both, assessment and monitoring approaches. The Lake Templin food web especially offers the opportunity to reliably investigate trophic magnification in German freshwater ecosystems.

Abbreviations
BCF: Bioconcentration factor; BMF: Biomagnification factor; BSAF: Biotasediment accumulation factor; CDD: Chlorinated dibenzodioxin; CDF: Chlorinated dibenzofuran; CI: Confidence interval; DDD/DDE/DDT: Dichlorodiphenyldichloroethane/dichlorodiphenyldichloroethylene/dichlorodiphenyltrichloroethane; dw: Dry weight (as normalization); EQS: Environmental quality standard; ESB: Environmental specimen bank; EU: European Union; GIT: Gastro-intestinal tract; HCB: Hexachlorobenzene; HCH: Hexachlorocyclohexane; Hg: Mercury; LOD: Limit of detection; LOQ: Limit of quantification; MeHg: Methylmercury; OECD: Organisation for Economic Co-operation and Development; (P)BDE: (Poly)brominated diphenyl ether; PCB: Polychlorinated biphenyl; PCDD/F: Polychlorinated dibenzodioxin/furan; QC/QA: Quality control/quality assurance; PFAS: Perfluoroalkyl substances; PFDoA: Perfluorododecanoic acid; PFOS: Perfluorooctanesulfonic acid; POP: Persistent organic pollutant; SD: Standard deviation; SIA: Stable isotope analysis; TMF: Trophic magnification factor; TP: Trophic position; WFD: Water framework directive; ww: Wet weight (as normalization); WWTP: Waste water treatment plant.

Supplementary Information
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> **Additional file 1:** Supporting information containing details regarding: A) Sampling site, B) QC/QA, C) Trophic position calculation, D) Additional calculation methods, E) Biota concentrations, F) TMF normalization, G) TMF literature values and comparison to data from this study.

Authors' contributions
HR, VK, CS, CR, and JK conceived the project; VK and HR performed the calculations and compiled and evaluated the TMF study database; VK wrote a first version of the manuscript; all authors contributed to the optimization of the manuscript and the discussion of the data. All authors read and approved the final manuscript.

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article and its additional file.

Declarations

Ethics approval and consent to participate
All samplings have been conducted by professionals who held the necessary permits of the responsible state agencies.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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