TEXTE
137/2022

# Food web on ice - Investigation of the bioaccumulation of chemicals in an exemplary food chain

Final report

German Environment Agency

Umwelt Bundesamt

TEXTE 137/2022

Ressortforschungsplan of the Federal Ministry for the Enviroment,
Nature Conservation, Nuclear Safety and Consumer Protection

Project No. (FKZ) 3717 65 416 0

Report No. (UBA-FB) FB000568/ENG

# Food web on ice - Investigation of the bioaccumulation of chemicals in an exemplary food chain

Final report

by

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On behalf of the German Environment Agency

April 2021

## Imprint

**Publisher**
Umweltbundesamt
Wörlitzer Platz 1
06844 Dessau-Roßlau
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Internet: www.umweltbundesamt.de

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**Study performed by:**
Fraunhofer Institute for Molecular Biology and Applied Ecology (Fraunhofer IME)
Auf dem Aberg 1
57392 Schmallenberg
Germany

**Report completed in:**
April 2021

**Edited by:**
German Environment Agency
Jan Koschorreck, Caren Rauert

Publication as pdf:
http://www.umweltbundesamt.de/publikationen

ISSN 1862-4804

Dessau-Roßlau, December 2022

The responsibility for the content of this publication lies with the author(s).

**Abstract: Investigation of the bioaccumulation of chemicals in an exemplary food chain**

Trophic magnification factors (TMFs) have been derived in a variety of different aquatic ecosystems worldwide to investigate accumulation patterns of environmentally relevant chemicals. The TMF is defined as a metric that describes the average trophic magnification of a chemical through the analyzed food web under realistic environmental conditions. Not only is the TMF interesting for chemicals' risk assessment related questions, but also for monitoring aspects under the European Water Framework Directive (WFD). This study is the first TMF study conducted in a German freshwater ecosystem, that is, Lake Templin near Potsdam. Aim of the study was to investigate the food web magnification following existing guidance to derive reliable TMFs that could be used for regulatory purposes. A sampling campaign yielded 15 biota samples covering about three trophic levels, which have been processed and cryo-preserved following standardized protocols of the German Environmental Specimen Bank (ESB). The samples remain available for future analysis and, thus, form a "food web on ice". These large-scale food web samples are ready-to-use for a broad variety of analyses. In a first step, a plausibility check was performed. Different persistent organic pollutants (POPs), which are known to magnify in food webs and are not readily metabolized, serve as benchmarks. It could be shown that for nearly all of the POPs analyzed, the TMFs are significantly above 1. In a few cases, an enrichment is also seen, but not statistically relevant. Since not only POPs with lipophilic accumulation properties were analyzed, it could be concluded that the food web on ice samples from Lake Templin can be used to characterize the trophic magnification potential of further substances with less investigated bioaccumulation properties present in the samples. To this end, several PFAS, pharmaceuticals, pesticides and methyl siloxanes were investigated in the samples to derive their TMFs.

**Kurzbeschreibung: Bioakkumulation von Chemikalien in einer exemplarischen Nahrungskette**

Um das Akkumulationsverhalten umweltrelevanter Chemikalien unter realen Bedingungen zu untersuchen, wurden in vielen verschiedenen aquatischen Ökosystemen weltweit trophische Magnifikationsfaktoren (TMFs) abgeleitet. Der TMF ist definiert als eine Metrik, die die durchschnittliche trophische Anreicherung einer Chemikalie durch das analysierte Nahrungsnetz unter realistischen Umweltbedingungen beschreibt. TMFs sind nicht nur für Fragen der Risikobewertung von Chemikalien interessant, sondern auch für bestimmte Aspekte der Überwachung von Stoffen im Kontext der Wasserrahmenrichtlinie (WRRL). Diese Untersuchung ist die erste TMF-Studie, die in einem deutschen Süßwasser-Ökosystem - dem Templiner See bei Potsdam - durchgeführt wurde. Ziel der Studie war es, die Nahrungsnetzanreicherung von Stoffen zu untersuchen und zuverlässige TMFs abzuleiten, die für regulatorische Zwecke verwendet werden können. Es wurde ein Satz von Nahrungsnetzproben gewonnen, der 15 Biota-Proben aus etwa drei trophischen Ebenen umfasst. Die Proben wurden nach standardisierten Protokollen der Umweltprobenbank des Bundes (UPB) aufgearbeitet und tiefgekühlt gelagert. Die erhaltenen Nahrungsnetzproben bilden somit ein „Nahrungsnetz auf Eis" und sind nun für eine Vielzahl von Analysen einsetzbar. In einem ersten Schritt wurde eine Plausibilitätsprüfung durchgeführt. Als Vergleichsmaßstab dienen persistente organische Schadstoffe (POPs), von denen bekannt ist, dass sie sich in Nahrungsnetzen anreichern und zudem nicht leicht metabolisiert werden. Von den hier analysierten POPs zeigen die meisten Stoffe TMFs signifikant über 1. In wenigen Fällen ist zwar auch eine Anreicherung zu erkennen, die jedoch statistisch nicht signifikant ist. Da nicht nur POPs mit lipophilen Akkumulationseigenschaften analysiert wurden, kann der Schluss gezogen werden, dass die archivierten Proben des „Nahrungsnetzes auf Eis" aus dem Templiner See zur Charakterisierung des trophischen Magnifikationspotentials weiterer in den Proben vorhandener Substanzen mit weniger untersuchten Bioakkumulationseigenschaften verwendet werden können. Zu diesem Zweck wurden mehrere PFAS, Arzneimittelwirkstoffe, Pestizide und Methylsiloxane in den Proben analysiert, um für diese TMFs abzuleiten.

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| Attachment 1: | Report by IfB (Institut für Binnenfischerei e.V.) on the Lake Templin biota sampling (2018) - in German language |
| Attachment 2: | Report by LimPlan on supporting plankton samplings and plankton analyses (2018) - in German language |
| Attachment 3: | Report by agroisolab GmbH on bulk stable isotope analysis - in German language |
| Attachment 4: | Report by Isodetect GmbH on amino acid-specific stable isotope analysis - in German language |
| Attachment 5: | Report by Eurofins GfA Lab Service GmbH on biota analyses for organic chemicals (2020) - in German language |
| Attachment 6: | Report by Eurofins GfA Lab Service GmbH on PCB analysis of passive sampler extracts (2021) - in German language |
| Attachment 7: | Database on TMF studies in freshwaters (Microsoft Excel file; 2020) |
| Attachment 8: | Results from analyses of food web samples at Fraunhofer IME (Microsoft Excel files in a zip archive file; 2019-2021) |
| Attachment 9: | Lake Templin TMF evaluations (Microsoft Excel file; 2020) |
| Attachment 10: | Documentation of the ‘Food web on ice’ Workshop in March 2021 (zip archive file with invitation, all presentation files and workshop summary) |

## List of abbreviations

| | |
|---|---|
| **BAF** | bioaccumulation factor |
| **BCF** | bioconcentration factor |
| **BMF** | biomagnification factor |
| **CI** | confidence interval |
| **cVMS** | cyclic volatile methyl siloxanes |
| **dw** | dry weight |
| **d$^{15}$N, d$^{13}$C or δ$^{15}$N, δ$^{13}$C** | ratio of a heavier isotope in relation to a lighter one; for nitrogen (N) and carbon (C) |
| **D4** | octamethylcyclotetrasiloxane |
| **D5** | decamethylcyclopentasiloxane |
| **dl-PCB** | dioxin-like polychlorinated biphenyls |
| **DMA** | Direct Mercury Analyzer |
| **dSPE** | dispersed solid phase extraction |
| **GIT** | gastrointestinal tract |
| **GPC** | gel permeation chromatography |
| **Hg** | mercury |
| **IME** | Fraunhofer Institute for Molecular Biology and Applied Ecology |
| **lw** | lipid weight |
| **MeHg** | mono-methylmercury |
| **n.a.** | not available |
| **ndl-PCB** | non-dioxin-like polychlorinated biphenyls |
| **PBDE** | polybrominated diphenylethers |
| **PBT** | persistent, bioaccumulative, toxic |
| **PCDD** | polychlorinated dibenzodioxins and dibenzofurans |
| **PDMS** | polydimethylsiloxane |
| **PFAS** | per- and polyfluoroalkyl substances |
| **PFOS** | perfluorooctanesulfonate |

| | |
|---|---|
| **PRC** | performance reference compounds |
| **rpm** | round per minute |
| **RSD** | relative standard deviation |
| **SD** | standard deviation |
| **SID-GC/ICP-MS** | stable isotope dilution-gas chromatography coupled to inductively coupled plasma-mass spectrometry |
| **THg** | total mercury |
| **TL** | trophic level (for generic classification as, e.g., as primary consumer) |
| **TMAH** | tetramethylammonium hydroxide |
| **TMF** | trophic magnification factor |
| **TP** | trophic position (determined for an organism in a certain ecosystem) |
| **UBA** | Umweltbundesamt (German Environment Agency) |
| **vPvB** | very persistent, very bioaccumulating |
| **WFD** | Water Framework Directive |
| **WP** | work package |
| **ww** | wet weight |

# Summary

In risk assessment of chemicals, the aquatic bioaccumulation potential of compounds is assessed as a critical property. Various parameters are determined experimentally in laboratory or field studies or estimated based on experimental data. In particular, bioconcentration factors, bio-accumulation factors and biomagnification factors (BCFs, BAFs, BMFs) serve as measures of this potential. A still relatively new approach is the determination of so-called trophic magnification factors (TMFs), which integrate enrichment processes in a food web. The TMF is defined as a metric that describes the average trophic magnification of a chemical through the analyzed food web under realistic environmental conditions. So far, TMFs are mainly available for legacy chemicals in a variety of different aquatic ecosystems worldwide and only for a few current-use compounds TMFs have been derived. Not only is the TMF interesting for chemicals' risk assess-ment related questions (e.g., for assessments with regard to substances that are potentially persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB)), but also for certain aspects of the biota monitoring implemented under the European Water Framework Directive (WFD). However, there is a lack of sufficient practical experience in protocol standardization and the use of derived TMFs for regulatory substance evaluations. To this end, a field study to collect, freeze and store food web samples to form a 'Food web on ice' was initiated by the German Environment Agency allowing to investigate bioaccumulation and biomagnification of chemicals in a freshwater ecosystem. If possible, methods already used for the German Environmental Specimen Bank (ESB), an environmental monitoring program involving long-term storage of samples in a cryo-archive at ultra-low temperatures, should be applied.

In order to achieve an international exchange of knowledge and experiences, a scientific advisory board was built for the project by inviting renowned international scientists with expertise in bioaccumulation research and first-hand experience in trophic magnification studies. During the project period, several meetings with the seven members of the advisory board were organized. During these meetings, the project team shared the status of the project and invited comments from the advisory board members. Several recommendations by board members were considered and followed, e.g., the inclusion of passive sampling into the study and the removal of the gastrointestinal tracts from the sampled larger individual fish. The kind support of the members of the scientific advisory board is gratefully acknowledged.

At the start of the project, a literature evaluation of relevant TMF-studies was conducted. The evaluation focused on organic substances. Metals appeared less relevant due to their low bio-magnification potential as many studies confirmed. The only exceptions known so far are mercury and methylmercury compounds. For these substances, however, a comprehensive meta-evaluation of biomagnification research is available. Since the experimental TMF study was planned to be implemented in an inland water body, the literature evaluation of biomagni-fication / trophic magnification focused on investigations in rivers and lakes. Freshwater TMF studies were evaluated with regard to the following aspects: geographical region, taxonomic groups, range of trophic levels, amount of sample material, period of sampling, measured bio-metric parameters, baseline organisms for trophic classifications, source of pollution burden, and other possible relevant aspects (e.g., normalization, statistical methods, handling of non-detects). About 60 publications on TMF investigations in inland waters have been evaluated in a structured way and about 1100 TMFs for about 400 chemicals (including isomers/congeners) have been aggregated. PCB- and PBDE congeners have been studied most often. The data, which cover the time period 1995–2020, were gathered in an overview table.

The compiled data were used for the preparation of a publication covering the selection and application of TMFs for priority substances to normalize freshwater fish monitoring data under the WFD. In the peer-reviewed contribution the usage of TMFs for the normalization of fish burden to a certain trophic level (top predator, here trophic level 4) was explored, an issue that was raised in a guidance document on biota monitoring by the EU Commission. The substances covered in the publication are WFD priority substances considered for a monitoring in biota (preferably fish).

Aim of the experimental part of the 'Food web on ice' project was to plan and conduct a sampling campaign following existing guidance for TMF studies to collect a set of food web samples, which can be used to derive reliable TMFs that are appropriate for application in regulatory purposes.

An important decision to be made at the beginning of the experimental part was the selection of a suitable water body for the field sampling. Several water bodies have been considered as study site. The main criteria for assessing the suitability of a water body were: accessibility for sampling, a certain water body size, presence of appropriate abundances of plankton, invertebrate and different fish species and presence of a certain level of pollution (so that relevant chemicals can be detected at all trophic levels of the food web). These criteria reduced the number of appropriate water bodies in Germany considerably. For example, most lakes considered only had low levels of pollution so that it would be difficult to quantify interesting chemicals especially in low trophic level organisms. Other lakes would not have allowed the sampling of sufficient amounts of plankton for the planned analyses.

In joint discussions between the German Environment Agency and the project team at Fraunhofer IME and after consultation of the members of the international scientific advisory board, it was finally decided to conduct the study at Lake Templin near Potsdam. This lake is flown through by the Havel River and affected by the discharge of the effluents of a major sewage treatment plant upstream the Havel River.

Favorable aspects of Lake Templin include a sufficient occurrence of plankton (however, phytoplankton is only expected to be available in lower quantities due to decreasing eutrophication), the occurrence of mussels as part of the food web (here the zebra mussel *Dreissena polymorpha*) and the exposure of lake biota to pollutants carried by the Havel River.

The main focus for the investigation was put on the southern part of Lake Templin, which is less urbanized in the shore area (only few buildings). In addition, in this part of the lake also less (leisure) shipping traffic could be expected and the shore of the water body was easier accessible.

For the sampling of the Lake Templin food web, the following requirements were identified:

- The selected food web items should be, as far as possible, representative of the entire food web allowing a comprehensive evaluation of a substance's trophic magnification.
- The organisms are sampled as representatives of the respective trophic level since it is not possible to cover all parts of the lake food web.
- The selected organisms should have dietary relationships so that it can be assumed that the substance concentrations determined in organisms of higher trophic levels derive from trophic levels represented in the sample set.
- To fulfil the steady state requirement, no migratory species should be included in the sampling list, as these species may also be influenced from other habitats as well.

- ► The selected species should not be endangered (no Red List species).

Since pelagic food webs are in general less complex in comparison to benthic ones, it was intended to sample organisms that feed preferably in the water phase. However, due to the expected usage of different carbon sources by organisms, a purely pelagic food chain can probably not be expected in Lake Templin.

Before the sampling campaign was conducted, information about the species abundant in the target ecosystem Lake Templin had been collated with the help of local experts. Thus, information about specific lifestyles could be obtained which in turn allowed for a better understanding of the dietary relationships.

In cooperation with an external expert team, a passive sampling campaign was conducted to examine whether water concentrations of potential target substances change over time. The water concentrations may also be used to calculate BCFs/BAFs for the sampled biota. Passive samplers were deployed at three different locations in the lake. Each passive sampler cage was equipped with three different types of sorbents: Empore disks, Atlantic disks and polydimethyl-siloxane (PDMS) sheets. The Empore and Atlantic disks, which bind more polar compounds, were exposed in Chemcatcher housings. Exposure periods for the Chemcatcher disks were one month and for the PDMS sheets, which target at non-polar chemicals, about two months (end of June - August; August - October).

The extraction and quantitative analysis of the passive samplers for most of the benchmark substances could not be performed during the project period due to a limited budget. Only for PCB, an analysis of a set of PDMS sheets was possible. Therefore, the PDMS passive samplers were carefully cleaned for removal of biofilm residues, air-dried in a clean bench for several hours and extracted with acetone using a Soxhlet device. The extracts were analyzed for the full set of PCB congeners. However, at the end of the project only preliminary data were available. Due to different loadings of the applied isotope-labeled PCB-performance reference compounds in the PDMS sheets for the two exposure periods, not for all PCB congeners concentrations could be determined. Nevertheless, the preliminary data (presented as sums for differently high chlorinated PCB groups) support the assumption that the PCB concentrations at the two sampling sites in the southern part of Lake Templin were similar during the two exposure periods. No significant difference of the total PCB sums between the two sites and between the August and October samples were detected ($p < 0.05$).

All biota samplings at Lake Templin that were later used for the 'Food web on ice' sample set were conducted mid-September 2018.

For sampling of plankton fractions, two different techniques were tested and applied in the southern part of Lake Templin. The first method utilized a stacked cascade of nets that allowed a fractionation of the filtered water. Water pumps were operated and transported lake water to the cascade of three nets with mesh sizes of 250, 100 and 40 µm, respectively. These types of plankton fractions could only be used for temporal comparisons of plankton properties because the collected biomass was too low for the planned chemical analyses.

The second plankton sampling method utilized an approximately 5 m long coned net with 200 µm mesh size and was applied to gain a large plankton fraction for the food web analyses. The net was exposed in Lake Templin near the shore and lake water was pumped through the

net. The moist plankton fraction sampled during one day was directly transferred into liquid nitrogen and subsequently stored in the vapor phase above liquid nitrogen (< -130°C). Due to the high water content the major part of the plankton fraction was freeze-dried. This process run over several days until the dry weight was constant on two successive days.

Mussels (*Dreissena polymorpha*) were sampled at four different spots in the southern part of Lake Templin on two days. Preferably, mussels growing on larger stones which had been exposed directly to the water phase were sampled. The sample mass was increased by including *D. polymorpha* from the upper levels of sediment-attached colonies using a dip net. The collected mussels were sorted and dead shells were removed from the bulk. The remaining mussels were kept in aerated tap water in glass aquaria for about 48 hours to clear their guts. The gut-cleared mussels were removed from the water, frozen in liquid nitrogen (including shells and respiration water) and stored in the ESB cryo-archive (< -130°C). Frozen mussels were divided into two groups with sizes of < 2 cm and > 2 cm. The frozen mussels were allowed to thaw to such a degree as that the soft tissue could be separated from the shells and again being transferred into the ESB cryo-archive. The prepared zebra mussel composite samples were cryo-milled and homogenized following established ESB protocols. The zebra mussels from Lake Templin were also used as the baseline species for the trophic level determination of biota samples with stable isotopes.

About ten different fish species were sampled at Lake Templin during the sampling campaign. Two different fishing methods were applied, electrofishing and an overnight deployment of gillnets with different mesh sizes. Fish were sorted by size and species, stored on ice and transported to the laboratory. Biometric data of each fish were recorded (size, weight, sex). Fish smaller than 20 cm were treated as one composite sample per species, transferred into clean stainless-steel containers filled with liquid nitrogen and stored in the ESB cryo-archive. Fish larger than 20 cm were dissected under a clean bench within 72 hours after sampling. From each individual fish, one skinless fillet and the gastrointestinal tract (GIT) were removed. Approximately 20 scales of each fish were collected for age determination. Fillet and GIT were weighed and transferred into separate containers filled with liquid nitrogen. In some cases, the fish stomach content was analyzed for remainders of food items to gain additional information on the trophic level of the respective fish. The remaining carcass was cut into small pieces and also transferred into a marked stainless-steel container with liquid nitrogen. Commercially available frozen fillet of Alaska pollock was used as field blank for fish. The fillet was removed from the packaging and exposed just like the fish catch after sampling and later openly in the room where the fish were dissected. After several hours, the fish was frozen again and further treated like the Lake Templin fish samples. Finally, all fish samples were transferred to the ESB cryo-archive. Larger individual fish (fillet and carcass separately) and the prepared composite samples of small fish were manually crushed, cryo-milled and homogenized.

Nitrogen and carbon stable isotope determinations ($^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios) of Lake Templin food web samples were performed after extraction of lipids. Data are expressed as ‰ $\delta^{15}N$ and ‰ $\delta^{13}C$ and were used to evaluate the fish trophic positions (TP). The zebra mussels were used as baseline organisms with an assigned TP of 2.0.

After completion of the cryo-milling for all food web samples and a first rough check of individual samples for stable isotope signatures, fillet and remaining fish samples were re-combined

proportionally to obtain whole fish samples again (without GIT). However, fractions of fillet and remaining fish were kept separately for possible additional analyses.

The reduced set of food web items was characterized based on the stable isotope data. In a next step, the food web items' stable isotope signatures were checked for coherence. This step was necessary to create a basis for decision making for the preparation of appropriate composite samples. By forming representative composite samples, the number of samples could be reduced without losing the informative value of the analysis results. It was also tried to avoid a dominance of individuals of one species. In total, about 30 whole fish samples, five mussel samples, seven plankton fractions and one field blank were analyzed for stable isotopes of carbon and nitrogen. The data set was examined to characterize the feeding behavior of the sampled organisms or species (e.g., pelagic feeding relations).

Contrary to expectations, larger differences in the $\delta^{13}C$-signatures were found, which may indicate the use of heterogeneous carbon sources in the food web (benthic vs. pelagic feeding, possible inputs from terrestrial sources). In case of the plankton fractions, unknown proportions of suspended particulate matter in the samples may also have influenced the $\delta^{13}C$-signatures.

The increments in the $\delta^{15}N$ signatures over the whole sample set are much smaller than expected. If the standard increment of 3.4 ‰ $\delta^{15}N$ per trophic level is applied, lower trophic positions for the fishes would result than those given in the literature (e.g., www.fishbase.org). Alternatively, a value of 2.3 ‰ was chosen as the increment for the trophic enrichment of $^{15}N$. This value was determined in a published meta-analysis as the mean trophic shift between aquatic consumers and their diet. With this lower increment selected here, the TPs are in the range of the literature TPs. Larger deviations are found, e.g., for small perch. However, the lower TP can be explained by a probably less piscivorous diet of the juvenile fish.

Since, on the basis of the stable isotope data, some fish samples do not appear to be components of a common food web, they were not considered for the further investigation. These samples were mixed samples of small asps as well as those of small and large rudds and an additional perch composite sample. After these samples with diverging signatures were removed, the expected correlation of the $\delta^{15}N$ and $\delta^{13}C$ data improved clearly.

The final set of food web samples comprised of 15 biota samples covering about three trophic levels (one plankton fraction, two samples of differently sized mussels, and twelve whole fish samples from seven different species). Additionally, the field blank sample was included. About 50 - 80 subsamples of each of these samples were prepared following an ESB protocol. Finally, more than 1200 subsamples of the final food web samples were stored in the cryo-archive of the German ESB in the gas phase above liquid nitrogen at temperatures < -130°C. Afterwards, individual subsamples were taken from the archive for the analyses of stable isotopes, parameters such as lipid and protein content, and selected target chemicals for TMF determinations.

As next step, parameters for sample characterization were determined (lipid, water and protein contents). For normalizations of pollutant burdens, the lipid content of the food web items was determined gravimetrically by applying the Smedes-method. The water content was determined by weighing of samples before and after freeze-drying. For the sampled zebra mussels, it has to be considered that these contained respiration water, which increases the water content of the tissue homogenate. The respiration water content has to be considered when applying the wet weight data of the zebra mussels for TMF evaluations or comparisons with mussel data from other sources. For the determination of the protein contents, which also can be applied for normalizations, an internally documented protocol was applied.

In the selected food web items, including the field blank sample, a set of chemicals was analyzed in order to determine the possible biomagnification and finally the trophic magnification factors. By this means, the plausibility of the TMF derived from the selected food web items should be demonstrated. The applied methods had mostly already been used for other investigations and descriptions were published in peer-reviewed journals.

Polybrominated diphenylethers (PBDE), chlorinated legacy compounds, chlorinated dioxins/furans and polychlorinated biphenyls (PCB) were analyzed by German ESB standard methods after extraction of the biota samples with appropriate solvents.

For the group of per- and polyfluoroalkyl substances (PFAS), a set of 38 PFAS was analyzed applying a previously published method. Aliquots of homogenized samples were extracted using tetrabutylammonium as an ion pair reagent and methyl-*tert.*-butyl ether as solvent. Analysis was performed using ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) applying isotope-labeled internal standards. Each sample was analyzed in duplicate. Limits of quantification (LOQ) of 0.5 ng/g wet weight for each substance were validated by recovery experiments at this concentration level. No PFAS levels above the LOQs were detected in the procedural and solvent blanks. Relative standard deviations (RSDs) between duplicate sample measurements showed a suitable reproducibility.

The selected food web items were also analyzed for the cyclic volatile methyl siloxanes (cVMS) octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6). The cVMS were extracted with acetonitrile and n-hexane. For quantification, tetrakis-(trimethylsilyloxy)silane was added to the samples as internal standard before the extraction procedure. Speciation and quantitative measurements were performed with a GC-ICP-MS/MS coupling method, which was published previously. For method validation and quality control, fortification experiments with fish samples were performed. Blanks were measured along each measurement series and were uncritical. Wet weight-based LOQs were in the ranges 6.50 - 22.1 ng/g for D4, 2.90 - 20.8 ng/g for D5 and 12.5 - 117 ng/g for D6.

Diclofenac was analyzed with other pharmaceutical compounds (canrenone, carbamazepine and the transformation product 10-Hydroxy-10,11-dihydrocarbamazepine). Additionally, caffeine was included as a possible indicator substance for communal wastewater. For analysis of these substances, samples were extracted and cleaned up with gel permeation chromatography and dispersed solid phase extraction before measurement on a UHPLC-HRMS system. The method was validated for whole fish according to the requirements of guideline SANCO 3029 by spiking a blank matrix at LOQ and 10 x LOQ levels. Recoveries and their relative standard deviations were within the set criteria (recovery: 70 - 110 %, RSD: < 20 %). For caffeine and canrenone, minor blank subtractions were necessary as no background free matrix was available. The resulting LOQ was 10 ng/g wet weight for each analyte.

The analysis of pyrethroids followed a published protocol with slight modifications. The covered substances were tefluthrin, transfluthrin, λ-cyhalothrin, permethrin, cypermethrin and deltamethrin. For analysis, freeze-dried samples were extracted with a mixture of n-hexane and dichloromethane. After centrifugation, the supernatant was removed and the extraction process was repeated twice. The extracts were combined, evaporated to dryness and reconstituted in acetonitrile. The sample was cleaned up by solid phase extraction. The eluate was evaporated and reconstituted in n- hexane. GC-MS/MS analysis was performed on a triple quadrupole mass

spectrometer with negative chemical ionization and methane as a reagent gas. For the individual analytes specific quantifier and qualifier mass transitions were used. Quantification was conducted with a seven-point calibration including internal standards. Dry weight-based LOQs for the biota analysis were 0.033 ng/g for tefluthrin, transfluthrin and λ-cyhalothrin, 8.33 ng/g for permethrin and cypermethrin, and 16.7 ng/g for deltamethrin.

The analysis of chlorinated paraffins in the food web samples by a sub-contractor could only start end of 2020. The report will be provided after finalization of the study as publication draft for a peer-reviewed journal. The method is similar to the procedure that was used for the analysis of German ESB samples (manuscript already submitted to a peer-reviewed journal). The preliminary data for the concentrations of the chlorinated paraffins in the food web samples were presented during the final project workshop in March 2021.

Measurement of total mercury was performed by a dedicated atomic absorption spectrometry method applying a Direct Mercury Analyzer (DMA) for previously freeze-dried samples. The LOQs of the previously published protocol were 0.145 - 0.245 ng/g dry weight. Verification of measurements were performed with certified reference materials. Measured method blanks were always negligible.

Analysis of methylmercury (MeHg) was performed with SID-GC/ICP-MS (stable isotope dilution-gas chromatography with detection by inductively coupled plasma-mass spectrometry). After addition of a certified $^{201}$Hg-enriched MeHg standard, tissue samples were extracted by a microwave assisted procedure with tetramethylammonium hydroxide. Afterwards, samples were derivatized with sodium tetrapropylborate. Volatile mercury species were then extracted in n-hexane and analyzed by SID-GC/ICP-MS as published elsewhere. The respective LOQs were in the range of 0.2 - 1.7 ng/g dry weight (derived from blanks measured along with each set of samples). Verifications of measurements were performed with certified reference materials.

For the TMF evaluations, the data from the food web analyses for organic chemicals and for PFAS, mercury/methylmercury and cVMS were applied. Due to the uncertain water content of the plankton fraction (frozen together with an unknown amount of water) and mussels (including an unknown amount of respiration water), all TMF for polar compounds were calculated on a dry weight basis. TMF for non-polar compounds were based on lipid-normalized concentrations. For most of these benchmark chemicals, TMF determinations could be performed. For this evaluation, only data sets were considered where at maximum two samples had levels below the LOQs (these were mainly the plankton fraction and the zebra mussels). In these cases, the concentration below the LOQ was substituted by a concentration of 0.5 * LOQ.

Unfortunately, for some compounds no evaluation was possible because too many biota samples had concentrations below the LOQ. This holds true for most PFAS and the analyzed set of pharmaceuticals and pyrethroids.

For the substances where sufficient data were available, the derived TMFs from the Lake Templin food web were compared to TMFs reported in published meta-analyses. However, these literature evaluations deliver mean TMFs with very broad ranges due to large range of different ecosystems covered.

TMFs were derived for the whole set of Lake Templin food web samples (n = 15), the set of samples without the plankton fraction (n = 14), and the set of samples without the plankton

fraction and the two zebra mussel samples (n = 12, fish only). For the calculated TMFs, mean and 95 % confidence intervals are given. While for the whole food web most correlations are significant (two-tailed test of significance of Pearson's correlation coefficient), especially for the 'fish only' evaluations some correlations were not significant. Most TMFs for the whole set of food web items are in a quite narrow range of about 1.5 - 2.5. These values were at the lower end of the ranges of the respective TMFs reported in the literature. The highest TMF was found for 4,4'-DDT, a legacy insecticide, for the 'fish only' evaluation (about 10). For the investigated cVMS only D5 was present in all biota samples. Interestingly, a significant TMF < 1 was found for D5 in Lake Templin. Literature reports different TMFs (< 1 / > 1) for D5 with data for (shallow) lakes - such as Lake Templin - tending to show also low TMFs.

Since for the successful plausibility check not only POPs with lipophilic accumulation properties were analyzed, it could be concluded that the archived 'Food web on ice' samples from Lake Templin can be used to characterize the trophic magnification potential of further substances with less investigated bioaccumulation properties present in the samples.

Finally, the 'Food web on ice' project was introduced comprehensively to the scientific community by researchers from Fraunhofer IME, German Environment Agency and project partners during a two-day online workshop on March 16/17, 2021. An invited keynote presentation on TMF research built the basis for the scientific exchange. Examples from other studies on trophic magnification presented by scientists from Italy and France provided a broader picture. About 75 scientists from regulatory agencies, research institutes and industry participated in the workshop. Participants came from 12 countries including Canada and the USA.

During the general discussion of the workshop, the following issues were covered:

- Criteria for the selection of baseline organisms for the calculation of trophic positions;
- Possible use of passive sampling data to support the TMF evaluations (by allowing the estimation of dissolved water concentrations to be used for BAF calculations for comparisons/plausibility checks);
- Possible trophic magnification of polar compounds (bioconcentration by gill uptake vs. biomagnification by diet uptake);
- Use of TMFs in regulatory context: For the EU Water Framework Directive often BAFs derived from field studies are used for quality standards deviations instead of a combination of BCFs derived from laboratory tests and TMFs;
- It was emphasized that TMFs for higher tier assessment (e.g., to support the 'bioaccumulation' classification) can only to be applied for chemicals already in use (retrospective assessment).

# Zusammenfassung

Bei der Risikobewertung von Chemikalien wird das aquatische Bioakkumulationspotenzial von Chemikalien als eine kritische Eigenschaft bewertet. Verschiedene Parameter werden experimentell in Labor- oder Feldstudien bestimmt oder es werden Bioakkumulationsdaten auf der Grundlage von Versuchsergebnissen abgeschätzt. Insbesondere Biokonzentrationsfaktoren, Bioakkumulationsfaktoren und Biomagnifikationsfaktoren (BCFs, BAFs, BMFs) dienen als Maß für dieses Potenzial. Ein noch relativ neuer Ansatz ist die Bestimmung von sogenannten trophischen Magnifikationsfaktoren (TMFs), die Anreicherungsprozesse in einem Nahrungsnetz integrieren. Der TMF ist definiert als eine Metrik, welche die durchschnittliche trophische Anreicherung einer Chemikalie durch das analysierte Nahrungsnetz unter realistischen Umweltbedingungen beschreibt. Bisher sind TMFs hauptsächlich für bereits lange im Gebrauch befindliche umweltrelevante Chemikalien verfügbar. Diese TMFs wurden in einer Vielzahl verschiedener aquatischer Ökosysteme weltweit abgeleitet, um Akkumulationsmuster von Chemikalien zu untersuchen. Dagegen sind nur für wenige aktuell verwendete Verbindungen TMFs verfügbar. TMFs sind aber nicht nur für Fragen der Risikobewertung von Chemikalien interessant (beispielsweise für Bewertungen von Substanzen, die potenziell persistent, bioakkumulierend und toxisch (PBT) oder sehr persistent und sehr bioakkumulierend (vPvB) sind), sondern auch für bestimmte Aspekte des Biota-Monitorings, das unter der Europäischen Wasserrahmenrichtlinie (WRRL) umgesetzt wird. Es mangelt jedoch bislang an ausreichender praktischer Erfahrung sowohl in der Standardisierung von TMF-Untersuchungen als auch bei der Verwendung der abgeleiteten TMFs für regulatorische Stoffbewertungen. Vor diesem Hintergrund wurde vom Umweltbundesamt eine Feldstudie zum Sammeln, Einfrieren und Lagern von repräsentativen Nahrungsnetzproben für ein „Nahrungsnetz auf Eis" initiiert, um so die Bioakkumulation und Biomagnifikation von Chemikalien in einem Süßwasserökosystem untersuchen zu können. Dabei sollte soweit wie möglich auf Methoden zurückgegriffen werden, die bereits für die Umweltprobenbank des Bundes (UPB), ein Umweltmonitoring-Programm unter Einbeziehung der Langzeitlagerung von Proben in einem Kryoarchiv bei Tiefsttemperaturen, angewandt werden.

Um einen internationalen Wissens- und Erfahrungsaustausch zu erreichen, wurde für das Projekt ein wissenschaftlicher Beirat gebildet, in den renommierte Wissenschaftler aus verschiedenen Staaten mit Expertise in der Bioakkumulationsforschung und praktischer Erfahrung in Studien zur trophischen Magnifikation eingeladen wurden. Während der Projektlaufzeit wurden mehrere Sitzungen mit den sieben Mitgliedern des Beirats organisiert. Bei diesen Treffen informierte das Projektteam über den Stand des Projekts und bat die Beiratsmitglieder um Kommentare. Mehrere Empfehlungen der Beiratsmitglieder wurden berücksichtigt und umgesetzt, wie zum Beispiel die Einbeziehung von Passivsammler-Probenahmen in die Studie und das Entfernen der Gastrointestinaltrakte der beprobten größeren Einzelfische vor der Untersuchung. Den Mitgliedern des wissenschaftlichen Beirats wird für ihre engagierte Unterstützung herzlich gedankt.

Zu Beginn des Projekts wurde eine Literaturauswertung relevanter TMF-Studien durchgeführt. Der Schwerpunkt der Auswertung lag auf organischen Stoffen. Metalle erschienen aufgrund ihres geringen Biomagnifikationspotenzials weniger relevant, wie viele Studien bestätigen. Die einzigen bisher bekannten Ausnahmen sind Quecksilber und Methylquecksilber-Verbindungen. Für diese Stoffe liegt jedoch eine umfassende Meta-Auswertung von Biomagnifikationsuntersuchungen vor. Da die experimentelle TMF-Studie in einem Binnengewässer durchgeführt werden sollte, konzentrierte sich die Literaturauswertung zur Biomagnifikation / trophischen Magnifikation auf Untersuchungen in Flüssen und Seen. TMF-Studien in Süßwasserökosystemen wurden hinsichtlich folgender Aspekte ausgewertet: geographische Region, taxonomische

Gruppen, Spanne der Trophiestufen, Menge des Probenmaterials, Zeitraum der Probenahme, gemessene biometrische Parameter, Basislinien-Organismen für die Bestimmung der trophischen Positionen, Quelle der Schadstoffbelastung und andere mögliche relevante Aspekte (z.B. Normalisierung, statistische Methoden, Umgang mit Konzentrationen unterhalb der Bestimmungsgrenzen). Es wurden ca. 60 Publikationen zu TMF-Untersuchungen in Binnengewässern strukturiert ausgewertet und ca. 1100 TMFs für ca. 400 Chemikalien (einschließlich Isomeren/Kongeneren) aggregiert. Viele Daten beziehen sich beispielsweise auf PCB- und PBDE-Kongenere. Die erfassten Daten aus dem Zeitraum 1995-2020 wurden in einer Übersichtstabelle zusammengestellt.

Die gesammelten Daten wurden für die Vorbereitung einer Veröffentlichung verwendet, die die Auswahl und Anwendung von TMFs für prioritäre Stoffe zur Normalisierung von Süßwasserfisch-Monitoringdaten unter der WRRL behandelt. In dem vor der Veröffentlichung wissenschaftlich begutachteten Beitrag in einer Fachzeitschrift wurde die Verwendung von TMFs zur Normalisierung der Fischbelastung auf ein bestimmtes trophisches Niveau (Spitzenprädatoren, hier Trophiestufe 4) untersucht. Dieses Vorgehen wird in einem Leitfaden der EU-Kommission zum Biota-Monitoring vorgeschlagen. Bei den in der Veröffentlichung behandelten Chemikalien handelt es sich um prioritäre Stoffe der WRRL, die für ein Monitoring in Biota (vorzugsweise in Fischen) vorgesehen sind.

Ziel des experimentellen Teils des Projekts „Nahrungsnetz auf Eis“ war es, eine Probenahmekampagne zu planen und durchzuführen, die den bestehenden Richtlinien für TMF-Studien folgt, um einen Satz geeigneter Nahrungsnetzproben zu sammeln. Damit soll die Bestimmung zuverlässiger TMFs ermöglicht werden, die für die Anwendung in regulatorischen Kontexten geeignet sind.

Eine wichtige Entscheidung, die zu Beginn des experimentellen Teils getroffen werden musste, war die Auswahl eines geeigneten aquatischen Ökosystems für die Probenahme. Es wurden mehrere Wasserkörper als Untersuchungsort in Betracht gezogen. Die Hauptkriterien für die Eignung eines Wasserkörpers waren: Zugänglichkeit für die Probenahme, eine Mindestgröße des Wasserkörpers, angemessene Abundanzen von Plankton, Wirbellosen und verschiedenen Fischarten und eine ausreichende anthropogene Belastung, so dass relevante Chemikalien auf allen trophischen Ebenen des Nahrungsnetzes nachgewiesen werden können. Diese Kriterien reduzierten die Anzahl der geeigneten Wasserkörper in Deutschland erheblich. So wiesen die meisten der betrachteten Seen nur eine geringe Schadstoffbelastung auf, so dass es schwierig wäre, relevante Chemikalien vor allem in Organismen der unteren Trophiestufen zu quantifizieren. Andere Seen erschienen als nicht geeignet, da es in diesen nicht möglich wäre, ausreichende Mengen an Plankton für die geplanten Analysen zu beproben.

In gemeinsamen Gesprächen zwischen dem Umweltbundesamt und dem Projektteam am Fraunhofer IME und nach Konsultation der Mitglieder des internationalen wissenschaftlichen Beirats wurde schließlich entschieden, die Studie am Templiner See bei Potsdam durchzuführen. Dieser See wird von der Havel durchflossen und ist durch die Einleitungen einer Großkläranlage im Oberlauf der Havel beeinflusst.

Zu den vorteilhaften Aspekten des Templiner Sees gehören ein ausreichendes Planktonvorkommen (Phytoplankton war jedoch aufgrund der abnehmenden Eutrophierung nur in geringeren Mengen zu erwarten), das Vorkommen von Muscheln als Teil des Nahrungsnetzes (hier die Dreikantmuschel *Dreissena polymorpha*) und die Exposition von Biota im Seewasser gegenüber Schadstoffen, die durch die Havel eingetragen werden.

Das Hauptaugenmerk bei der Untersuchung wurde auf den südlichen Teil des Templiner Sees gelegt, der im Uferbereich weniger erschlossen ist (nur wenige Gebäude). Außerdem war in

diesem Teil des Sees auch weniger (Freizeit-)Schiffsverkehr zu erwarten und das Ufer des Gewässers war leichter zugänglich.

Für die Beprobung des Nahrungsnetzes des Templiner Sees wurden die folgenden Anforderungen ermittelt:

- Die ausgewählten Nahrungsnetzelemente sollten repräsentativ für das gesamte Nahrungsnetz sein, um eine umfassende Bewertung der trophischen Magnifikation einer Substanz zu ermöglichen.
- Die Organismen werden als Repräsentanten der jeweiligen Trophiestufe beprobt, da es nicht möglich ist, alle Elemente des Nahrungsnetzes des Sees abzudecken.
- Die ausgewählten Organismen sollten in Nahrungsbeziehung stehen, so dass davon ausgegangen werden kann, dass die ermittelten Stoffkonzentrationen in Organismen höherer Trophiestufen von den Organismen niedriger trophischer Stufen stammen, die im Probensatz repräsentiert sind.
- Um die Gleichgewichtsanforderung zu erfüllen, sollten nur sesshafte Spezies bei der Probenahme berücksichtigt werden, da nicht-sesshafte Arten auch durch Konditionen anderer Lebensräume beeinflusst sein können.
- Die ausgewählten Arten sollten nicht gefährdet sein (keine Rote-Liste-Arten).

Da pelagische Nahrungsnetze im Allgemeinen weniger komplex sind als benthische, sollten vor allem Organismen beprobt werden, die sich bevorzugt in der Wasserphase ernähren. Aufgrund der zu erwartenden Nutzung unterschiedlicher Kohlenstoffquellen durch die Organismen ist das Vorhandensein einer rein pelagischen Nahrungskette im Templiner See jedoch nicht zu erwarten.

Vor Beginn der Probenahmekampagne wurden mit Hilfe von lokalen Experten Informationen über die im Zielökosystem Templiner See vorkommenden Arten zusammengetragen. So wurde ein besseres Verständnis der Nahrungsbeziehungen ermöglicht.

In Zusammenarbeit mit einem externen Expertenteam wurde eine Passivsammler-Probenahmekampagne durchgeführt, um zu untersuchen, ob sich die Wasserkonzentrationen potenzieller Zielsubstanzen über den Untersuchungszeitraum ändern. Die Wasserkonzentrationen können auch zur Berechnung von BCFs/BAFs der Zielstoffe für die beprobten Organismen verwendet werden. Passivsammler wurden an drei verschiedenen Stellen im See eingesetzt. Jeder Passivsammler-Käfig war mit drei verschiedenen Sorptionsmitteln ausgestattet: Empore- und Atlantic-Membranen sowie Folien aus Polydimethylsiloxan (PDMS). Die Empore- und Atlantic-Membranen, die eher polarere Verbindungen binden, wurden in Chemcatcher-Gehäusen exponiert. Die Expositionszeit für die Chemcatcher betrug einen Monat und für die PDMS-Folien, die unpolare Chemikalien binden, etwa zwei Monate (Ende Juni - August; August - Oktober).

Für die meisten Chemikalien, die zur Plausibilitätsprüfung der Anreicherung im Nahrungsnetz untersucht wurden, konnte die Extraktion und quantitative Analyse der Passivsammler während der Projektlaufzeit aufgrund des begrenzten Budgets nicht durchgeführt werden. Nur für PCB war die Analyse eines Satzes von PDMS-Folienextrakten möglich. Dazu wurden die PDMS-Passivsammler zunächst zur Entfernung von Biofilmrückständen sorgfältig gereinigt, mehrere Stunden an der Luft getrocknet und dann mit Aceton in einer Soxhlet-Apparatur extrahiert. Die Extrakte wurden auf alle PCB-Kongenere analysiert. Zum Ende des Projekts lagen jedoch nur vorläufige Daten vor. Aufgrund der unterschiedlichen Beladung der PDMS-Folien mit isotopenmarkierten PCB-Referenzverbindungen für die beiden Expositionszeiträume konnten nicht für

alle PCB-Kongenere Konzentrationen bestimmt werden. Dennoch stützen die vorläufigen Daten (dargestellt als Summen für unterschiedlich stark chlorierte PCB-Gruppen) die Annahme, dass die PCB-Konzentrationen an den beiden Probenahmestandorten im südlichen Teil des Templiner Sees während der beiden Expositionszeiträume ähnlich waren. Es wurde kein signifikanter Unterschied der Gesamt-PCB-Summen zwischen den beiden Standorten sowie zwischen den August- und Oktober-Proben festgestellt ($p < 0,05$).

Alle Probenahmen der Biota-Proben, die später den „Nahrungsnetz auf Eis"-Probensatz bildeten, wurden Mitte September 2018 am Templiner See durchgeführt.

Für die Probenahme von Planktonfraktionen wurden zwei verschiedene Techniken getestet und im südlichen Teil des Templiner Sees angewandt. Bei der ersten Methode wurde eine Kaskade von Netzen verwendet, die eine Fraktionierung des gefilterten Wassers ermöglichte. Wasserpumpen transportierten Seewasser zu der Kaskade aus drei Netzen mit Maschenweiten von 250, 100 bzw. 40 µm. Die so gewonnenen Planktonfraktionen konnten nur für zeitliche Vergleiche von Planktoneigenschaften verwendet werden, da die gesammelten Biomassen zu gering für die geplanten chemischen Analysen waren.

Die zweite Methode zur Planktonprobenahme nutzte ein ca. 5 m langes konisches Netz mit 200 µm Maschenweite und wurde eingesetzt, um eine große Menge der Planktonfraktion für die Nahrungsnetzanalysen zu gewinnen. Das Netz wurde im Templiner See in Ufernähe ausgebracht und Seewasser wurde durch das Netz gepumpt. Die wasserhaltige Planktonfraktion, die während eines Tages gesammelt wurde, wurde direkt in flüssigen Stickstoff überführt und anschließend im UPB-Kryoarchiv (< -130°C) gelagert. Aufgrund des hohen Wassergehalts wurde der größte Teil der Planktonfraktion gefriergetrocknet. Dieser Prozess lief über mehrere Tage, bis das Trockengewicht an zwei aufeinanderfolgenden Tagen konstant war.

Muscheln (*Dreissena polymorpha*) wurden an vier verschiedenen Stellen im südlichen Teil des Templiner Sees an zwei Tagen beprobt. Bevorzugt wurden solche Muscheln abgesammelt, die auf größeren Steinen wuchsen und direkt der Wasserphase ausgesetzt waren. Die Probenmasse wurde vergrößert, indem auch *D. polymorpha* aus der oberen Schicht von am Sediment lebenden Kolonien mit einem Kescher beprobt wurden. Die gesammelten Muscheln wurden sortiert, um leere Schalen zu entfernen. Die verbliebenen Muscheln wurden in Glasaquarien mit belüftetem Leitungswasser für ca. 48 Stunden gehältert, damit die Darminhalte ausgeschieden wurden. Anschließend wurden die Muscheln aus dem Wasser genommen, in flüssigem Stickstoff eingefroren (einschließlich Schalen und Atemwasser) und im UPB-Kryoarchiv eingelagert (Temperatur < -130°C). Die gefrorenen Muscheln wurden dann in zwei Gruppen mit Größen von < 2 cm und > 2 cm unterteilt. Danach wurden die gefrorenen Muscheln so weit aufgetaut, dass das Weichgewebe von den Schalen getrennt werden konnte, und anschließend sofort wieder in das UPB-Kryoarchiv überführt. Die größensortierten Dreikantmuschel-Mischproben wurden nach etablierten UPB-Protokollen kryogemahlen und homogenisiert. Für die Trophiestufen-Bestimmung von Biota-Proben mit stabilen Isotopen dienten die Dreikantmuscheln aus dem Templiner See auch als Basislinienspezies.

Während der Probenahmekampagne wurden etwa zehn verschiedene Fischarten im Templiner See beprobt. Es wurden zwei verschiedene Fangmethoden angewandt, das Elektrofischen und ein nächtlicher Einsatz von Stellnetzen mit unterschiedlichen Maschenweiten. Die Fische wurden nach Größe und Art sortiert, auf Eis gelagert und ins Labor transportiert. Die biometrischen Daten jedes Fisches wurden erfasst (Größe, Gewicht, Geschlecht). Fische, die kleiner als 20 cm waren, wurden als eine Mischprobe je Art verwendet und in sauberen, mit flüssigem Stickstoff gefüllten Edelstahlbehältern eingefroren und dann in das UPB-Kryoarchiv überführt. Fische, die größer als 20 cm waren, wurden als Individualproben innerhalb von 72 Stunden nach

der Probenahme unter einer Reinluftwerkbank seziert. Von jedem der einzeln aufgearbeiteten Fische wurden ein Filet ohne Haut und der Gastrointestinaltrakt (GIT) entnommen. Weiterhin wurden von diesen Fischen jeweils ca. 20 Schuppen für die Altersbestimmung beprobt. Filet und GIT wurden gewogen und zum Einfrieren in separate, mit flüssigem Stickstoff gefüllte Behälter überführt. In einigen Fällen wurden Mageninhalte dieser Fische auf Nahrungsreste untersucht, um zusätzliche Informationen über die trophische Position des untersuchten Fisches zu erhalten. Der jeweils verbleibende Restfisch wurde in kleine Stücke geschnitten und ebenfalls in einen Edelstahlbehälter mit flüssigem Stickstoff überführt. Kommerziell erhältliches, gefrorenes Alaska-Seelachsfilet wurde als Feldblindprobe für die Fischuntersuchungen verwendet. Das Seelachsfilet wurde dazu aus der Verpackung genommen, zunächst wie die gefangenen Fische und später im Arbeitsbereich, wo die Fische seziert wurden, offen exponiert. Nach einigen Stunden wurde der Fisch wieder eingefroren und später wie die Fischproben aus dem Templiner See weiterbehandelt. Alle Fischproben wurden dann in das UPB-Kryoarchiv eingelagert. Die größeren individuell aufgearbeiteten Fische (Filet und Restfische getrennt) und die aufbereiteten Mischproben der kleinen Fische wurden dann jeweils manuell zerkleinert, kryogemahlen und homogenisiert.

Stabilisotopenmessungen für Stickstoff und Kohlenstoff ($^{15}N/^{14}N$- und $^{13}C/^{12}C$-Verhältnisse) der Proben aus dem Nahrungsnetz des Templiner Sees wurden nach Fettextraktion durchgeführt. Die Daten werden als ‰ $\delta^{15}N$ und ‰ $\delta^{13}C$ ausgedrückt und zur Bewertung der trophischen Positionen (TP) der Fische verwendet. Die Dreikantmuscheln wurden als Basislinienorganismen mit einer festgelegten TP von 2,0 verwendet.

Nach Abschluss der Kryomahlung der Nahrungsnetzproben und einer ersten groben Überprüfung der Stabilisotopensignaturen der einzelnen Proben wurden Filet- und Restfischproben anteilig neu kombiniert, um wieder ganze Fischproben, die den ursprünglich beprobten Organismen (ohne GIT) entsprachen, zu erhalten. Teilmengen der Filet- und Restfische wurden jedoch für mögliche weitere Untersuchungen separat aufbewahrt.

Die Nahrungsnetzelemente wurden auf der Grundlage der Stabilisotopendaten charakterisiert. Im nächsten Schritt wurden die Isotopensignaturen auf Kohärenz geprüft. Dieser Schritt war notwendig, um eine Entscheidungsgrundlage für die Erstellung von geeigneten Mischproben zu schaffen. Durch die Bildung repräsentativer Mischproben konnte die Anzahl der Proben reduziert werden, ohne die Aussagekraft der Analyseergebnisse zu verlieren. Es wurde auch versucht, eine Dominanz von Individuen einer Art im endgültigen Satz der Nahrungsnetzproben zu vermeiden. Insgesamt wurden Stabilisotopendaten von etwa 30 Ganzfischproben, fünf Muschelproben, sieben Planktonfraktionen und einem Feldblindwert bestimmt. Mit Hilfe der Stabilisotopendaten wurde versucht, die Ernährungsweise der beprobten Organismen bzw. Arten zu charakterisieren (beispielsweise pelagische Nahrungsbeziehungen).

Entgegen den Erwartungen wurden größere Unterschiede in den $\delta^{13}C$-Signaturen der beprobten Organismen gefunden. Dies könnte auf die Nutzung heterogener Kohlenstoffquellen im Nahrungsnetz hinweisen (benthische bzw. pelagische Ernährung, mögliche Einträge aus terrestrischen Quellen). Die $\delta^{13}C$-Signaturen der Planktonfraktionen könnten auch durch einen unbekannten Anteil von Schwebstoff in den Proben beeinflusst worden sein.

Die Inkremente der $\delta^{15}N$-Signaturen über den gesamten Probensatz waren viel kleiner als erwartet. Bei Anwendung des Standardinkrements von 3,4 ‰ $\delta^{15}N$ pro Trophiestufe würden sich niedrigere trophische Positionen für die Fische ergeben als in der Literatur berichtet (beispielsweise bei www.fishbase.org) angegeben. Alternativ wurde ein Wert von 2,3 ‰ als Inkrement für die trophische Anreicherung von $^{15}N$ gewählt. Dieser Wert wurde in einer

publizierten Meta-Analyse als mittlere trophische Verschiebung zwischen aquatischen Konsumenten und ihrer Nahrung ermittelt. Mit diesem hier gewählten niedrigeren Inkrement liegen die berechneten TPs im Bereich Referenzwerte aus Literaturquellen. Größere Abweichungen finden sich aber beispielsweise für die kleinen Flussbarsche, deren niedrigere TP sich durch die wahrscheinlich weniger piscivore Ernährung der Jungfische erklären lässt.

Da einige Fischproben auf der Grundlage der stabilen Isotopendaten nicht Bestandteile eines gemeinsamen Nahrungsnetzes zu sein scheinen, wurden sie für die weitere Untersuchung nicht berücksichtigt. Bei diesen Proben handelte es sich um Mischproben aus kleinen Rapfen sowie aus kleinen und großen Rotfedern und um eine zusätzliche Flussbarsch-Mischprobe. Nachdem diese Proben mit divergierenden Signaturen entfernt wurden, verbesserte sich die erwartete Korrelation der $\delta^{15}N$- und $\delta^{13}C$-Daten deutlich.

Der endgültige Satz von Nahrungsnetzproben umfasste 15 Biota-Proben, die etwa drei trophische Stufen abdeckten (eine Planktonfraktion, zwei Proben unterschiedlich großer Muscheln und zwölf Ganzfischproben von sieben verschiedenen Arten). Zusätzlich ist eine Feldblindprobe enthalten. Von jeder dieser Proben wurden nach einem UPB-Protokoll etwa 50 - 80 Teilproben abgefüllt. Insgesamt wurden mehr als 1200 Teilproben der endgültigen Nahrungsnetzproben im Kryoarchiv der UPB in der Gasphase über flüssigem Stickstoff bei Temperaturen von unter -130°C eingelagert. Für die Analysen von stabilen Isotopen, Parametern wie Fett- und Proteingehalt und ausgewählten Zielchemikalien für TMF-Bestimmungen wurden anschließend einzelne Teilproben aus dem Archiv entnommen.

Im nächsten Schritt wurden Parameter zur Probencharakterisierung bestimmt (Fett-, Wasser- und Proteingehalte). Zur späteren Normalisierung der Schadstoffbelastungen wurde der Fettgehalt der Nahrungsnetzproben gravimetrisch nach der Smedes-Methode gemessen. Der Wassergehalt wurde durch Wiegen der Proben vor und nach der Gefriertrocknung bestimmt. Bei den beprobten Dreikantmuscheln ist zu beachten, dass diese Atemwasser enthalten, das den Wassergehalt der Weichkörper-Mischproben erhöht. Der Atemwassergehalt muss berücksichtigt werden, wenn Frischgewichtsdaten der Dreikantmuscheln für TMF-Auswertungen oder Vergleiche mit Muscheldaten aus anderen Quellen verwendet werden. Für die Bestimmung der Proteingehalte der Nahrungsnetzproben, die auch für Normalisierungen genutzt werden können, wurde eine intern dokumentierte Methode genutzt.

In den ausgewählten Nahrungsnetzelementen, einschließlich der Feldblindprobe, wurde ein Satz von Referenzchemikalien analysiert, um die mögliche Biomagnifikation und schließlich die trophischen Magnifikationsfaktoren zu bestimmen. Auf diese Weise sollte die Plausibilität der aus den ausgewählten Nahrungsnetzelementen abgeleiteten TMFs belegt werden. Die angewandten Methoden wurden größtenteils bereits für andere Untersuchungen eingesetzt und Beschreibungen in begutachteten Beiträgen in Fachzeitschriften veröffentlicht.

Polybromierte Diphenylether (PBDE), chlorierte organische Schadstoffe, chlorierte Dioxine/ Furane und polychlorierte Biphenyle (PCB) wurden nach Extraktion der Biota-Proben mit geeigneten Lösungsmitteln nach UPB-Standardmethoden analysiert.

Für die Gruppe der Per- und Polyfluoralkylsubstanzen (PFAS) wurde ein Satz von 38 PFAS unter Anwendung einer bereits veröffentlichten Methode analysiert. Teilproben der homogenisierten Nahrungsnetzproben wurden mit Tetrabutylammonium als Ionenpaar-Reagenz und Methyl-*tert.*-butylether als Lösungsmittel extrahiert. Die Analyse erfolgte mittels Ultra-Hochleistungsflüssigkeitschromatographie gekoppelt mit hochauflösender Massenspektrometrie (UHPLC-HRMS) unter Verwendung von isotopenmarkierten internen Standards. Jede Probe wurde zweifach analysiert. Bestimmungsgrenzen (BGs) von 0,5 ng/g Frischgewicht für jede Substanz wurden durch Wiederfindungsversuche auf diesem Konzentrationsniveau validiert. Es wurden

keine PFAS-Gehalte oberhalb der BGs in den prozeduralen und Lösungsmittel-Blindwerten nachgewiesen. Die relativen Standardabweichungen (RSA) von doppelt gemessenen Proben zeigten eine akzeptable Reproduzierbarkeit.

Die ausgewählten Nahrungsnetzproben wurden auch auf die cyclischen flüchtigen Methylsiloxane (cyclic volatile methyl siloxanes, cVMS) Octamethylcyclotetrasiloxan (D4), Decamethylcyclopentasiloxan (D5) und Dodecamethylcyclohexasiloxan (D6) untersucht. Die cVMS wurden mit Acetonitril und n-Hexan extrahiert. Um die Quantifizierung der cVMS zu ermöglichen, wurde den Proben vor der Extraktion Tetrakis-(trimethylsilyloxy)silan als interner Standard zugesetzt. Die Speziation und die quantitativen Messungen wurden mit einer GC-ICP-MS/MS-Kopplungsmethode durchgeführt, die schon veröffentlicht wurde. Zur Methodenvalidierung und Qualitätskontrolle wurden Wiederfindungsversuche mit Fischproben durchgeführt. Blindwerte wurden bei jeder Messreihe mitgemessen und waren unauffällig. Die auf Frischgewicht bezogenen BGs lagen für die Messserien in den Bereichen 6,50 - 22,1 ng/g für D4, 2,90 - 20,8 ng/g für D5 und 12,5 - 117 ng/g für D6.

Der Arzneimittelwirkstoff Diclofenac wurde mit weiteren pharmazeutischen Verbindungen (Canrenon, Carbamazepin und dem Transformationsprodukt 10-Hydroxy-10,11-dihydrocarbamazepin) analysiert. Zusätzlich wurde Koffein als mögliche Indikatorsubstanz für kommunale Abwässer mit untersucht. Für die Analyse dieser Substanzen wurden die Biota-Proben extrahiert und mittels Gelpermeationschromatographie und dispergierender Festphasenextraktion aufgereinigt, bevor die quantitative Messung mit einem UHPLC-HRMS erfolgte. Die Methode wurde gemäß den Anforderungen der Richtlinie SANCO 3029 für Ganzfische validiert, indem eine Blindwertmatrix bei BG- und 10 x BG-Konzentrationen aufgestockt wurde. Die Wiederfindungen und ihre relativen Standardabweichungen lagen innerhalb der festgelegten Kriterien (Wiederfindung: 70 - 110 %, RSA: < 20 %). Für Koffein und Canrenon waren geringfügige Blindwertabzüge notwendig, da keine blindwertfreie Matrix zur Verfügung stand. Die resultierenden BGs lagen für alle Analyten bei 10 ng/g Frischgewicht.

Die Analyse der Pyrethroide folgte einem veröffentlichten Protokoll mit leichten Modifikationen. Die erfassten Substanzen waren Tefluthrin, Transfluthrin, λ-Cyhalothrin, Permethrin, Cypermethrin und Deltamethrin. Für die Analyse wurden die gefriergetrockneten Proben mit einer Mischung aus n-Hexan und Dichlormethan extrahiert. Nach dem Zentrifugieren wurde der Überstand entfernt und die Extraktion zweimal wiederholt. Die Extrakte wurden kombiniert, zur Trockne eingedampft und in Acetonitril rekonstituiert. Die Probe wurde durch Festphasenextraktion aufgereinigt. Das Eluat wurde eingedampft und in n-Hexan rekonstituiert. Die GC-MS/MS-Analyse wurde auf einem Triple-Quadrupol-Massenspektrometer mit negativer chemischer Ionisation und Methan als Reagenzgas durchgeführt. Für die einzelnen Analyte wurden spezifische Quantifizierer- und Qualifizierer-Massenübergänge verwendet. Die Quantifizierung wurde mit einer Sieben-Punkte-Kalibrierung einschließlich interner Standards durchgeführt. Die auf Trockengewicht gezogenen BGs für die Biota-Analyse betrugen 0,033 ng/g für Tefluthrin, Transfluthrin und λ-Cyhalothrin, 8,33 ng/g für Permethrin und Cypermethrin sowie 16,7 ng/g für Deltamethrin.

Die Analyse der chlorierten Paraffine in den Proben des Nahrungsnetzes durch einen Unterauftragnehmer konnte erst Ende 2020 beginnen. Der Bericht wird nach Abschluss der Studie als Entwurf für eine begutachtete Publikation in einer Fachzeitschrift zur Verfügung gestellt. Die Methode entspricht dem Verfahren, das für die Analyse von chlorierten Paraffinen in Proben aus der UPB verwendet wurde (Manuskript bereits bei einer Fachzeitschrift zur Begutachtung eingereicht). Die vorläufigen Daten für die chlorierten Paraffine in den Nahrungsnetzproben wurden während des abschließenden Projektworkshops vorgestellt.

Die Messung von Gesamtquecksilber in den zuvor gefriergetrockneten Nahrungsnetzproben erfolgte mittels Atomabsorptionsspektrometrie unter Verwendung eines „Direct Mercury Analyzer“. Die auf Basis eines bereits veröffentlichten Protokolls durchgeführten Messungen erreichten BGs von 0,145 - 0,245 ng/g Trockengewicht. Die Richtigkeit der Messungen wurde mit zertifizierten Referenzmaterialien überprüft. Die gemessenen Methodenblindwerte waren immer vernachlässigbar gering.

Die Analyse von Methylquecksilber (MeHg) wurde mittels SID-GC/ICP-MS (Stabilisotopen-verdünnung-Gaschromatographie mit Detektion durch Massenspektrometrie mit induktiv gekoppeltem Plasma) durchgeführt. Nach Zugabe eines zertifizierten $^{201}$Hg-angereicherten MeHg-Standards wurden die gefriergetrockneten Biota-Proben durch ein mikrowellenunterstütztes Verfahren mit Tetramethylammoniumhydroxid extrahiert. Nach der Extraktion wurden die Proben mit Natriumtetrapropylborat derivatisiert. Die flüchtigen Quecksilberspezies wurden dann mit n-Hexan extrahiert und mittels SID-GC/ICP-MS, wie an anderer Stelle bereits beschrieben, analysiert. Die erreichten BGs lagen im Bereich von 0,2 - 1,7 ng/g Trockengewicht (abgeleitet von Blindwerten, die zusammen mit jedem Probensatz gemessen wurden). Die Überprüfung der Richtigkeit der Messungen erfolgte mit zertifizierten Referenzmaterialien.

Für die TMF-Auswertungen wurden die Daten der Analysen der Nahrungsnetzproben auf die untersuchten lipophilen organischen Chemikalien sowie auf PFAS, Quecksilber/Methylquecksilber und cVMS verwendet. Aufgrund des unsicheren Wassergehalts der Planktonfraktion (zusammen mit einer unbekannten Menge Wasser eingefroren) und der Muschelproben (enthalten eine unbekannte Menge Atemwasser) wurden alle TMFs für polare Verbindungen auf Basis des Trockengewichts berechnet. Die TMF-Werte für unpolare Verbindungen wurden auf Basis der fettnormierten Konzentrationen berechnet. Für die meisten der untersuchten Vergleichschemikalien konnten TMF-Bestimmungen durchgeführt werden. Für diese Auswertung wurden nur Datensätze berücksichtigt, bei denen maximal zwei der Nahrungsnetzproben Werte unterhalb der BGs aufwiesen (dies betraf hauptsächlich die Planktonfraktion und die Dreikantmuscheln). In diesen Fällen wurde die Konzentration unterhalb der BG durch eine Konzentration von 0,5 * BG ersetzt.

Leider war für einige Verbindungen keine Auswertung möglich, da zu viele Biota-Proben Konzentrationen unterhalb der BG aufwiesen. Dies gilt für die meisten PFAS und den analysierten Satz von Pharmazeutika und Pyrethroiden.

Für die Substanzen, deren Datengrundlage ausreichend war, wurden die aus dem Nahrungsnetz des Templiner Sees abgeleiteten TMFs mit TMFs verglichen, die in veröffentlichten Meta-Studien berichtet wurden. Diese Literaturauswertungen liefern aufgrund der großen Bandbreite der verschiedenen erfassten Ökosysteme allerdings gemittelte TMFs mit sehr großen Spannweiten.

Die TMFs wurden für den gesamten Probensatz des Nahrungsnetzes des Templiner Sees (n = 15), den Probensatz ohne die Planktonfraktion (n = 14) und den Probensatz ohne die Planktonfraktion und die beiden Dreikantmuschelproben (n = 12, nur Fische) abgeleitet. Für die berechneten TMFs werden Mittelwerte und 95 %-Konfidenzintervalle angegeben. Während für den gesamten Satz der Nahrungsnetzproben die meisten Korrelationen signifikant sind (zweiseitiger Test auf Signifikanz des Pearson-Korrelationskoeffizienten), waren insbesondere für die „nur Fisch“-Auswertungen einige Korrelationen nicht signifikant. Die meisten TMFs für den gesamten Satz von Nahrungsnetzelementen liegen in einem recht engen Bereich von etwa 1,5 - 2,5. Diese Werte lagen am unteren Ende der Bereiche der entsprechenden in der Literatur berichteten TMFs. Der höchste TMF-Wert wurde für 4,4’-DDT, ein früher verwendetes Insektizid, bei der „nur Fisch“-Auswertung gefunden (ca. 10). Für die untersuchten cVMS war nur D5 in allen Biota-Proben vorhanden. Interessanterweise wurde für das Nahrungsnetz des Templiner Sees für D5

ein signifikanter TMF < 1 gefunden. In der Literatur werden unterschiedliche TMFs (< 1 / > 1) für D5 berichtet, wobei die Daten für (flache) Seen - wie den Templiner See - tendenziell auch niedrigere TMFs zeigen.

Da bei der erfolgreichen Plausibilitätsprüfung nicht nur TMFs für POPs mit lipophilen Akkumulationseigenschaften bestimmt wurden, kann gefolgert werden, dass die archivierten Proben des „Nahrungsnetzes auf Eis“ aus dem Templiner See genutzt werden können, um das trophische Magnifikationspotenzial weiterer in den Proben vorhandener Substanzen mit weniger untersuchten Bioakkumulationseigenschaften zu charakterisieren.

Am Projektende wurde das „Nahrungsnetz auf Eis“-Projekt von Forschenden des Fraunhofer IME, des Umweltbundesamts und Projektpartnern der wissenschaftlichen Öffentlichkeit im Rahmen eines zweitägigen Online-Workshops ausführlich vorgestellt. Eine eingeladene Keynote-Präsentation zur TMF-Forschung bildete die Grundlage für den wissenschaftlichen Austausch. Beispiele aus anderen Studien zur Untersuchung der trophischen Magnifikation, präsentiert von Forschenden aus Italien und Frankreich, erlaubten eine Einordnung der Ergebnisse. Etwa 75 Forschende aus Behörden, Forschungsinstituten und der Industrie nahmen an dem Workshop am 16./17. März 2021 teil. Die Teilnehmenden kamen aus 12 Staaten, darunter auch aus Kanada und den USA.

In der allgemeinen Diskussion während des Workshops wurden die folgenden Themen behandelt:

- Kriterien für die Auswahl von Basislinienorganismen für die Berechnung von trophischen Positionen;
- Mögliche Verwendung von Daten aus Passivsammler-Probenahmen zur Unterstützung der TMF-Bewertungen (indem die abgeschätzten Wasserkonzentrationen für BAF-Berechnungen für Vergleiche/Plausibilitätsprüfungen verwendet werden können);
- Mögliche trophische Magnifikation von polaren Verbindungen (Biokonzentration durch Kiemenaufnahme im Gegensatz zu Biomagnifikation durch Nahrungsaufnahme);
- Verwendung von TMFs im regulatorischen Kontext: Für die EU-Wasserrahmenrichtlinie werden oft aus Feldstudien abgeleitete BAFs für die Ableitung von Qualitätsstandards verwendet anstelle einer Kombination von BCFs aus Labortests und TMFs;
- Einige Teilnehmende betonten, dass TMFs für höherstufige Bewertungen (beispielsweise zur Unterstützung der Einstufung „bioakkumulierend“) nur für Chemikalien angewandt werden können, die bereits in Gebrauch sind (retrospektive Bewertung).

# 1 Introduction

## 1.1 Project background

The project 'Investigation of the bioaccumulation of chemicals in an exemplary food chain' (project code FKZ 3717 65 416 0, UBA Az: Z 6 - 97 327/13) was funded by the German Environment Agency (UBA) in the period 02.06.2017 - 30.04.2021.

This research was initiated to investigate bioaccumulation and biomagnification of chemicals in the field. In risk assessment of chemicals, the bioaccumulation potential of compounds is assessed as a critical property. Various parameters are determined experimentally in laboratory or field studies or estimated on the basis of experimental data. In particular bioconcentration factors, bioaccumulation factors and biomagnification factors (BCFs, BAFs, BMFs) serve as measures of this potential.

A still relatively new approach is the determination of so-called trophic magnification factors (TMFs), which integrate enrichment processes in a food web. So far, TMFs are mainly available for legacy chemicals and only a few current-use compounds (reviews: Borgå et al. (2012), Conder et al. (2012), Walters et al. (2016)). Moreover, there is a need for the use of TMFs, for example in the context of the Water Framework Directive (EC 2014, Kidd et al. 2019), or for substance evaluation with regard to substances that are potentially persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB). However, there is a lack of sufficient practical experience to standardize the procedure and use derived TMFs for regulatory substance evaluations.

In order to achieve an international exchange of knowledge and experiences, a scientific advisory board was built by inviting renowned international scientists with expertise in bioaccumulation research (see section 11).

The outcome of this project should help to gain experience in the determination of TMFs and their use for regulatory purposes.

## 1.2 Project work packages

The project was structured in several work packages (WP):

- WP I - Literature evaluation of relevant TMF-studies;
- WP II - Coordination of and communication with the scientific advisory board;
- WP III - Selection of the sampling site and organization of the sampling;
- WP IV - Sampling campaign for food web samples;
- WP V - Sample transport, sample preparation and sample storage;
- WP VI - Analysis of stable isotopes ($d^{15}N$, $d^{13}C$) by agroisolab GmbH, Jülich.

In August 2019, the project budget was increased to fund additional analyses of the food web samples gathered by a successful sampling campaign. The analyses were partly sub-contracted to external partners:

- lipid content (method according to Smedes (1999)): conducted by Fraunhofer IME;
- protein content: conducted by Fraunhofer IME;
- PCDD/F and ndl- and dl-PCB: conducted by Eurofins GfA Lab Service GmbH, Hamburg;
- organochlorine pesticides: conducted by Eurofins GfA Lab Service GmbH, Hamburg;

- PBDE: conducted by Eurofins GfA Lab Service GmbH, Hamburg;
- short-, medium- and long-chained chlorinated paraffins: conducted by Stockholm University;
- PFAS: conducted by Fraunhofer IME;
- methyl mercury: conducted by Fraunhofer IME;
- cyclic volatile methyl siloxanes: conducted by Fraunhofer IME;
- diclofenac (and other pharmaceuticals): conducted by Fraunhofer IME;
- pyrethroid pesticides: conducted by Fraunhofer IME.

# 2 Literature evaluation of relevant TMF-studies

As agreed upon with the supervisors at UBA, the literature evaluation focused on organic substances. Metals appear less relevant due to their low biomagnification as many studies confirmed (e.g., evaluated in reviews by Suedel et al. (1994) and Gray (2002)). The only exception known so far are mercury and methyl mercury compounds. For these substances, however, a comprehensive meta-evaluation of biomagnification research is available (Lavoie et al. 2013), so that there was no need for further evaluations.

Focus: Since the TMF study was planned to be implemented in an inland water body, the literature evaluation of biomagnification / trophic magnification focuses on rivers and lakes. Publications covering studies with relatively high pressures on the investigated ecosystems (e.g., e-waste areas in China) were also mainly excluded.

Search for relevant published TMF studies: TMF studies of organic substances in lakes/rivers were searched for in scientific literature databases using suitable keywords (e.g., food web magnification, trophic magnification, TMF, freshwater, lake, river) or combinations of these.

Evaluation of the TMF studies with regard to the following questions: How many and which taxonomic groups were covered, which trophic levels, how much sample material, which temporal integration, which biometric parameters, which indicators for trophic classification, where does the pollution burden on the ecosystem come from, which other aspects are relevant (e.g., necessary parameters for normalization)?

Evaluation of the retrieved TMF studies with regard to the applied statistical methods: Which statistical methods/tools were used? How were concentration data below the analytical limits of determination included, if applicable?

Evaluation of the TMF-studies with regard to the regional selection of areas: Where were TMF studies conducted (geographical regions)? Which water body types (running waters vs. lakes)?

About 60 publications on TMF investigations in inland waters have been evaluated in a structured way and about 1100 TMFs for about 400 chemicals (including isomers/congeners) have been aggregated. Many data refer to PCB and PBDE congeners. The time period covered was 1995–2020. The data were collected in an Excel file.

The data base is provided as a Microsoft Excel file with this final report (digital Attachment 7; file Fraunhofer-IME_TMF-Werte_Literatur_18082020.xlsx).

The compiled TMFs were used for the preparation of the publication ‘Selection and application of trophic magnification factors for priority substances to normalize freshwater fish monitoring data under the European Water Framework Directive: a case study’ (Rüdel et al. 2020). In this work the usage of TMF for the normalization of fish burden to a certain trophic level (here trophic level = 4) was explored. This issue was raised in a guidance document by the EU

Commission (EC 2014) in the context of the Water Framework Directive (WFD) and further discussed in an article by Kidd et al. (2019). The substances covered are WFD priority substances considered for a monitoring in biota (preferably fish). The abstract of the peer-reviewed manuscript is presented in the following textbox.

**Abstract of the article: 'Selection and application of trophic magnification factors for priority substances to normalize freshwater fish monitoring data under the European Water Framework Directive: a case study' (Rüdel et al. 2020); published as open access in Environmental Sciences Europe (https://enveurope.springeropen.com/articles/10.1186/s12302-020-00404-8)**

**Background:** The European Water Framework Directive (WFD) requires the monitoring of biota - preferably fish - to check the compliance of tissue concentrations of priority substances (PS) against substance-specific environmental quality standards (EQSs). In monitoring programs, different fish species are covered, which often are secondary consumers with a trophic level (TL) of about 3. For harmonization, a normalization of monitoring data to a common trophic level is proposed, i.e., TL 4 (predatory fish) in freshwaters, so that data would be sufficiently protective. For normalization, the biomagnification properties of the chemicals can be considered by applying substance-specific trophic magnification factors (TMFs). Alternatively, TL-corrected biomagnification factors ($BMF_{TL}$s) may be applied. Since it is impractical to derive site-specific TMFs or $BMF_{TL}$s, often data from literature will be used for normalization. However, available literature values for TMFs and $BMF_{TL}$s are quite varying. In the present study, the use of literature derived TMFs and $BMF_{TL}$s in data normalization is studied more closely.
**Results:** An extensive literature evaluation was conducted to identify appropriate TMFs for the WFD PS polybrominated diphenyl ethers (PBDE), hexachlorobenzene, perfluorooctane sulfonate (PFOS), dioxins and dioxin-like compounds (PCDD/F + dl-PCB), hexabromocyclododecane, and mercury. The TMFs eventually derived were applied to PS monitoring data sets of fish from different trophic levels (chub, bream, roach, and perch) from two German rivers. For comparison, PFOS and PBDE data were also normalized using literature-retrieved $BMF_{TL}$s.
**Conclusions:** The evaluation illustrates that published TMFs and $BMF_{TL}$s for WFD PS are quite variable and the selection of appropriate values for TL 4 normalization can be challenging. The normalized concentrations partly included large uncertainties when considering the range of selected TMFs, but indicated whether an EQS exceedance at TL 4 can be expected. Normalization of the fish monitoring data revealed that levels of substances accumulating in the food web (TMF or BMF > 1) can be underestimated when relying on fish with TL < 4 for EQS compliance assessment. The evaluation also revealed that TMF specifically derived for freshwater ecosystems in Europe would be advantageous. Field-derived $BMF_{TL}$s seemed to be no appropriate alternative to TMFs, because they can vary even stronger than TMFs.

# 3 Selection of the sampling site and organization of the sampling

An important decision to be taken at the beginning of the experimental part of the project was the selection of a suitable water body for the field sampling. Several water bodies have been considered as study site. Main criteria for the suitability of a water body were:

- accessibility for sampling,
- a certain water body size,
- presence of appropriate plankton, invertebrate and fish species and
- presence of a certain level of pollution (so that relevant chemicals can be detected at all trophic levels of the food web).

These criteria reduced the number of appropriate water bodies in Germany. For example, most lakes considered had only low levels of pollution so that it would be difficult to quantify interesting chemicals especially in low trophic level organisms. Other lakes would not allow the sampling of sufficient amounts of plankton for the planned analyses.

One lake initially considered as appropriate was Lake Großer Wannsee near Berlin, a shallow lake (9 m depth), which is a part of the Havel River. The surface area is about 2.7 $km^2$. It is influenced by sewage treatment plants (STPs) in the upstream area (about 15 km distance) so that a certain level of pollution could be expected. Especially in summer, the lake is also used for water sports and leisure boat traffic, which cause additional emissions. There are several marinas around the lake. Recent monitoring data for WFD priority substances in fish from the Großer Wannsee show a moderate contamination level in comparison to river sites more influenced by STP emissions (Radermacher et al. 2019). It is therefore likely that a range of compounds would be detectable in biota from all trophic levels of interest. Previous studies showed that up to 10 fish species are living in the Großer Wannsee. Zebra mussels are also abundant as well as zooplankton. Phytoplankton was less abundant in recent years due to lower nutrients loads. A further advantage of the site was that a cooperation with local water authorities would be possible.

However, after further discussion with local experts it came out that the lake is not continuously exposed to STP effluents. While in the winter half-year the effluents are discharged into the Havel upstream of Großer Wannsee, in the summer half-year the effluents of the STP Ruhleben are discharged via the Teltow canal downstream of Großer Wannsee. This seasonal change of the pollution level was assessed as drawback (no continuous exposure of biota to pollutants can be expected). There were also concerns that the high leisure traffic on Lake Großer Wannsee during the summer period may interfere with the sampling campaign.

Therefore further lakes in the region, which seem comparable to Lake Großer Wannsee, were considered. In joint discussions between UBA and the project team at Fraunhofer IME and after consultation of members of the international scientific advisory board, it was finally decided that the sampling would be carried out at Lake Templin near Potsdam. This lake, like Lake Großer Wannsee, is also part of the lower Havel River. However, it is larger and only slightly affected by the seasonal change in the discharge of the effluents of the STP Ruhleben which are discharged into the Havel River.

Many aspects found favorable for Lake Großer Wannsee in the previous planning could be transferred to Lake Templin. These points include exposure to pollutants carried by the Havel River, a sufficient occurrence of plankton (substantiated by data from the Brandenburg State

Environmental Agency in recent years; however, phytoplankton is only expected to be available in lower quantities due to decreasing eutrophication) and the occurrence of mussels as part of the food web (here the zebra mussel *Dreissena polymorpha*).

The main focus for the investigations was put on the southern part of Lake Templin, which is less frequented in the shore area (only few buildings). In addition, in this part of the lake also less (leisure) shipping traffic was expected and the accessibility of the shore of the water body was better.

For the plankton sampling either an access for a small boat by means of a car trailer was required or a landing stage with power supply for the installation of a pump and a large net and accessibility for a van to transport the freezing equipment to the landing stage. Both requirements were fulfilled at Lake Templin. A large number of landing stages allowed the flexible choice of sampling points, which were also accessible for larger equipment.

Lake Templin also revealed infrastructural advantages regarding the exposure of passive samplers. Since the Water and Shipping Authority (WSA) Potsdam operates a site at Lake Templin, it was approached to support the sampling campaign. To this end, the cages for the passive sampler exposure were attached to buoys which were removed from the water by means of a crane installed on a vessel operated by the WSA staff. The kind support by WSA Potsdam is gratefully acknowledged.

Another advantage of Lake Templin was the possibility to involve the near-by Institute of Inland Fisheries (Institut für Binnenfischerei, IfB) located at Potsdam-Sacrow as partner. Since the IfB regularly samples and examines Lake Templin, a lot of information was available on the occurring fish and other species, their feeding behavior, population size and optimal sampling locations. Thus, the IfB experts were contracted for the fish sampling campaign as well as for the collection of mussels and the large-scale plankton sampling.

# 4 Sampling campaign for food web samples

## 4.1 Considerations

With regard to the planned sampling, considerations were made as to where, what, how, when and how much organisms and biomass should be sampled.

Food webs in shallow lakes often rely on different energy sources, which are either based on pelagic or on benthic feeding organisms. The pelagic food web is based on carbon stemming from primary producers in the water phase (phytoplankton), and the benthic one on carbon taken up by organisms from the sediment. Additionally, relevant terrestrial detritus inputs (e.g., from leaves or surface runoff) may occur (Cole & Solomon 2012). However, the feeding relations are not completely separated but interconnected. During their life cycle organisms may change between energy sources (e.g., young fish first feeding on pelagic plankton and then switching to benthic insects as adults). Higher trophic level organisms may rely indirectly on benthic energy sources when using benthically feeding prey as food. Generally, a coupling between benthic and pelagic food webs is assumed where fish act as integrators (Vander Zanden & Vadeboncoeur 2002). In a recent study at two Chinese lakes it was shown that benthic-pelagic coupling occurred on multiple trophic levels (Wang et al. 2020). While lower trophic level consumers contributed the largest proportion (> 90%), top consumers were of limited importance. The energy exchange was highly asymmetric: the amount of energy exported from benthic organisms to pelagic ones was four times higher than for the opposite direction (Wang et al. 2020). To which extent the described mechanisms influence the nutrient distribution in Lake Templin biota is not known. However, it can be expected that these effects occur in this comparatively shallow lake, too.

For the sampling of the Lake Templin food web, the following requirements were identified:

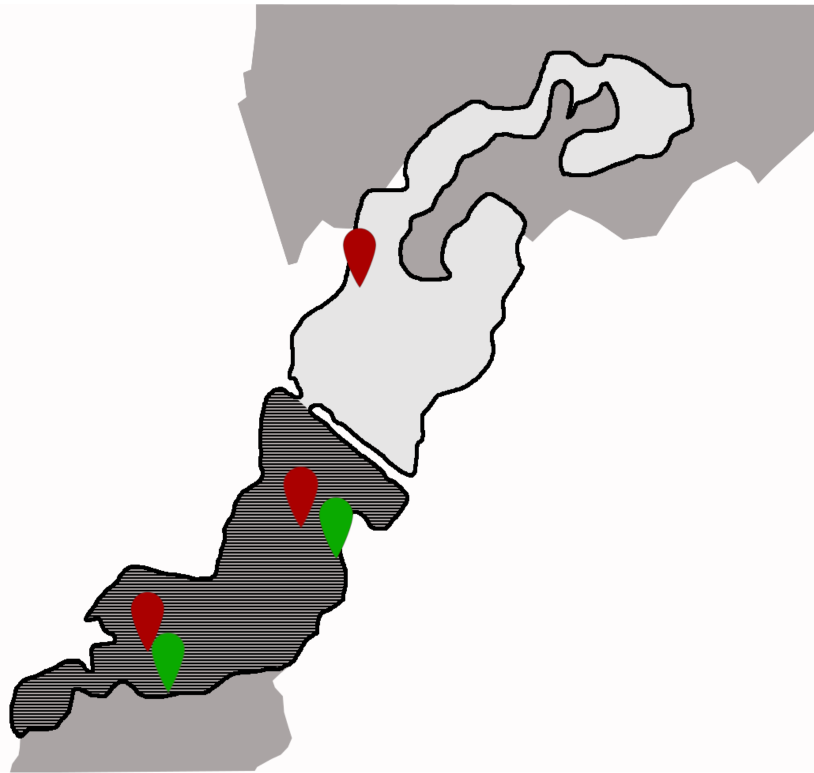
- The selected food web items should be, as far as possible, representative of the entire food web allowing a comprehensive evaluation of a substance's trophic magnification.
- The organisms are sampled as representatives of the respective trophic level since it is not possible to cover all parts of the lake food web.
- The selected organisms should have dietary relationships so that it can be assumed that the substance concentrations determined in organisms of higher trophic levels derive from trophic levels represented in the sample set.
- To fulfil the steady state requirement, no migratory species should be included in the sampling list, as these species may also be influenced from other habitats as well.
- The selected species should not be endangered.

Since pelagic food webs are in general less complex in comparison to benthic ones, it was intended to sample organisms which feed preferably in the water phase. However, due to the expected usage of different carbon sources by organisms, a purely pelagic food chain can probably not be found in Lake Templin as outlined above.

Before the sampling campaign was conducted, information about the species abundant in the target ecosystem Lake Templin had been collated with the help of local experts. Thus, information about specific lifestyles could be obtained which in turn allowed for a better understanding of the dietary relationships.

The different locations for the organisms and the passive samplers covered in the sampling campaign are shown in Figure 1.

**Figure 1: Sampling areas for plankton fractions, mussels, fish and passive samplers at Lake Templin**



Explanation: Red dots mark the locations of the passive samplers, green dots mark the locations of the plankton fraction sampling. In the hatched area, the main focus was on collecting mussels and fish. The light grey area in the north marks the Potsdam city area, the grey area in the south indicates the area of the city of Caputh.

## 4.2 Passive sampler exposure

In cooperation with UFZ Leipzig research center a passive sampling campaign was conducted to examine whether concentrations of potential target substances change over time (Becker et al. 2020, Petrie et al. 2016). End of June 2018 passive samplers were deployed at three different locations in the lake (compare Figure 1). Each passive sampler cage was equipped with three different types of sorbents: Empore disks SDB-RPS (Sigma-Aldrich) with PES-membrane (Pall Supor-450; 0.45 µm/47 mm diameter; Pali Corp., USA), Atlantic HLB-L disks (Horizon Technology, USA) with PES-membrane (Pall Supor-450; 0.45 µm/50 mm diameter; Pall Corp., USA), and 250 µm polydimethylsiloxane (PDMS) sheets (Shielding Solutions Ltd., England). Six replicates of each sorbent material were placed in one passive sampler cage. The Empore and Atlantic disks were exposed in Chemcatcher housings. Exposure periods for the Chemcatcher membranes were one month and for the PDMS sheets about two months (end of June - August; August - October).

The geo-coordinates (UTM; z = 33U, y = northing, x= easting) of the three sites were: site 1, y = 365363.335, x = 5804811.396 (Potsdamer Havel, km 21.893, in the Northern part of the lake); site 2, y = 364913.761, x = 5803171.038 (km 20.186, in the southern part of the lake); site 3, y = 363693.224, x = 5802231.977 (km 18.638, also in the southern part of the lake).

A first non-target analysis of Empore and Atlantic disks, which were exposed parallel to the passive samplers applied here, was conducted by UFZ. The results confirm that a broad spectrum of anthropogenically emitted chemicals (herbicides, other plant protection products, transformation products and other organic micropollutants including biocides, surfactants and industrial chemicals) was present in the water body (Grodtke et al. 2021).

However, the extraction and quantitative analysis of all passive samplers for selected benchmark substances could not be performed during the project period due to limited budget. Only for PCB, an analysis of a set of 15 PDMS sheets was possible (extraction at Fraunhofer IME, see section 5.5; analysis by Eurofins GfA Lab Service GmbH). Additionally, a set of PDMS sheet extracts were provided to the University of Stockholm for the analysis of chlorinated paraffins.

## 4.3 Plankton sampling

Two different techniques for sampling of plankton fractions were tested and applied in the southern part of Lake Templin (see Figure 1).

The first method, applied by LimPlan company (Dr. W. Arp, in cooperation with B. Koppelmeyer from enviteam company), utilized a stacked cascade net that allowed a fractioning of the filtered water. Two water pumps were operated for about 5 hours and transported the lake water to the cascade net (total pumped volume 32 $m^3$). Latter consisted of three nets with mesh sizes of 250, 100, and 40 µm, respectively. The inflowing water passed the nets in the denoted order. The nets were regularly washed with lake water to prevent clogging of the nets. The sampled material, which was suspended in lake water, was stored in cooled brown glass flasks until the samples were frozen in liquid nitrogen (< -130°C) 1 - 6 hours after sampling. From a non-frozen subsample a rough determination of sampled species was performed for each size fraction obtained. One pre-test and two regular samplings at Lake Templin and another sampling at a reference lake (Lake Großer Lienewitzsee, near Caputh) were conducted. These types of plankton fractions were used for temporal comparisons of plankton properties. The plankton sampling and analysis report of LimPlan is provided as digital Attachment 2.

The second method was applied to gain a large plankton fraction for the TMF determinations and utilized an approx. 5 m long coned net with 200 µm mesh size. The net was exposed in the lake near the shore (water depth 3 m) and lake water was pumped through the net (pump Aquahandy, LINN Gerätebau; approx. 50 $m^3$/h pump volume). Sampling was conducted in collaboration with staff of the Institute of Inland Fisheries (IfB; Potsdam-Sacrow) over a period of about 6 hours on September 19, 2018. In intervals of 30 - 60 minutes the net was cleaned from larger debris and the sampled material carefully pressed in the net to remove excess water. The moist plankton fraction was directly transferred into liquid nitrogen and subsequently stored in the vapor phase of liquid nitrogen (< -130°C).

The biota sampling report of IfB is provided as digital Attachment 1.

## 4.4 Mussel sampling

Mussels (*Dreissena polymorpha*) were sampled at four different spots in southern part of Lake Templin (see Figure 1) by IfB staff on September 17 - 18, 2018. Preferably, mussels growing on larger stones, which are directly exposed to the water phase, were sampled. However, since the water level was low due a drought period in the summer of 2018, mussels from this source were not available in large quantities. Therefore, the sample was increased by including *D. polymorpha* from the upper levels of sediment-attached colonies, using a dip net. Zebra mussels are known to feed on algae and bacteria by filtering the ambient water (e.g., as cited in Cole and Solomon (2012)).

The collected mussels were transferred to the near-by IfB facility. There, the mussels were sorted and dead shells were removed from the bulk and the remaining mussels kept in fresh, aerated tap water in glass aquaria for about 48 hours to clear their guts. Then, a portion of the mussels was removed from the tanks, put on ice, and dissected. The soft tissue was removed and frozen in liquid nitrogen (< -130°C). The removed shells were stored and used for age determinations.

The biota sampling report of IfB is provided as digital Attachment 1.

The major fraction of the gut-cleared mussels was removed from the water, frozen in liquid nitrogen (including shells and respiration water), transported to the Fraunhofer IME site and stored under cryogenic conditions in the ESB archive (< -130°C). Soft tissues from these mussels were retrieved as well, but samples were roughly divided into a group of mussels with a size of < 2 cm and a group with a size of > 2 cm. Finally, the frozen mussels were allowed to thaw to such a degree as that the soft tissue could be separated from the shells and again being transferred into liquid nitrogen (Teubner et al. 2018).

Zebra mussels from Lake Templin were also used as the baseline species for the trophic level determination of biota samples with stable isotopes (see section 9.1).

## 4.5 Fish sampling

Different fish species (see Table 1) were sampled in the period September 17 - 18, 2018. Two different fishing methods were applied, electrofishing (FEG 5000, electrode diameter approx. 45 cm, EFKO), and an overnight deployment of gillnets with different mesh sizes. Fish were sorted by size and species, stored on ice and transported to the ESB laboratory. The biota sampling report of IfB is provided as digital Attachment 1.

At the ESB, the biometric data of each fish was recorded (size, weight, sex). Fish smaller than 20 cm were treated as one composite sample per species and transferred into clean stainless-steel containers filled with liquid nitrogen. Fish larger than 20 cm were dissected under a clean bench within 72 h after sampling.

From each individual fish, one skinless fillet, the gastrointestinal tract (GIT), and approx. 20 scales were removed. Scales were carefully cleaned with distilled water, slowly dried overnight on paper towels and stored in paper bags at room temperature. An age determination by examination of the scales was performed by IfB staff. Fillet and GIT were weighed and transferred into separate containers filled with liquid nitrogen. In some cases the fish stomach content was analyzed for remainders of food items to gain additional information on the trophic level of the respective fish. The remaining carcass was cut into smaller pieces (approx. 6 cm per dimension) and also transferred into a marked stainless-steel container with liquid nitrogen. For the final sample set, carcass and fillet samples were re-combined in the documented ratio to yield a composite sample similar to the original whole fish.

Some fish samples (small asp, small and large rudd, additional perch) were not considered for inclusion in the food web set due to unclear feeding behavior and/or stable isotope signatures not fitting to the other fish species (see section 9.1).

**Table 1: Final set of sampled fish species**

| Sample / Species | Sample details | Feeding characterization# |
|---|---|---|
| White bream *(Blicca bjoerkna)* | Individuals > 20 cm (fillet & carcass & GIT) | benthopelagic / phyto- and zooplankton, makrozoobenthos |
| Roach *(Rutilus rutilus)* | Composite sample of small individuals | pelagic / phyto- and zooplankton |
| Roach *(Rutilus rutilus)* | Individuals > 20 cm (fillet & carcass & GIT) | benthopelagic / zooplankton, detritus, makrozoobenthos |
| Bleak *(Alburnus alburnus)* | Composite sample of small individuals | pelagic / phyto- and zooplankton |
| Perch *(Perca fluviatilis)* | Composite sample of small individuals | pelagic / phyto- and zooplankton, small fish |
| Perch *(Perca fluviatilis)* | Individuals > 20 cm (fillet & carcass & GIT) | pelagic / piscivorous |
| Pike *(Esox lucius)* | Individuals > 20 cm (fillet & carcass & GIT) | pelagic / piscivorous, amphibians |
| Asp *(Aspius aspius)* | Composite sample of small individuals | pelagic / piscivorous |
| Pikeperch *(Sander lucioperca)* | Individuals > 20 cm (fillet & carcass & GIT) | pelagic / piscivorous |

# Data from fishbase.org (Froese & Pauly 2019).

## 4.6 Field blank

Commercially available frozen fillet of Alaska pollock (*Gadus chalcogrammus*), obtained from a local supermarket, was used as field blank for fish. The fillet was removed from the packaging and exposed openly in the room where the fish were dissected. After several hours the fish was frozen again and further treated as the Lake Templin fish samples.

# 5 Sample preparation and sample storage

## 5.1 Cryo-milling

The prepared zebra mussel composite samples were cryo-milled and homogenized following established ESB protocols (Rüdel et al. 2008).

Larger individual fish (fillet and carcass separately) and the prepared composite samples of small fish were manually crushed, cryo-milled and homogenized in the same manner (Fliedner et al. 2018, Rüdel et al. 2008).

## 5.2 Freeze-drying

Due to the high water content, the major part of the plankton fraction was freeze-dried (Alpha 1-2LDplus, Christ, Osterode, Germany). The process was run over several days until the weight between weightings on two successive days was constant.

However, for the analysis of cVMS freeze-drying was inappropriate as sample pre-treatment due to the high vapor pressure of the target compounds.

In some cases, also other samples were freeze-dried to allow a higher amount of sample material for extractions (e.g., zebra mussel).

## 5.3 Subsample preparation

The processing of the samples was completed in the year 2019. After cryo-milling of food web samples and a first rough check of individual samples for stable isotope signature, fillet and remaining fish samples were re-combined proportionally to obtain whole fish samples again. However, fractions of fillet and remaining fish were kept separately for possible additional analyses.

Subsequently, each whole fish was re-examined for the stable isotope signature to characterize the sampled food web. After an examination of which species could be considered as part of the final food web set, about 50 - 80 subsamples of each of these samples were prepared following an ESB protocol (Rüdel et al. 2008).

## 5.4 Sample storage

Samples were stored in the gas phase above liquid nitrogen at temperatures < -130°C in the cryo-archive of the German ESB (Rüdel & Weingärtner 2008). More than 1200 subsamples were finally stored under cryogenic conditions. Individual subsamples were taken from the archive for the analyses of stable isotopes, parameters such as lipid and protein content, and selected chemicals afterwards.

## 5.5 Extraction of PDMS sheets

The PDMS passive samplers were carefully cleaned to remove biofilm residues, air-dried in a clean bench for several hours and extracted using a Soxhlet device. Three PDMS sheets from one sampling site/date were extracted with 100 mL of acetone for a period of 24 - 30 hours. Before extraction, an internal standard was spiked into the solvent (100 µL of a solution of 100 ng/mL 4,4'-DDD, ring-$D_8$ = 10.0 ng). After extraction, 100 µL of a γ-HCH standard ($^{13}C_6$, 100 ng/mL) were added (= 10.0 ng).

# 6 Stable isotope analyses and calculation of trophic positions

## 6.1 Stable isotope analyses of bulk food web samples

Nitrogen and carbon stable isotope determinations ($^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios) of Lake Templin food web samples were performed by agroisolab GmbH (Jülich, Germany) after extraction of lipids. Details of the protocol are described elsewhere (Radermacher et al. 2019). The compiled report files are provided as digital Attachment 3.

Data are expressed as ‰ $\delta^{15}N$ and ‰ $\delta^{13}C$ and evaluated for fish trophic positions (TP) as described by Post (2002) and Fliedner et al. (2018). Dreissenid mussels were applied as baseline organisms with an assigned TP of 2.0 (Post 2002)). The following equation was applied for the TP calculation (Δ - trophic enrichment increment):

$$TP_{secondary\ consumer} = TP_{baseline\ organism} + ((\delta^{15}N_{secondary\ consumer} - \delta^{15}N_{baseline\ organism}) / \Delta)$$

First, a $\delta^{15}N$ trophic enrichment increment Δ of 3.4 ‰ for a TP difference of 1 was applied (Post 2002). However, a better agreement of fish TP with fish reference data was gained when applying a Δ of 2.3 ‰ (McCutchan et al. 2003).

## 6.2 Mixing model for considering two carbon sources

To consider a feeding of organisms on two (e.g., pelagic and benthic) carbon sources, the mixing model proposed by Post (2002) was applied exemplarily. The value α characterizes the benthic fraction in the diet of a consumer. Assuming that the transfer of nitrogen and carbon through the food web is similar, α can be estimated using the $\delta^{13}C$ signature (Post 2002):

$$\alpha = (\delta^{13}C_{secondary_consumer} - \delta^{13}C_{baseline_organism_pelagic}) / (\delta^{15}N_{baseline_organism_benthic} - \delta^{13}C_{baseline_organism_pelagic}).$$

For applying the mixing model, zebra mussels were assumed to rely on benthic energy sources (lowest $\delta^{13}C$ value of the consumer organisms; however, the low $\delta^{13}C$ values may also be caused by terrestrial sources). The $\delta^{13}C$ signature of the 40 - 100 µm plankton fraction sampled in July (highest $\delta^{13}C$ level of all food web items sampled) was assumed as 100 % pelagic (α = 0). The α values of all food web items were then between 0 and 1.

The trophic positions are the calculated as:

$$TP_{secondary_consumer} = TP_{baseline_organism} + ((\delta^{15}N_{secondary_consumer} - [\delta^{15}N_{baseline_organism_benthic} * \alpha + \delta^{15}N_{baseline_organism_pelagic} * (1 - \alpha]) / \Delta).$$

## 6.3 Amino-acid-specific stable isotope analyses of food web samples

In recent years it was suggested that amino-acid-specific stable isotope analyses of food web samples could allow a better determination of the trophic position of organisms. In principle, the determination of the trophic position of an organism is performed by using one amino acid which is essential (phenylalanine, short PHE; cannot be synthesized by most organisms) and one which is non-essential (glutamic acid, short GLU; is synthesized by organisms). PHE represents the source $\delta^{15}N$ signature while GLU represents $\delta^{15}N$ of the actual trophic position. Thus, the $\delta^{15}N$ of phenylalanine serves as basis for the TP calculation using the equation:

$$TP = ((\delta^{15}N_{GLU} - \delta^{15}N_{PHE}) - \beta) / \Delta_{AA} + 1$$

As in previous studies, for the TP calculation based on amino acids a $\delta^{15}N$ trophic enrichment increment for amino acids $\Delta_{AA}$ of 7.6 ‰ for a TP difference of 1 was applied. The difference β between glutamic acid $\delta^{15}N$ and phenylalanine $\delta^{15}N$ in primary consumers was assumed to be 3.4 ‰.

Isodetect GmbH (Leipzig, Germany) was contracted to determine amino-acid-specific stable isotope ratios of Lake Templin food web samples ($\delta^{15}N$ ratios for all and $\delta^{13}C$ ratios for a selection of samples) The extraction of the amino acids was performed by an in-house procedure following protocols described in the literature. A gas chromatograph with coupled mass spectrometer was used to identify and quantify the amino acids. The amino acids were identified by means of the recorded mass spectra and by comparing the retention times with an amino acid standard mixture. To determine the amino acid concentrations, two internal standards were added to each sample at the beginning of the extraction. The amino-acid-specific stable isotope data are provided as digital Attachment 4 (report files of Isodetect GmbH).

Finally, the amino-acid-specific stable isotope ratios could not be evaluated since the calculated trophic positions of the fish samples were not consistent (large deviations from the TPs derived on basis of the bulk stable isotope data; see section 6.1).

# 7 Determination of target chemicals in food web items

## 7.1 General

In the selected food web items, including one field blank sample, a set of chemicals was analyzed in order to determine the possible biomagnification and finally the trophic magnification factors. By this means, the plausibility of the TMF derived from the selected food web items should be demonstrated. Furthermore, parameters for sample characterization have been determined (lipid, water and protein contents). The applied methods had mostly already been used for other investigations and descriptions were published in peer-reviewed journals. Thus, here only brief descriptions are given and references for more detailed protocols are provided.

The data generated by Fraunhofer IME are provided as Microsoft Excel files in a zip archive file as digital Attachment 8.

## 7.2 Lipid, water and protein contents

The lipid content of the food web items was determined gravimetrically by applying the method of Smedes (1999).

The water content was determined by weighing of samples before and after freeze-drying. For the sampled zebra mussels, it has to be considered that these contained respiration water, which increases the water content of the tissue homogenate. An evaluation of zebra mussel data of the German ESB revealed that the average respiration water fraction is 27.6 % (n = 57). There was no difference in respiration water content observed between smaller and larger mussels. The respiration water content has to be considered when applying the wet weight data of the zebra mussels for TMF evaluations or comparisons with mussel data from other sources.

For the protein determinations, an internally documented protocol was applied following a protein isolation with 20 mL Tissue T-PER Tissue Protein Extraction Reagent (Thermo Scientific) from 1 g of sample.

## 7.3 PFAS[1]

For the group of per- and polyfluoroalkyl substances (PFAS), a set of 38 PFAS was analyzed. The analytical method has been described previously by Kotthoff et al. (2020). In brief, 0.5 g aliquots of homogenized samples were extracted using tetrabutylammonium as an ion pair reagent and methyl-*tert.*-butyl ether as solvent. For freeze-dried plankton, the samples weight was reduced to 0.25 g. Extracts from fish samples were cleaned up with an additional freezing step. Analysis was performed using ultra-high performance liquid chromatography coupled to a high-resolution mass spectrometer (UHPLC-HRMS). Each sample was analyzed in duplicate. The analyte spectrum including the corresponding internal standard (IS) used for quantification is presented in .

**Table 2: List of PFAS analyzed in the samples including their CAS number and the corresponding internal standard (IS)**

| Analyte | Acronym | CAS no. | Selected IS |
|---|---|---|---|
| Perfluoropropanoic acid | PFPrA | 422-64-0 | $^{13}C_4$-PFBA |
| Perfluorobutanoic acid | PFBA | 375-22-4 | $^{13}C_4$-PFBA |
| Perfluoropentanoic acid | PFPA | 2706-90-3 | $^{13}C_5$-PFPeA |

[1] This section was contributed by Dr. Bernd Göckener (Fraunhofer IME) who supervised these analyses.

| Analyte | Acronym | CAS no. | Selected IS |
|---|---|---|---|
| Perfluorohexanoic acid | PFHxA | 307-24-4 | $^{13}C_2$-PFHxA |
| Perfluoroheptanoic acid | PFHpA | 375-85-9 | $^{13}C_4$-PFHpA |
| Perfluorooctanoic acid | PFOA | 335-67-1 | $^{13}C_4$-PFOA |
| Perfluorononanoic acid | PFNA | 375-95-1 | $^{13}C_5$-PFNA |
| Perfluorodecanoic acid | PFDA | 335-76-2 | $^{13}C_2$-PFDA |
| Perfluoroundecanoic acid | PFUnA | 2058-94-8 | $^{13}C_2$-PFUnA |
| Perfluorododecanoic acid | PFDoA | 307-55-1 | $^{13}C_2$-PFDoA |
| Perfluorotridecanoic acid | PFTrDA | 72629-94-8 | $^{13}C_2$-PFDoA |
| Perfluorotetradecanoic acid | PFTeDA | 376-06-7 | $^{13}C_2$-PFDoA |
| Perfluorohexadecanoic acid | PFHxDA | 67905-19-5 | $^{13}C_2$-PFDoA |
| Perfluorooctadecanoic acid | PFODA | 16517-11-6 | $^{13}C_2$-PFDoA |
| Perfluorobutane sulfonic acid | PFBS | 375-73-5 | $^{13}C_3$-PFBS |
| Perfluorohexane sulfonic acid | PFHxS | 355-46-4 | $^{18}O_2$-PFHxS |
| Perfluoroheptane sulfonic acid | PFHpS | 375-92-8 | $^{13}C_4$-MPFOS |
| Linear perfluorooctane sulfonic acid | PFOS-linear | 4021-47-0 | $^{13}C_4$-MPFOS |
| Branched perfluorooctane sulfonic acid | PFOS-branched | n.a. | $^{13}C_4$-MPFOS |
| Perfluorodecane sulfonic acid | PFDS | 333-77-3 | $^{13}C_4$-MPFOS |
| 4:2 Fluorotelomer sulfonic acid | 4:2-FtS | 757124-72-4 | $^{13}C_2$-6:2 FtS |
| 6:2 Fluorotelomer sulfonic acid | 6:2-FtS | 27619-97-2 | $^{13}C_2$-6:2 FtS |
| 8:2 Fluorotelomer sulfonic acid | 8:2-FtS | 27619-96-1 | $^{13}C_2$-6:2 FtS |
| 6:2 Fluorotelomer phosphate diester | 6:2-diPAP | 57677-95-9 | $^{13}C_4$-6:2-diPAP |
| 8:2 Fluorotelomer phosphate diester | 8:2-diPAP | 678-41-1 | $^{13}C_4$-8:2-diPAP |
| 6:2/8:2 Fluorotelomer phosphate diester | 6:2/8:2-diPAP | 943913-15-3 | $^{13}C_4$-8:2-diPAP |
| Perfluorooctane sulfonamide | FOSA | 754-91-6 | d3-MeFOSA |
| N-Methyl perfluorooctane sulfonamide | MeFOSA | 31506-32-8 | d3-MeFOSA |
| N-Ethyl perfluorooctane sulfonamide | EtFOSA | 4151-50-2 | d3-MeFOSA |
| Perfluorooctane sulfonamidoacetic acid | FOSAA | 2806-24-8 | d3-MeFOSAA |
| N-Methyl perfluorooctane sulfonamidoacetic acid | MeFOSAA | 2355-31-9 | d3-MeFOSAA |
| N-Ethyl perfluorooctane sulfonamidoacetic acid | EtFOSAA | 4151-50-2 | d3-MeFOSAA |
| Heptafluoropropoxy propanoic acid (GenX) | HFPO-DA | 13252-13-6 | $^{13}C_3$-HFPO-DA |
| Perfluoroethylcyclohexane sulfonic acid | PFECHS | 67584-42-3 | $^{13}C_4$-MPFOS |
| 9Cl-Perfluoro-3-oxononane sulfonic acid | 9Cl-PF3ONS | 756426-58-1 | $^{13}C_4$-MPFOS |
| 11Cl-Perfluoro-3-oxoundecane sulfonic acid | 11Cl-PF3OUdS | 763051-92-9 | $^{13}C_4$-MPFOS |
| 7H-Perfluoroheptanoic acid | 7H-PFHpA | 1546-95-8 | $^{13}C_4$-PFHpA |
| Ammonium perfluoro-4,8-dioxa-3H-nonanoic acid | ADONA | 919005-14-4 | $^{13}C_4$-PFOA |

n.a. - not available.

Limits of quantification (LOQ) of 0.5 ng/g ww for each substance were validated by recovery experiments at this concentration level. Limits of detection (LOD) were not derived in this procedure. For each day of sample preparation, two procedural blanks were prepared with the samples to detect cross-contaminations alongside with blank solvent injections. No PFAS levels above the LOQs were detected in the procedural and solvent blanks. Relative standard deviations (RSDs) between duplicate sample measurements showed a suitable reproducibility: with an exception of one value in one sample (PFDoA, RSD: 24%) all RSD values were less than 20%

(overall mean: 4.9%). For fish and mussel tissue, the method was already used to participate successfully in the recent Quasimeme Laboratory Performance Studies (www.quasimeme.org).

## 7.4 PBDE, chlorinated legacy compounds, chlorinated dioxins/furans and dl-PCB and ndl-PCB

PBDE, chlorinated legacy compounds, chlorinated dioxins/furans and dl-PCB and ndl-PCB were analyzed by Eurofins GfA Lab Service GmbH (Hamburg, Germany). Descriptions of the methods are given elsewhere (Fliedner et al. 2016, Radermacher et al. 2019).

The data are provided as digital Attachment 5 (report file; Eurofins GfA Lab Service GmbH).

## 7.5 cVMS[2]

The selected food web items were analyzed for the cyclic volatile methyl siloxanes (cVMS) octamethylcyclotetrasiloxane (D4, CAS no. 556-67-2), decamethylcyclopentasiloxane (D5, CAS no. 541-02-6) and dodecamethylcyclohexasiloxane (D6, CAS no. 540-97-6). The cVMS were extracted with acetonitrile and n-hexane. For this purpose, the samples were first subjected to a solid/liquid extraction with acetonitrile and n-hexane. For quantification, tetrakis-(trimethyl-silyloxy)silane (M4Q, CAS no. 3555-47-3, NEOCHEMA GmbH, Bodenheim, Germany) was added to the samples as internal standard before the extraction procedure. Speciation and measurements were performed with a GC-ICP-MS/MS coupling method. A mixed stock solution of commercially available D4, D5 and D6 single compound standards (NEOCHEMA GmbH, Bodenheim, Germany) was prepared and further diluted to appropriate calibration standards. Details of the extraction and analytical method are reported in Radermacher et al. (2020).

Limits of detection (LOD) were calculated according to the blank value method according to standard DIN 32645 (DIN 2008). Further, possible background concentrations were taken into account for determining the LODs. LODs ranged between 2.17 ng/g wet weight (ww) and 7.36 ng/g ww for D4, between 0.966 ng/g ww and 6.95 ng/g ww for D5 and between 4.16 ng/g ww and 38.9 ng/g ww for D6. The corresponding limits of quantification (LOQ) were calculated with LOQ = 3 × LOD. Accordingly, the LOQ are in the ranges 6.50 - 22.1 ng/g ww for D4, 2.90 - 20.8 ng/g ww for D5 and 12.5 - 117 ng/g ww for D6.

To identify possible contamination during extraction and analysis or by chemicals and equipment used, method blanks, which were treated like the samples but without weighing sample material, were included in each measurement series. For D5 and D6, all method blanks (n = 26) were below the respective LOD. For D4, 10 method blanks were below the respective LOD. In one measurement series of two different extractions, five method blanks were below LOQ and eleven method blanks above the LOQ (6.50 ng/g ww) in a range of 7.20 - 11.9 ng/g ww (calculated based on the mean weighing of fish tissue in the samples). Furthermore, analytical blanks were measured along each measurement series in order to identify possible contaminations of the GC-ICP-MS/MS system. For D4 as well as for D5 and D6, all analytical blanks were below the respective LOD.

For method validation and quality control, fortification experiments were performed and various quality control and recalibration standards were measured. Since no certified reference material for cVMS is available, one batch of bream muscle tissue and one batch of bream carcass tissue was used as laboratory internal reference material and was analyzed along the measurement series.

[2] This section was contributed by Georg Radermacher (Fraunhofer IME) who supervised these analyses.

Fortification experiments (n = 24) showed average recoveries ± standard deviation (SD) of 97.8 ± 16.0 % for D4, 98.8 ± 10.0 % for and 88.8 ± 14.7 % for D6. The spikes ranged from 164 ng/g to 250 ng/g for all three investigated cVMS.

The commercially available mixture of siloxanes including D4, D5 and D6 (NEOCHEMA GmbH) and a recalibration standard were analyzed multiple times along each measurement series at different concentrations as quality control standards. The mean recoveries are compiled in Table 3.

**Table 3: Mean recoveries ± standard deviation (SD) of quality control and recalibration standards**

| Quality control standard / Concentration | Mean recovery in % ± SD | | |
|---|---|---|---|
| | D4 | D5 | D6 |
| Siloxane mixture / 50 µg/L (n = 13) | 91.5 ± 1.4 | 85.7 ± 1.5 | 90.0 ± 5.4 |
| Siloxane mixture / 200 µg/L (n = 19) | 94.6 ± 3.1 | 97.1 ± 2.3 | 96.1 ± 3.1 |
| Recalibration standard / 300 µg/L (n = 13) | 101 ± 5 | 92.7 ± 2.0 | 94.3 ± 3.8 |
| Recalibration standard / 400 µg/L (n = 19) | 98.3 ± 2.0 | 97.9 ± 2.2 | 98.2 ± 2.6 |
| Siloxane mixture / 600 µg/L (n = 18) | 95.8 ± 1.6 | 99.4 ± 1.9 | 98.8 ± 2.4 |
| Siloxane mixture / 750 µg/L (n = 18) | 97.9 ± 1.0 | 96.6 ± 1.3 | 99.1 ± 4.4 |

For the bream muscle tissue laboratory internal reference material, the concentrations of D4 and D6 were mostly < LOQ or < LOD. Hence, only the recovery for D5 was determined and ranged between 79.7 % and 100 %, the average recovery was 91.5 ± 8.1 % (n = 8). For the bream carcass tissue laboratory internal reference material, the concentrations of D6 were mostly < LOQ or < LOD. The recoveries for D4 and D5 ranged from 105 to 152 % and from 67.5 to 143 %, respectively. The average recoveries were 123 ± 14 % for D4 and 96.5 ± 28.4 % for D5 (both n = 8).

## 7.6 Diclofenac and other pharmaceuticals[3]

The analysis of diclofenac was amended by the analysis of other common pharmaceuticals and caffeine as an indicator substance for communal wastewater. Table 4 gives an overview of substances that were analyzed in parallel with diclofenac including their CAS number and the corresponding internal standard used for quantification.

**Table 4: List of pharmaceuticals and caffeine analyzed including their CAS number and internal standards**

| Analyte | CAS no. | Selected IS |
|---|---|---|
| Diclofenac | 15307-86-5 | $D_4$-diclofenac |
| Caffeine | 58-08-2 | $^{13}C$-caffeine |
| Canrenone | 976-71-6 | $^{13}C$-caffeine |
| Carbamazepine | 298-46-4 | $D_{10}$-carbamazepine |
| 10-Hydroxy-10,11-dihydrocarbamazepine | 29331-92-8 | $^{13}C$-10-Hydroxy-10,11-dihydrocarbamazepine |
| Triamcinolone acetonide | 76-25-5 | $^{13}C$-10-Hydroxy-10,11-dihydrocarbamazepine |

For analysis of these substances, samples were extracted and cleaned up with gel permeation chromatography (GPC) and dispersed solid phase extraction (dSPE) before measurement on a UHPLC-HRMS system.

[3] This section was contributed by Dr. Bernd Göckener (Fraunhofer IME) who supervised these analyses.

0.5 g of sample were weighed into 15 mL centrifuge tubes and 50 µL of an internal standard solution in methanol (1 mg/L of each target compound) were added. After addition of 5 mL acetonitrile, samples were treated in an ultrasonic bath for 15 min and subsequently shaken on a horizontal shaker for 15 min. Samples were centrifuged for 5 min at 4700 rpm and the supernatant was transferred into another 15 mL centrifuge tube. The extraction was repeated twice. The combined extracts were evaporated to dryness in a stream of nitrogen and were resolved in 1 mL dichloromethane. After treatment in an ultrasonic bath for 5 min, 1.5 mL ethyl acetate were added and tubes were shaken by hand. The samples were again centrifuged (4700 rpm, 5 min) and the supernatant was transferred to a GPC vial. Size-exclusion chromatography was performed on a Gilson (Middleton, Wisconsin) GPC system (Liquid handler GX271, Pump 307, Syringe-Pump 402, Interface-Module 508) with a packed column (45 x 1.5 cm) filled with BioBeads S-X3 (Bio-Rad Laboratories, Hercules, California). The injection volume was 2 mL at an isocratic flow rate of 1.5 mL ethyl acetate/cyclohexane (50/50, V/V) per minute. The total run time was 60 min. The extracts were collected within a window from 25 – 50 min for further analysis. The collected GPC extracts were evaporated to dryness in a stream of nitrogen and the residue was resolved in 2 mL methanol in an ultrasonic bath for 5 min. 1 mL was transferred to a 2 mL dSPE tube (150 mg magnesium sulfate, 50 mg Z-Sep) and shortly shaken by hand. After centrifugation at 4700 rpm for 5 min, 750 µL of the supernatant were transferred to a 1.5 mL autosampler vial and were evaporated to dryness again. The residue was resolved in 750 µL of methanol/water (50/50, V/V) and analyzed via UHPLC-HRMS.

Analysis was performed on a UPLC Acquity System (Waters Corporation, Milford, Massachusetts) coupled to a Q Exactive Plus high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts). Water with 0.1% formic acid and 2 mM ammonium acetate was used as mobile phase (MP) A and methanol with 0.1% formic acid and 2 mM ammonium acetate was used as MP B. Chromatographic separation was performed on a Phenomenex Kinetex Biphenyl (100 x 2.1 mm, 1.7 µm) column heated to 40 °C. The injection volume was 20 µL. The following gradient was applied with a constant flow rate of 0.3 mL/min: initial conditions of 5% MP B were held constant for 1 min followed by a linear increase to 100% MP B from 1 – 20 min . After 24 min, initial conditions were reset within 1 min and were kept constant for another 5 min. The HRMS system was operated in positive electrospray ionization (ESI+) mode and the following source parameters were applied: sheath gas flow rate: 35; aux gas flow rate: 10; sweep gas flow rate: 2; spray voltage: 3.5 kV; capillary temperature: 320 °C; aux gas heater temperature: 350 °C; S-lens RF level: 55.0. Data were acquired in Fullscan mode with a scan range from m/z 50 – 600 at a resolution of 70,000. The automatic gain control target was set to 1e6 with a maximum injection time of 200 ms.

For quantification, the corresponding $[M+H^+]^+$ ions of all substances and internal standards were used with a mass accuracy of 5 ppm. A linear nine-point calibration curve ranging from 5.0 – 500 µg/L per analyte and 100 µg/L per internal standard, respectively, was used. Two quality control samples at levels of 30 and 300 µg/L, respectively, were used for ongoing verification of the calibration. Both, procedural and solvents blanks showed no quantifiable amounts of background contamination. The method was validated for whole fish according to the requirements of guideline SANCO 3029 (European Commission 2000) by spiking a blank matrix at LOQ and 10 x LOQ level (five replicates per level). All recoveries and their relative standard deviations were within the set criteria (recovery: 70 - 110 %, RSD: < 20 %) and are presented in Table 5 for each analyte. For caffeine and canrenone, minor blank subtractions were necessary as no background free matrix was available. The resulting LOQ was 10 ng/g ww for each analyte. LODs were estimated to be one third of the LOQ, i.e. 3.33 ng/g.

**Table 5: Validation results for the analysis of diclofenac, other pharmaceuticals, and caffeine (for each level, a fivefold determination was performed)**

| Analyte | Mean recovery LOQ level | RSD LOQ level | Mean recovery 10xLOQ level | RSD 10xLOQ level |
|---|---|---|---|---|
| Diclofenac | ± 81 % | ± 4 % | ± 104 % | ± 3 % |
| Caffeine* | ± 96 % | ± 6 % | ± 105 % | ± 1 % |
| Canrenone* | ± 99 % | ± 10 % | ± 96 % | ± 1 % |
| Carbamazepine | ± 105 % | ± 2 % | ± 105 % | ± 1 % |
| 10-Hydroxy-10,11-dihydrocarbamazepine | ± 103 % | ± 4 % | ± 103 % | ± 1 % |
| Triamcinolone acetonide | ± 76 % | ± 4 % | ± 82 % | ± 2 % |

* results after background subtraction

## 7.7 Pyrethroids[4]

The analysis of pyrethroids was conducted as described by Corcellas et al. (2015) after slight modifications. Table 6 gives an overview of all analyzed substances including their CAS numbers and the corresponding internal standard used for quantification.

**Table 6: List of analyzed pyrethroids including their CAS number and internal standards**

| Analyte | CAS No. | IS |
|---|---|---|
| Tefluthrin | 79538-32-2 | $d_6$-*trans*-permethrin |
| Transfluthrin | 118712-89-3 | $d_6$-*trans*-permethrin |
| λ-Cyhalothrin | 91465-08-6 | $d_6$-*trans*-permethrin |
| Permethrin | 52645-53-1 | $d_6$-*trans*-permethrin |
| Cypermethrin | 52315-07-8 | $d_6$-*trans*-cypermethrin |
| Deltamethrin | 52918-63-5 | $d_6$-*trans*-cypermethrin |

For analysis, approximately 300 mg of freeze-dried sample was spiked with 20 µL of a solution of internal standards ($d_6$-*trans*-permethrin and $d_6$-*trans*-cypermethrin; 500 µg/L each) and 20 mL of a mixture of n-hexane and dichloromethane (2:1, volume:volume) was added. The samples were shaken for 5 min on a horizontal shaker and treated in an ultrasonic bath for 15 min. After centrifugation at 3500 rpm for 5 min, the supernatant was removed and the extraction process was repeated twice. The three extracts were combined, evaporated to dryness and reconstituted in 1 mL of acetonitrile by using an ultrasonic bath.

The sample was then cleaned up with a tandem solid phase extraction (SPE). Therefore, an Isolute C18 column (2 g/15 mL) and an Isolute AL-B column (5 g/25 mL) were combined and equilibrated with 25 mL of acetonitrile. After the sample was run through the cartridge, 30 mL of acetonitrile were used to elute the analytes from the cartridges. The eluate was then evaporated to a volume of 200 – 300 µL and was transferred to a micro-vial where the evaporation was continued until dryness was achieved. Afterwards the sample was reconstituted in 100 µL of hexane and analyzed by GC-MS/MS.

GC-MS/MS analysis was performed on an Agilent 8890 GC coupled to a 7000D triple quadrupole mass spectrometer. An Agilent HP-5MS Ultra Inert column (30 m x 250 µm x 0.25 µm) was used as a separation column with a flow of 1 mL Helium/min. A splitless injection of 1 µL was used with an inlet temperature of 275 °C and a Restek Topaz 4.0 mm ID Double Taper Inlet Liner. The

[4] This section was contributed by Dr. Bernd Göckener (Fraunhofer IME) who supervised these analyses.

temperature gradient was from 100 °C for 1 min followed by an increase of 15 °C/min until a temperature of 230 °C was achieved. The temperature was further increased with a rate of 10 °C/min to a temperature of 310 °C that was held for 5 min. Ionization was performed with an NCI (negative chemical ionization) source and methane as a reagent gas. The source temperature was 150 °C. The observed mass transitions for the individual analytes are listed in Table 7.

**Table 7: Mass transitions of pyrethroids (no qualifier mass transition was detected for transfluthrin)**

| Analyte | Use | Precursor ion [m/z] | Product ion [m/z] | Dwell time [ms] | Collision energy [V] |
|---|---|---|---|---|---|
| Tefluthrin | Quantifier | 240.8 | 205.0 | 100 | 5 |
| | Qualifier | 240.8 | 35.1 | 100 | 10 |
| Transfluthrin | Quantifier | 206.8 | 35.2 | 100 | 15 |
| | Qualifier | n.a. | - | - | - |
| λ-Cyhalothrin | Quantifier | 240.8 | 205.0 | 100 | 10 |
| | Qualifier | 204.8 | 121.1 | 100 | 20 |
| Permethrin | Quantifier | 206.7 | 35.1 | 50 | 10 |
| | Qualifier | 208.7 | 35.1 | 50 | 10 |
| Cypermethrin | Quantifier | 206.7 | 35.1 | 50 | 10 |
| | Qualifier | 208.7 | 35.1 | 50 | 10 |
| Deltamethrin | Quantifier | 297.1 | 78.9 | 100 | 10 |
| | Quantifier* | 297.1 | 80.9 | 100 | 10 |
| $d_6$-*trans*-permethrin | Quantifier | 213.0 | 35.1 | 50 | 10 |
| | Qualifier | 215.0 | 35.1 | 50 | 10 |
| $d_6$-*trans*-cypermethrin | Quantifier | 213.0 | 35.1 | 50 | 10 |
| | Qualifier | 215.0 | 37.1 | 50 | 10 |

n.a. - not available; * for deltamethrin, both observed mass transitions were summed up and the sum was used for quantification.

Quantification was conducted with a seven-point calibration including internal standards. The calibration ranges of analytes were from 0.02 – 2.0 µg/L (transfluthrin and tefluthrin), 0.2 - 20 µg/L (λ-cyhalothrin), 5.0 - 500 µg/L (permethrin and cypermethrin) and 10 - 1000 µg/L (deltamethrin), respectively. The concentration of the internal standards in the calibration samples was 100 µg/L each.

LOQs for the biota analysis were 0.033 ng/g dw for tefluthrin, transfluthrin and λ-cyhalothrin, 8.33 ng/g dw for permethrin and cypermethrin, and 16.7 ng/g dw for deltamethrin.

## 7.8 Chlorinated paraffins

Due to delays partly caused by the COVID-19 pandemic the analysis of chlorinated paraffins in the food web samples by Stockholm University could only start end of 2020. Additionally, in January 2021 PDMS passive sampler extracts prepared by Fraunhofer IME (section 5.5) were provided for the analysis of chlorinated paraffins in order to estimate water concentrations, if possible.

The report by Stockholm University will be prepared after finalization of the study (to be provided as publication draft for a peer-reviewed journal). The preliminary data for the chlorinated paraffins in the food web samples were already presented during the final project workshop in

March 2021 (Bo Yuan: Accumulation of chlorinated paraffins in the Lake Templin food webs; see section 12 and the digital Attachment 10 with the workshop documentation). The method is similar to that which was used for the analysis of German ESB samples (Bo Yuan et al. 2021: Long-Chain Chlorinated Paraffins Pose an Increasing Environmental Threat to German Ecosystems; to be submitted to a peer-reviewed journal in spring 2021).

## 7.9 Mercury/methylmercury[5]

Measurement of total mercury (THg) was performed with dedicated atomic absorption spectrometry (AAS) methods applying a Direct Mercury Analyzer (DMA) (DMA-80 instrument for solid samples; MLS GmbH, Leutkirch, Germany). The limits of quantification (LOQs) were 0.145 - 0.245 ng/g for the solid sample DMA. Verification of measurements were performed with the certified reference materials DORM-4 (Dogfish Muscle; Environmental Canada, Canada) and Mussel tissue NIST 2976 (National Institute of Standard and Technologies; Gaithersburg, Maryland, USA). Recovery of DORM-4 was 97.7 ± 3.6 % (RSD 3.7 %; n=30; nominal value 410 ± 55 ng/g) and 96.2 ± 2.4 % (RSD 2.5 %; n=9; nominal value 61.0 ± 3.6 ng/g) for Mussel tissue, respectively. Additionally, THg is measured in fish and mussel tissue samples within the scope of an interlaboratory comparison (Quasimeme, Wageningen, The Netherlands) two times per year (successful participation in the period where this samples set was analyzed). Measured method blanks were all below the LOD.

Analysis of mono-methylmercury (MeHg) was performed with SID-GC/ICP-MS (stable isotope dilution-gas chromatography coupled to inductively coupled plasma-mass spectrometry). After addition of a certified enriched MeHg standard ($^{201}$Hg-enriched Methylmercury, ISC Science, Oviedo, Spain) the freeze-dried tissue samples were extracted by a microwave assisted procedure with tetramethylammonium hydroxide (TMAH, Sigma-Aldrich, Taufkirchen, Germany). After extraction, samples were derivatized with sodium tetrapropylborate (ABCR, Karlsruhe, Germany). Volatile mercury species were then extracted in n-hexane and analyzed by SID-GC/ICP-MS based on protocols of Davis et al. (2007) and Monperrus et al. (2004). For details see Nguetseng et al. (2015). The respective LOQs were in the range of 0.2 - 1.7 ng/g dw (derived from blanks measured along with each set of samples). Verification of measurements were performed with the certified reference materials DORM-4, Mussel tissue NIST 2976 and NIST 1566b Oyster tissue. Recovery of DORM-4 was 978 ± 3.8 % (RSD 3.9 %; n=11; nominal value 355 ± 2.8 ng/g), 98.2 ± 8.6 % (RSD 8.7 %; n=6; nominal value 28.1 ± 0.3 ng/g) for Mussel tissue and 105 ± 6.9 % (RSD 6.6 %; n=6; nominal value 13.2 ± 1.7 ng/g) for Oyster tissue. Additionally, MeHg is measured in fish and mussel tissue samples within the scope of an interlaboratory comparison (Quasimeme, Wageningen, The Netherlands) at least once per year but due to the low number of participating laboratories for MeHg an evaluation was not possible so far.

[5] This section was contributed by Dr. Burkhard Knopf (Fraunhofer IME) who supervised these analyses.

# 8 PCB loads of passive sampler

The PDMS passive samplers were extracted (section 5.5) and analyzed for PCB (by Eurofins GfA Lab Service GmbH, Hamburg). However, at the end of the project only preliminary data were available. Due to apparently different loadings of the four applied PCB performance reference compounds (PRCs) in the PDMS sheets for the two exposure periods, not for all PCB congeners concentrations could be determined. Nevertheless, the preliminary data (here presented as sums for differently chlorinated PCB groups; full report by Eurofins GfA Lab Service provided as digital Attachment 6) support the assumption that the concentrations in the southern part of Lake Templin (see Figure 1) were similar at the sampling sites 2 / 3 and during the two periods (no significant difference of total PCB sum between sites 2 and 3 and between the August and October samples at $p < 0.05$; significant difference between site 1 and sites 2 / 3 at $p < 0.05$).

**Table 8: Summarized PCB loads of passive samplers (PDMS sheets, each sample consisted of three sheets)**

| Month / site (sample no.) | Sum DiCB ng/sample | Sum TriCB ng/sample | Sum TetraCB ng/sample | Sum PentaCB ng/sample | Sum HexaCB ng/sample |
|---|---|---|---|---|---|
| August, site 1 (1) | 0.996 | 37.5 | 66.8 | 67.4 | 76.0 |
| August, site 1 (2) | 1.42 | 43.9 | 78.4 | 75.7 | 86.6 |
| August, site 2 (24) | 1.87 | 32.7 | 58.2 | 49.6 | 57.6 |
| August, site 2 (25) | 0.991 | 30.3 | 54.4 | 49.0 | 57.0 |
| August, site 3 (9) | 1.10 | 32.3 | 56.5 | 54.1 | 61.1 |
| August, site 3 (10) | 0.708 | 26.5 | 49.9 | 46.4 | 55.4 |
| August, site 2 (blank) | < LOQ | 1.60 | 0.862 | 1.00 | 0.50 |
| October, site 1 (32) | 1.35 | < LOQ$ | 29.4$ | 49.3$ | 68.4 |
| October, site 1 (33) | 1.47 | 47.6 | 56.7 | 73.2 | 77.7 |
| October, site 2 (38) | 1.78 | 42.9 | 29.6$ | 10.0$ | 62.7 |
| October, site 2 (39) | 1.39 | 36.7 | 46.8 | 63.0 | 65.1 |
| October, site 3 (28) | 1.43 | 35.3 | 40.7 | 60.5 | 61.5 |
| October, site 3 (29) | 1.53 | 37.2 | 47.6 | 61.6 | 62.4 |

| Month / site (sample no.) | Sum HeptaCB ng/sample | Sum OctaCB ng/sample | Sum NonaCB ng/sample | PCB 209 (DecaCB) ng/sample | Total sum PCB ng/sample |
|---|---|---|---|---|---|
| August, site 1 (1) | 18.2 | 1.31 | < LOQ | < LOQ | **268** |
| August, site 1 (2) | 22.9 | 1.61 | < LOQ | < LOQ | **311** |
| August, site 2 (24) | 16.1 | 0.94 | < LOQ | < LOQ | **217** |
| August, site 2 (25) | 15.7 | 0.98 | < LOQ | < LOQ | **208** |
| August, site 3 (9) | 15.9 | 1.15 | < LOQ | < LOQ | **222** |
| August, site 3 (10) | 10.9 | 1.01 | < LOQ | < LOQ | **191** |
| August, site 2 (blank) | 0.155 | < LOQ | < LOQ | < LOQ | **4.1** |
| October, site 1 (32) | 17.9 | 1.10 | < LOQ | < LOQ | **(167)$** |
| October, site 1 (33) | 20.6 | 1.22 | < LOQ | < LOQ | **278** |
| October, site 2 (38) | 19.5 | 1.04 | < LOQ | < LOQ | **(168)$** |
| October, site 2 (39) | 17.8 | 1.04 | < LOQ | < LOQ | **232** |
| October, site 3 (28) | 15.1 | 0.87 | < LOQ | < LOQ | **215** |
| October, site 3 (29) | 16.6 | 1.02 | < LOQ | < LOQ | **228** |

Concentrations of the four PCB performance reference compounds (PCB 10, 30, 104, 145) were not used for the sum calculations. In some cases (especially in the October samples) some PCB had strikingly high concentrations. These PCB congeners were also removed from all sums. $ Concentrations for certain PCB congeners in this sample were identified as outliers and removed. < LOQ: no congener above the respective LOQs.

# 9 Evaluation of stable isotope signatures ($\delta^{15}N$, $\delta^{13}C$)

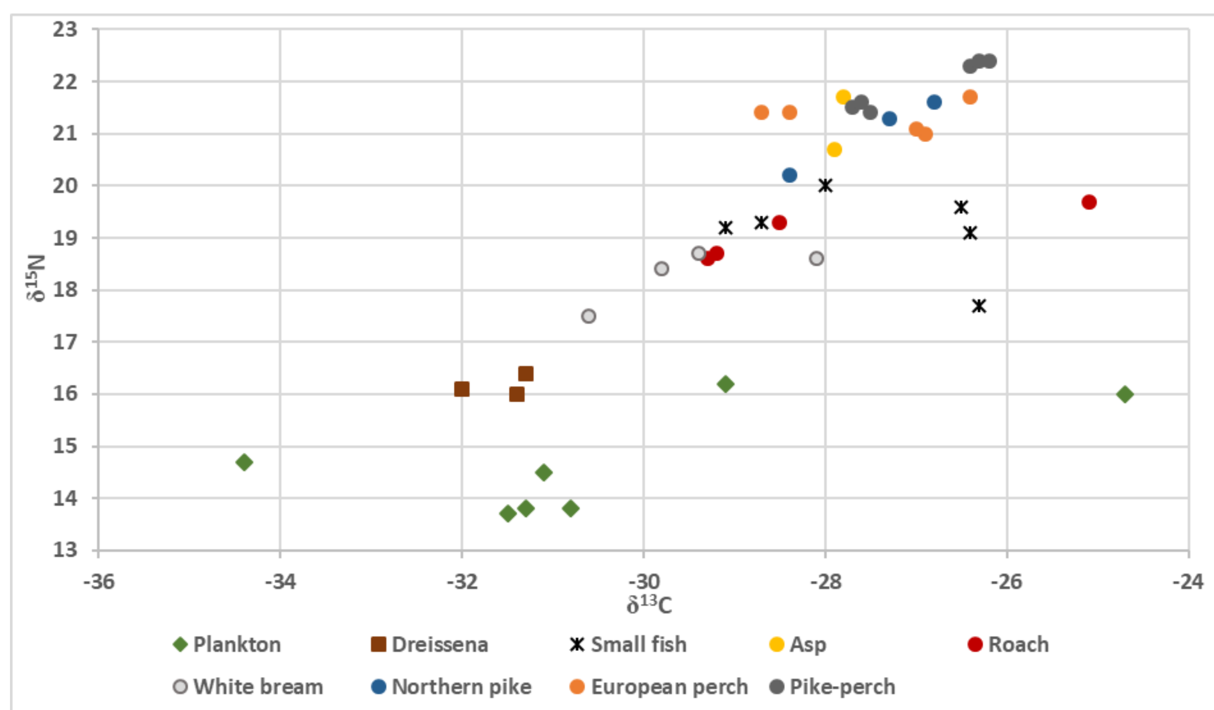
## 9.1 Food web structure

The sampled food web items were characterized based on the stable isotope data provided by agroisolab GmbH (see section 6.1). In a next step, the food web item stable isotope signatures were checked for coherence. This step was necessary to create a basis for decision making for the preparation of appropriate composite samples. By forming representative composite samples, the number of samples can be reduced without losing the informative value of analysis results. Also, a dominance of individuals of one species should be avoided. By means of the stable isotope signatures it was also tried to examine the feeding relations of the sampled organisms or species (e.g., pelagic feeding).

In total, about 30 fish samples, five mussel samples, seven plankton samples and two reference samples (field-blank samples) were analyzed for stable isotopes of carbon and nitrogen.

The biplot $\delta^{13}C$ vs. $\delta^{15}N$ for the sampled food web items from Lake Templin are shown in Figure 2. All fish stable isotope data refer to the finally prepared whole fish samples.

**Figure 2: Biplot of the stable isotope data for the sampled food web of Lake Templin.**




Source: own data. Fish data refer to whole fish samples.

Contrary to expectations, larger differences in the $\delta^{13}C$-signatures were found, which may indicate the use of heterogeneous carbon sources in the food web (benthic vs. pelagic feeding, possible inputs from terrestrial sources; see section 6.1). However, unknown proportions of suspended particulate matter may also have influenced the $\delta^{13}C$-signatures of the plankton fraction.

To explore whether the zebra mussels sampled from embankments and from the lake bottom are relying on different energy sources, additional mussels from two reference sites in the lake were also examined for their stable isotope signatures. It was found that the $\delta^{13}C$- and $\delta^{15}N$-signatures of the two reference sites (sampled from sediment and an embankment of Lake

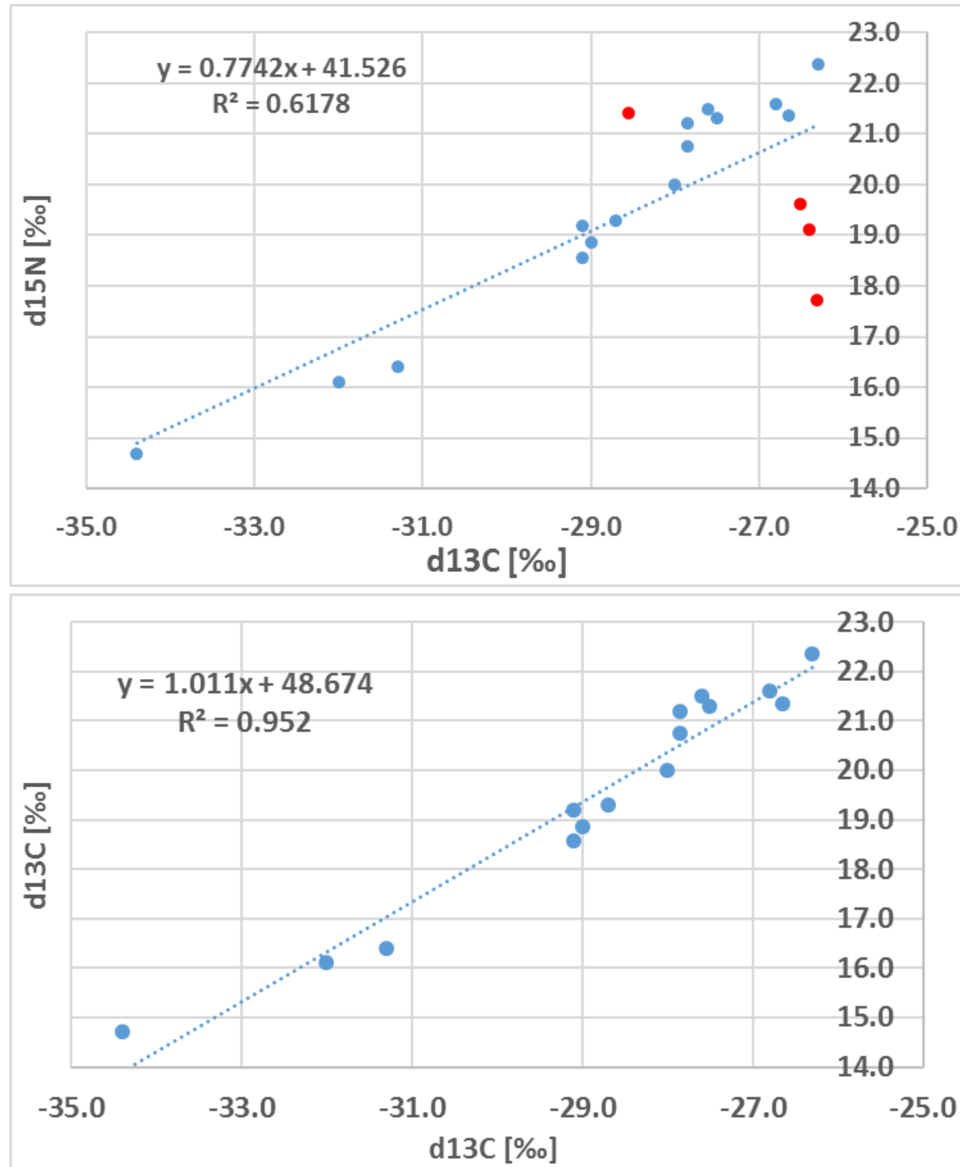
Templin; similar sample treatment) did not differ significantly. Thus, mussels from both types of sites seem to have comparable feeding behaviors. However, it could not be clarified why the $\delta^{13}C$-signatures are thus low in comparison to those of the fish samples.

The increments in the nitrogen signature over the whole sample set are much smaller than expected. If the standard increment of 3.4 ‰ $\delta^{15}N$ per trophic level (Post 2002) would be applied, lower trophic positions than those given in the literature would result (e.g. fishbase.org, Froese and Pauly (2019)).

Alternatively, a value of 2.3 ‰ was chosen as the increment for the trophic enrichment of $^{15}N$. This value was determined in a meta-analysis by McCutchan et al. (2003) as the mean trophic shift between aquatic consumers and their diet. With this lower increment selected here, the TPs are in the range of the TPs given by fishbase.org (Froese & Pauly 2019). Larger deviations are found for small perch (the lower TP can be explained by a probably less piscivorous diet) and bleak (for these also the high lipid content is striking and may support the finding of a higher TP in this ecosystem; see Table 11). The chosen increment of 2.3 ‰ also fits quite well to the trophic difference of the ecosystem of the upstream part of the Havel (Großer Wannsee), for which an increment of 1.9 ‰ can be derived using TPs from fishbase.org and stable isotope data provided in the study by Radermacher et al (2019).

Biplots of the remaining food web items of Lake Templin are shown in Figure 3. The upper biplot reveals four samples with diverging stable isotope signatures. Since, on the basis of the stable isotope data, some fish samples do not appear to be components of a common food web, they were also not considered for the further investigation. These samples are the mixed sample of small asps as well as small and large rudds. One perch composite sample was also not considered in the final sample set. After these samples with diverging signatures were removed (Figure 3, bottom biplot), the correlation of the $\delta^{15}N$ and $\delta^{13}C$ data was clearly improved.

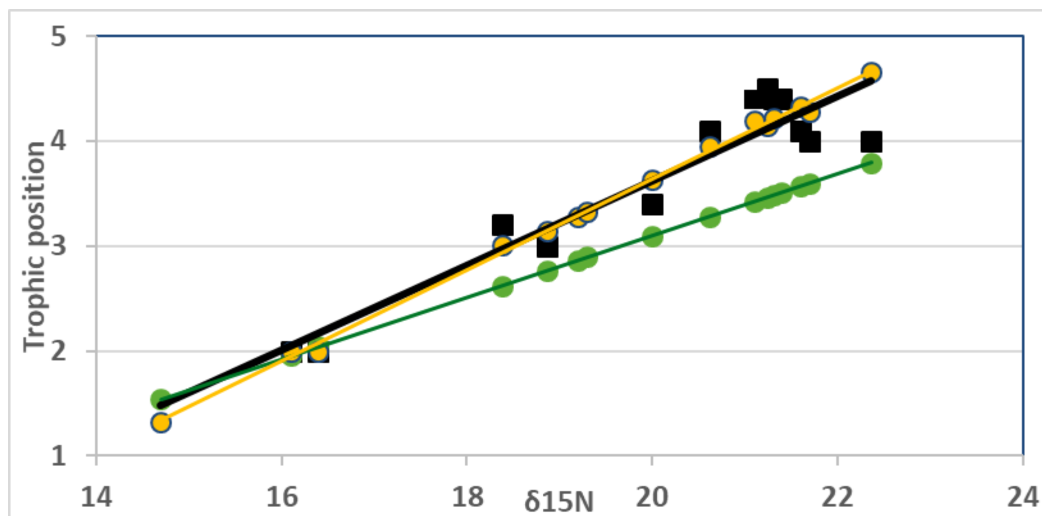
**Figure 3:** **Biplots of sampled food web items of Lake Templin (only relevant composite and/or individual samples are shown) before and after removal of samples with diverging signatures (samples marked red in the top biplot)**




Source: own data. Samples marked red (top biplot) were removed. The final food web items are shown in the bottom biplot (significant correlation at $p<0.0001$).

Figure 4 shows a comparison between the calculated TPs of the final food web items either with the standard increment of 3.4 ‰ $\delta^{15}$N per trophic level, or the here selected increment of 2.3‰. For comparison, the TPs obtained from the reference database (Froese & Pauly 2019) are shown, too. The chart reveals that the selected increment results in calculated fish TP which fit better to the trophic levels given for the fish in the reference database.

**Figure 4: Trophic positions (TPs) of the final set of food web items, either calculated with an increment of 3.4 ‰ or 2.3 ‰ $\delta^{15}N$ per TP, plotted versus the determined $\delta^{15}N$ values in relation to reference TP of fish species**




Source: own data. Green circles: TPs calculated from $\delta^{15}N$ data with an increment of 3.4; yellow circles: TPs calculated from $\delta^{15}N$ data with an increment of 2.3; black squares: TPs according to the reference data base (fishbase.org; Froese and Pauly (2019)).

The final set of biota samples is described in Table 9 where measured stable isotope data (for composite samples calculated from the data of the individual fished applied into the respective pool) and calculated trophic positions (TP) are listed. Thus, the sample set for the analyses for possible food web accumulation of chemicals consists of a total of 16 samples (including a field blank value, i.e. marine fish fillet exposed in the same way as the sampled fish). In addition, passive sampler extracts (Chemcatcher disks and PDMS sheets) can be analyzed for certain (water-soluble) substances, in order to be able considering water concentrations in the evaluation (see section 8 for PCB).

**Table 9: Characterization of the selected food web items of Lake Templin; the TP was calculated based on the measured $\delta^{15}N$ signatures assuming that all organisms are feeding predominantly on a common carbon source (see section 4.1)**

| No. | Sample | Description | | Age (a) | $\delta^{13}C$ [‰] | SD $\delta^{13}C$ [‰] | $\delta^{15}N$ [‰] | SD $\delta^{15}N$ [‰] | TL (fish-base) | TP (calcu-lated) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | plankton fraction | > 200 µm | composite sample | - | -34.4 | 0.2 | 14.7 | 0.1 | - | 1.3 |
| 2 | zebra mussel (small) | soft body | composite sample, < 2 cm | ca. 3 | -31.3 | 0.1 | 16.4 | 0.1 | - | 2.0# (2.1) |
| 3 | zebra mussel (large) | soft body | composite sample, > 2 cm | ca. 4 - 7 | -32.0 | 0.1 | 16.1 | 0.1 | - | 2.0# (1.9) |
| 4 | white bream (individuals no. 2,3,4) | whole fish | composite sample, 3 individuals | 7 / 7 / 6 | -29.2 | 0.9 | 18.6 | 0.2 | 3.2 | 3.0 |

| No. | Sample | Description | | Age (a) | $\delta^{13}C$ [‰] | SD $\delta^{13}C$ [‰] | $\delta^{15}N$ [‰] | SD $\delta^{15}N$ [‰] | TL (fish-base) | TP (calcu-lated) |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | roach (individuals no. 1,2,4) | whole fish | composite sample, 3 individuals | 11 / 10 / 11 | -29.0 | 0.4 | 18.9 | 0.4 | 3.0 | 3.1 |
| 6 | roach (small) | whole fish | composite sample | < 1 | -29.1 | 0.1 | 19.2 | 0.1 | 3.0 (2.0§) | 3.3 |
| 7 | bleak | whole fish | composite sample | < 1 | -28.7 | 0.1 | 19.3 | 0.1 | 2.7 | 3.3 |
| 8 | perch (small) | whole fish | composite sample | < 1 | -28.0 | 0.1 | 20.0 | 0.1 | 4.4 (3.4§) | 3.6 |
| 9 | pike (individuals no. 2,3) | whole fish | composite sample, 2 individuals | 3 / 3 | -28.0 | 0.8 | 20.6 | 0.8 | 4.1 | 3.9 |
| 10 | perch (individual no. 1) | whole fish | 1 individual | 11 | -27.0 | 0.1 | 21.1 | 0.1 | 4.4 | 4.1 |
| 11 | asp (individuals no. 1,2) | whole fish | composite sample, 2 individuals | 6 / 8 | -27.8 | 0.1 | 21.2 | 0.7 | 4.5 | 4.2 |
| 12 | perch (individuals no. 2,4) | whole fish | composite sample, 2 individuals | 7 / 7 | -26.7 | 0.4 | 21.3 | 0.5 | 4.4 | 4.2 |
| 13 | pikeperch A (individuals no. 1,5,6) | whole fish | composite sample, 3 individuals | 2 / 2 / 2 | -27.6 | 0.1 | 21.5 | 0.1 | 4.0 | 4.3 |
| 14 | pike (individual no. 1) | whole fish | 1 individual | 6 | -26.8 | 0.1 | 21.6 | 0.1 | 4.1 | 4.3 |
| 15 | pikeperch B (individuals no. 2,3,4) | whole fish | composite sample, 3 individuals | 2 / 2 / 2 | -26.3 | 0.1 | 22.4 | 0.1 | 4.0 | 4.7 |

SD - standard deviation; TL - generic trophic level; TP - trophic position; # the TP of zebra mussels (mean value of both mussel samples) was assumed to be 2.0; § since it is described for roach and perch that the food sources change during development, for young fish a TP was assumed which is 1 trophic level below the value given for adult fish at fishbase.org (for perch, this results in a clearly lower difference between assumed and calculated TP).

In an alternative approach to derive TP data for the selected food web items, it was tried to consider not only pelagic feeding but also influences of possible benthic feeding of the organisms. This evaluation assumes a benthic-pelagic coupling of the Lake Templin organisms (see section 4.1). To this end, the mixing model proposed by Post (2002) was applied (see section 6.2) assuming feeding on pelagic and benthic sources. However, possible influences of additional carbon inputs as, for example, by terrestrial sources could not be considered (no samples for comparison available, not enough different stable isotopes covered).

The benthic fraction ($\alpha$) is the proportion of nitrogen in the consumer ultimately derived from the base of the benthic food web of the prey of each food web item. It is estimated using the $\delta^{13}C$ signature (Post 2002). Zebra mussels had the lowest $\delta^{13}C$ value of the consumer organisms ($\delta^{13}C$ at least 3 ‰ lower than the fish values; $\alpha = 1$) and were assumed to be influenced by benthic sources (e.g., bacteria, detritus). The $\delta^{13}C$ signature of the 40 - 100 µm plankton fraction sampled

in July (2 months before the sampling of the final food web items) had the highest δ13C level of all food web items sampled. It was assumed to contain mainly phytoplankton and pelagic organisms (defined as 100 % pelagic, α = 0).

The data are compiled in Table 10. However, the consideration of possible benthic feeding sources did not change the calculated TP of the Lake Templin food web items significantly. The difference to the values calculated without consideration of different feeding sources (Table 7) is in the range -0.1 - 0.1 ‰. The reason is that the δ15N values of the assumed source organisms (plankton fraction from July, zebra mussels) are quite similar (16.0 ‰ vs. about 16.3 ‰).

**Table 10: Characterization of the selected food web items of Lake Templin; the TP was calculated with a mixing model (see section 6.2) assuming that all organisms are feeding on two carbon sources (directly or indirectly)**

| No. | Sample | Description | | $\delta^{13}C$ [‰] | SD $\delta^{13}C$ [‰] | α | $\delta^{15}N$ [‰] | SD $\delta^{15}N$ [‰] | TL (fish-base) | TP (calcu-lated) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | plankton fraction | > 200 µm | composite sample | -34.4 | 0.2 | 0.0 | 14.7 | 0.1 | - | 1.4 |
| 2 | zebra mussel (small) | soft body | composite sample, < 2 cm | -31.3 | 0.1 | 0.9 | 16.4 | 0.1 | 2.0# | 2.1 |
| 3 | zebra mussel (large) | soft body | composite sample, > 2 cm | -32.0 | 0.1 | 1.1 | 16.1 | 0.1 | 2.0# | 1.9 |
| 4 | white bream (individuals no. 2,3,4) | whole fish | composite sample, 3 individuals | -29.2 | 0.9 | 0.6 | 18.6 | 0.2 | 3.2 | 3.0 |
| 5 | roach (individuals no. 1,2,4) | whole fish | composite sample, 3 individuals | -29.0 | 0.4 | 0.6 | 18.9 | 0.4 | 3.0 | 3.2 |
| 6 | roach (small) | whole fish | composite sample | -29.1 | 0.1 | 0.6 | 19.2 | 0.1 | 3.0 (2.0§) | 3.3 |
| 7 | bleak | whole fish | composite sample | -28.7 | 0.1 | 0.6 | 19.3 | 0.1 | 2.7 | 3.4 |
| 8 | perch (small) | whole fish | composite sample | -28.0 | 0.1 | 0.5 | 20.0 | 0.1 | 4.4 (3.4§) | 3.7 |
| 9 | pike (individuals no. 2,3) | whole fish | composite sample, 2 individuals | -28.0 | 0.8 | 0.5 | 20.6 | 0.8 | 4.1 | 3.9 |
| 10 | perch (individual no. 1) | whole fish | 1 individual | -27.0 | 0.1 | 0.4 | 21.1 | 0.1 | 4.4 | 4.2 |
| 11 | asp (individuals no. 1,2) | whole fish | composite sample, 2 individuals | -27.8 | 0.1 | 0.5 | 21.2 | 0.7 | 4.5 | 4.2 |
| 12 | perch (individuals no. 2,4) | whole fish | composite sample, 2 individuals | -26.7 | 0.4 | 0.3 | 21.3 | 0.5 | 4.4 | 4.3 |
| 13 | pikeperch A (individuals no. 1,5,6) | whole fish | composite sample, 3 individuals | -27.6 | 0.1 | 0.4 | 21.5 | 0.1 | 4.0 | 4.3 |

| No. | Sample | Description | | $\delta^{13}C$ [‰] | SD $\delta^{13}C$ [‰] | α | $\delta^{15}N$ [‰] | SD $\delta^{15}N$ [‰] | TL (fish-base) | TP (calcu-lated) |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | pike (indivi-dual no. 1) | whole fish | 1 individual | -26.8 | 0.1 | 0.3 | 21.6 | 0.1 | 4.1 | 4.4 |
| 15 | pikeperch B (individuals no. 2,3,4) | whole fish | composite sample, 3 indivi-duals | -26.3 | 0.1 | 0.2 | 22.4 | 0.1 | 4.0 | 4.7 |

SD - standard deviation; TL - generic trophic level; TP - trophic position; # the TP of zebra mussels was assumed to be 2.0; § since it is described for roach and perch that the food sources change during development, for young fish a TP was assumed which is 1 trophic level below the value given for adult fish at fishbase.org (for perch, this results in a clearly lower difference between assumed and calculated TP); α - benthic fraction of prey (1 = 100 %); calculation as described in section 6.2.

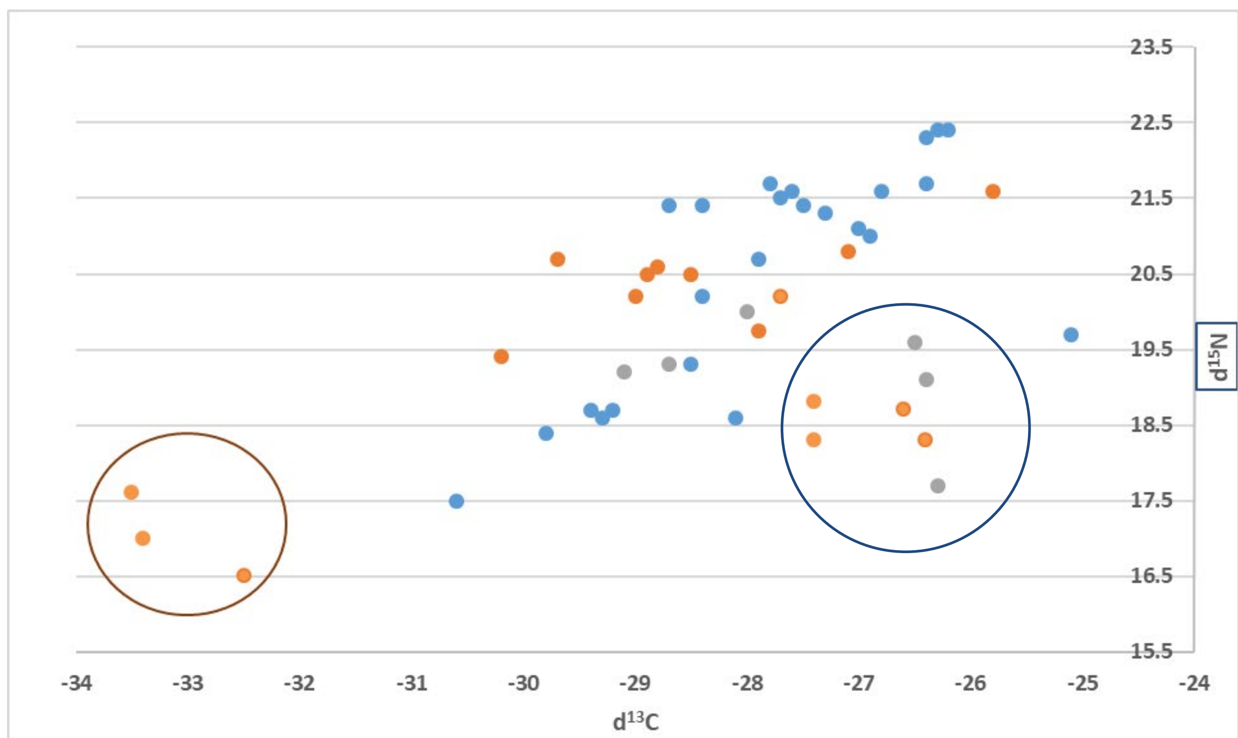
Finally, the TP data from the mixing model were not considered for the evaluations in this report. For the TMF evaluations presented here, the TP data from Table 7 were used.

## 9.2 Examination of fish gastrointestinal tract contents for stable isotope signatures

During the dissection of the larger individual fish, the gastrointestinal tract (GIT) was removed and stored separately at cryogenic temperatures. To get information about the possible nutrition of the sampled fish, the GIT was thawed and the contents were isolated. If different GIT contents could be identified in one fish, they were handled separately. The GIT contents were freeze-dried and analyzed for stable isotopes ratios by agroisolab GmbH.

Unfortunately, the results did not provide clear answers to the questions regarding the trophic positions of the fish. It can be assumed that the samples of the small fish of three species that were excluded from the analysis consumed macrophytes rather than plankton, since the respective isotope signatures are in similar ranges. Samples with shell fragments were treated with acid to remove carbonates before stable isotope analysis. By this means a bias of the isotope signatures by the carbon bound in the shells should be prevented. The results of these samples are forming a separate value group in the corresponding biplot (Figure 5). To obtain comparable results, however, other samples would have to be measured again with similar pre-treatment.

**Figure 5: Biplot of the stable isotope data for fish gastrointestinal tract contents and whole fish**




Source: own data. Blue dots: whole fish data; orange dots: GIT data; grey dots: small fish samples. Circles are marking samples which are apparently different from the remaining dataset.

# 10 Sample characterization results (lipid, water and protein contents)

For all samples, water contents were determined gravimetrically after freeze-drying. The lipid contents were determined according to Smedes (1999). Protein contents were measured following an internal method (see section 7.2). Data are shown in Table 11.

The lipid data are required to allow a normalization of the concentrations of lipophilic chemicals in samples to a uniform fat content. By this means the effect of different lipid contents of food web samples should be excluded. For some compounds (e.g., PFAS) a normalization to the protein content is discussed.

**Table 11: Lipid, water and protein content data for the selected set of food web items (in %)**

| sample description (no. in brackets: designation of individuals) | mean lipid content (n = 2 - 4) [%] | SD lipid content (n = 2 - 4) [%] | mean water content (n = 1 - 2) [%] | SD water content (n = 1 - 2) [%] | mean protein content (n = 2 - 3) [%] | SD protein content (n = 2 - 3) [%] |
|---|---|---|---|---|---|---|
| plankton fraction | 0.525$ | < 0.02 | 90.0# | - | 14.3 | 1.0 |
| zebra mussel (small) | 0.48 | < 0.01 | 97.0 (95.7)§ | < 0.1 | 2.2 | 0.1 |
| zebra mussel (large) | 0.31 | < 0.04 | 97.9 (97.0)§ | 0.1 | 1.6 | 0.0 |
| white bream (2,3,4) | 7.39 | 0.11 | 72.1 | - | 4.9 | 0.1 |
| roach (1,2,4) | 6.20 | 0.03 | 70.5 | - | 5.3 | 0.3 |
| roach (small) | 4.59 | 0.08 | 79.5 | - | 5.6 | 1.1 |
| bleak | 13.0 | 0.1 | 71.2 | 0.9 | 5.3 | 0.3 |
| perch (small) | 3.44 | 0.05 | 77.4 | - | 2.6 | 1.9 |
| pike (2,3) | 2.78 | 0.08 | 78.2 | - | 4.0 | 0.2 |
| perch 1 | 5.40 | 0.06 | 70.1 | - | 4.7 | 0.1 |
| asp (1,2) | 11.4 | 0.8 | 69.3 | - | 5.3 | 0.4 |
| perch (2,4) | 5.90 | 0.06 | 73.0 | - | 4.4 | 0.4 |
| pikeperch A (1,5,6) | 2.39 | 0.07 | 78.1 | - | 4.1 | 0.2 |
| pike 1 | 3.03 | 0.04 | 75.9 | - | 4.4 | 0.2 |
| pikeperch B (2,3,4) | 1.98 | 0.03 | 78.6 | - | 3.8 | 0.5 |

SD - standard deviation; $ determined for freeze-dried material and re-calculated as wet weight; # assumed water content; § calculated under assumption of a respiration water content of 27.6 % (calculated from data of the ESB data bank).

# 11 TMF determinations for benchmark chemicals

For the TMF evaluations, the data from the food web analyses for organic chemicals by Eurofins GfA Lab Service GmbH (digital Attachment 5) and for PFAS, mercury/methylmercury and cVMS by Fraunhofer IME (digital Attachment 9) were applied.

For most of these benchmark chemicals, TMF determinations could be performed. Some of the evaluations are presented here as examples.

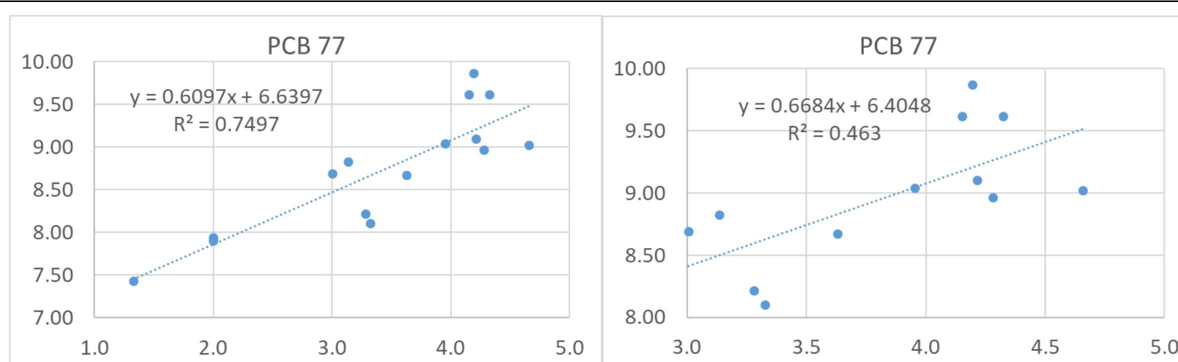
Due to the uncertain water content of plankton (frozen together with an undefined amount of water; see Table 11) and mussels (including an undefined amount of respiration water), all TMF for polar compounds were calculated on a dry weight basis. TMF for non-polar compounds were based on lipid-normalized concentrations.

Data are shown, e.g., for selected PCB (e.g., DL-PCB congeners 126 and 169 with the highest WHO TEQ factors; PCB 77 with high concentration and NDL-PCB congeners). Unfortunately, for some PCB no evaluation was possible because several biota samples had concentrations below the LOQ (especially the plankton fraction and/or the mussel samples). Further evaluations are shown for BDE congeners, legacy pesticides, mercury and methylmercury. The complete TMF evaluations are provided in a separate Microsoft Excel file (digital Attachment 9).

In the legend of the following tables derived TMFs are compared to TMFs derived from meta-analyses (e.g., Lavoie et al. (2013) for mercury, Walters et al. (2016) for organic compounds). These evaluations deliver mean TMFs with very broad ranges. In further evaluations, more focused comparisons with studies, which are more comparable with the present lake ecosystem, should be performed.

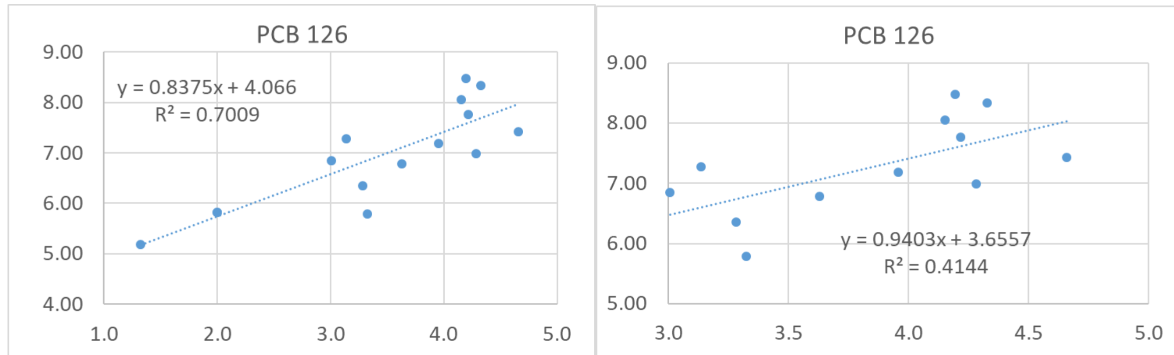
For TMFs, mean and 95 % confidence intervals (95 % CI) are given. While for the whole food web most correlations are significant (two-tailed test of significance of Pearson's correlation coefficient), especially for the 'fish-only' evaluations some correlations were not significant.

**Figure 6: TMF evaluation for DL-PCB 77: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



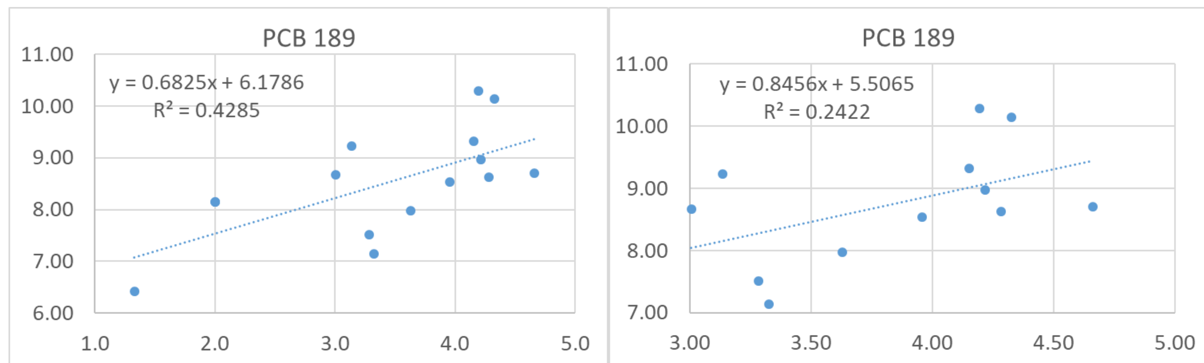

Source: own data. The TMF is 1.8 (95 % CI: 1.5 - 2.3; p<0.001) for all food web items (left diagram) and 1.9 (95 % CI: 1.1 - 3.3; p=0.019) for the fish only food web (right diagram); TMF literature data: 3.45 ± 2.46, n = 7 (Walters et al. 2016).

**Figure 7: TMF evaluation for DL-PCB 126: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



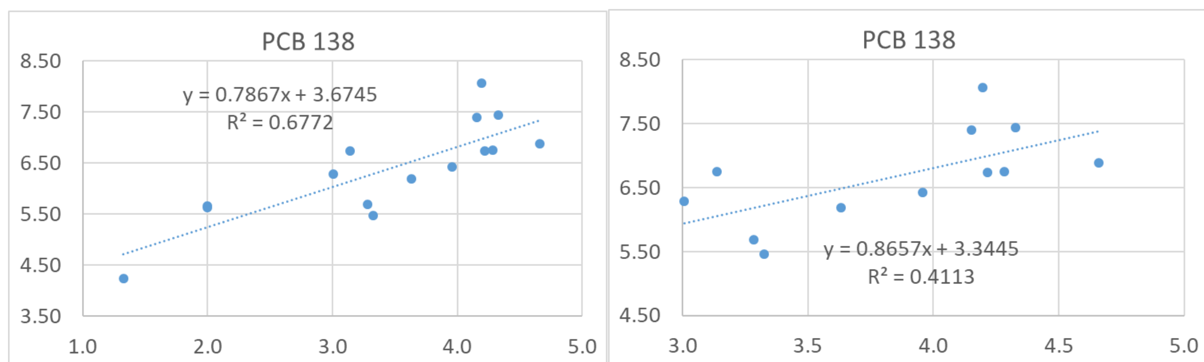

Source: own data. The TMF is 2.3 (95 % CI: 1.7 - 3.2; p<0.001) for all food web items (left diagram) and 2.5 (95 % CI: 1.1 - 5.7; p=0.028) for the fish only food web (right diagram); TMF literature data: 3.45 ± 2.46, n = 7 (Walters et al. 2016).

**Figure 8: TMF evaluation for DL-PCB 189: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



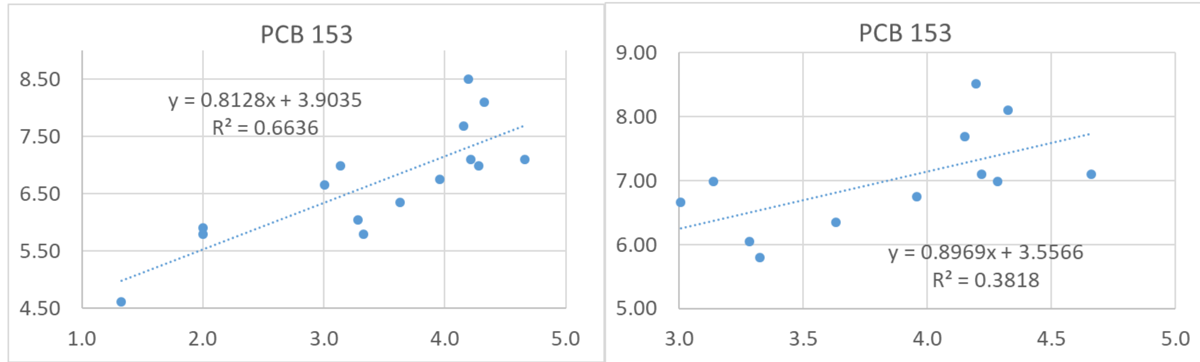

Source: own data. The TMF is 2.0 (95 % CI: 1.2 - 3.2; p=0.009) for all food web items (left diagram) and 2.3 (95 % CI: 0.8 - 6.7; p=0.116 not significant) for the fish only food web (right diagram); TMF literature data: 5.99 ± 5.1, n = 4 (Walters et al. 2016).

**Figure 9: TMF evaluation for NDL-PCB 138: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



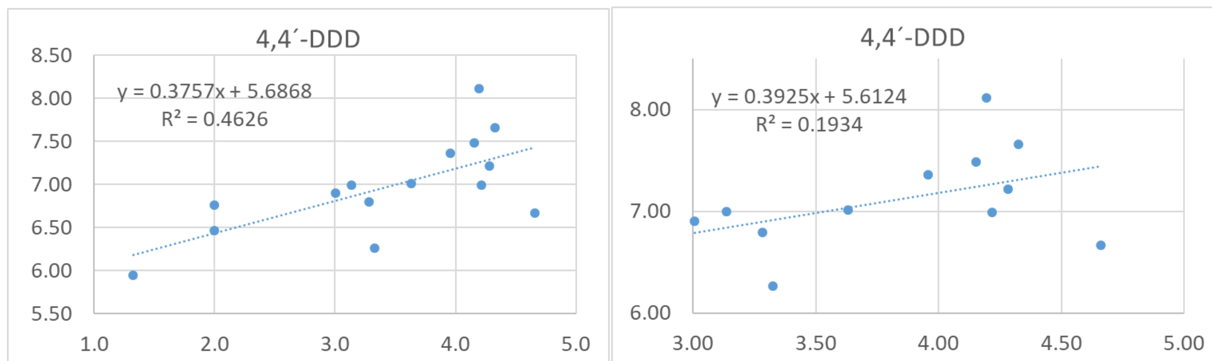

Source: own data. The TMF is 2.2 (95 % CI: 1.6 - 3.1; p<0.001) for all food web items (left diagram) and 2.4 (95 % CI: 1.1 - 5.0; p=0.030) for the fish only food web (right diagram); TMF literature data: 5.99 ± 8.58, n = 46 (Walters et al. 2016).

**Figure 10: TMF evaluation for NDL-PCB 153: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



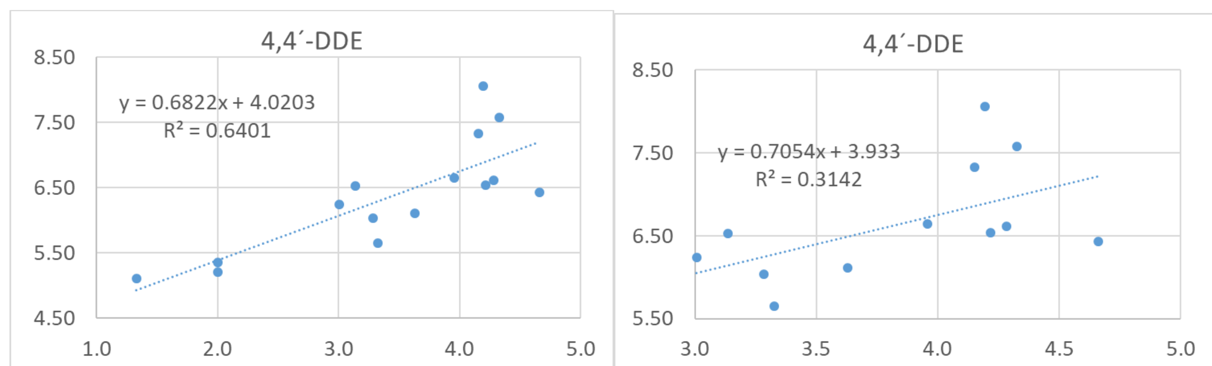

Source: own data. The TMF is 2.3 (95 % CI: 1.6 - 3.2; p<0.001) for all food web items (left diagram) and 2.4 (95 % CI: 1.8 - 5.5; p=0.038) for the fish only food web (right diagram); TMF literature data: 6 ± 6.74, n = 50 (Walters et al. 2016).

**Figure 11: TMF evaluation for 4,4´-DDD: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



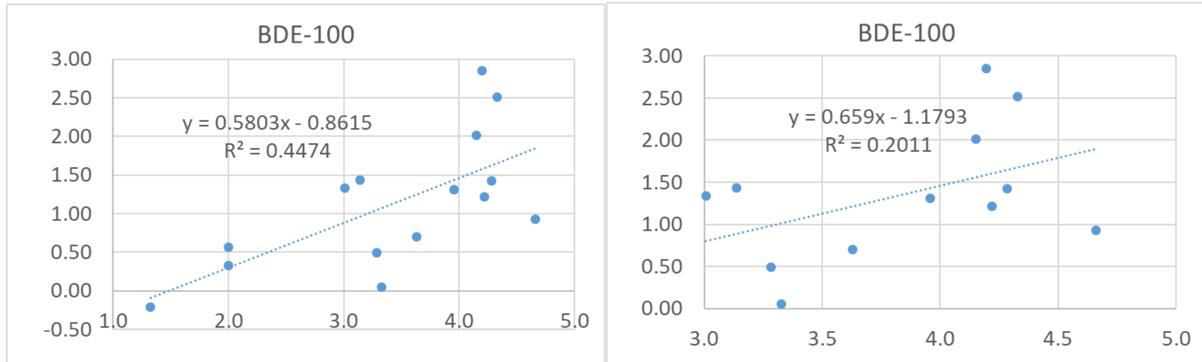

Source: own data. The TMF is 1.4 (95 % CI: 1.1 - 1.9; p=0.007) for all food web items (left diagram) and 1.4 (95 % CI: 0.8 - 2.6; p=0.185 not significant) for the fish only food web (right diagram); TMF literature data 1.81 ± 0.63, n = 7 (Walters et al. 2016).

**Figure 12: TMF evaluation for 4,4'-DDE: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



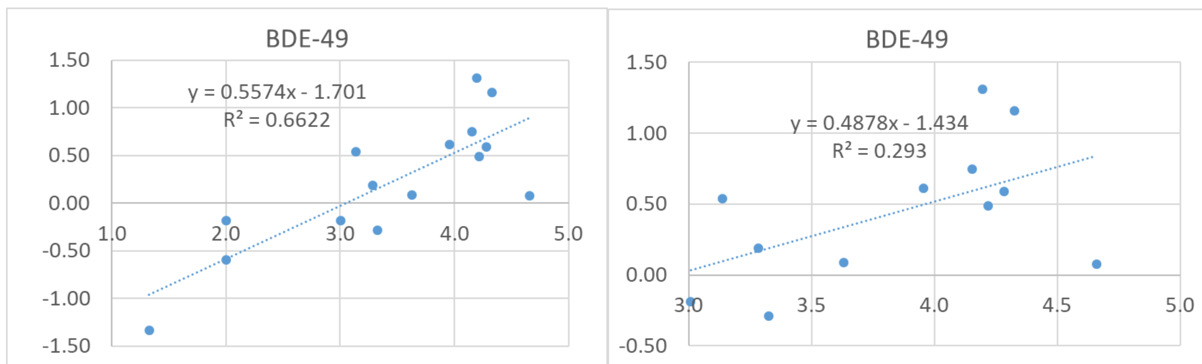

Source: own data. The TMF is 2.0 (95 % CI: 1.4 - 2.7; p<0.001) for all food web items (left diagram) and 2.0 (95 % CI: 0.93 - 4.2; p=0.071 not significant) for the fish only food web (right diagram); TMF literature data 4.32 ± 3.59, n = 40 (Walters et al. 2016).

**Figure 13: TMF evaluation for BDE-100: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



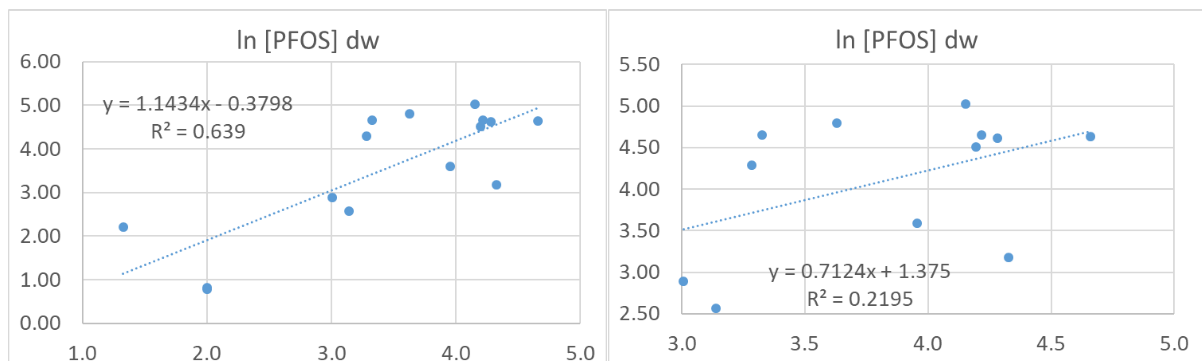

Source: own data. The TMF is 1.8 (95 % CI: 1.2 - 2.6; p=0.008) for all food web items (left diagram) and 1.9 (95 % CI: 0.7 - 4.9; p=0.164 not significant) for the fish only food web (right diagram); TMF literature data 2.98 ± 1.83, n = 17 (Walters et al. 2016).

**Figure 14: TMF evaluation for BDE-49: the logarithmic lipid-normalized concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



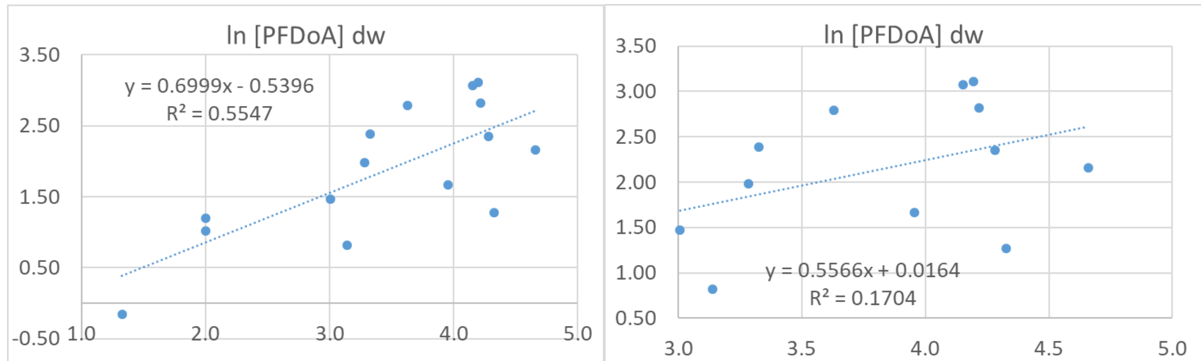

Source: own data. The TMF is 1.7 (95 % CI: 1.4 - 2.2; p<0.001) for all food web items (left diagram) and 1.6 (95 % CI: 0.93 - 2.8; p=0.084 not significant) for the fish only food web (right diagram); TMF literature data 2.99 ± 4.41, n = 4 (Walters et al. 2016).

**Figure 15: TMF evaluation for PFOS: the logarithmic dry weight concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



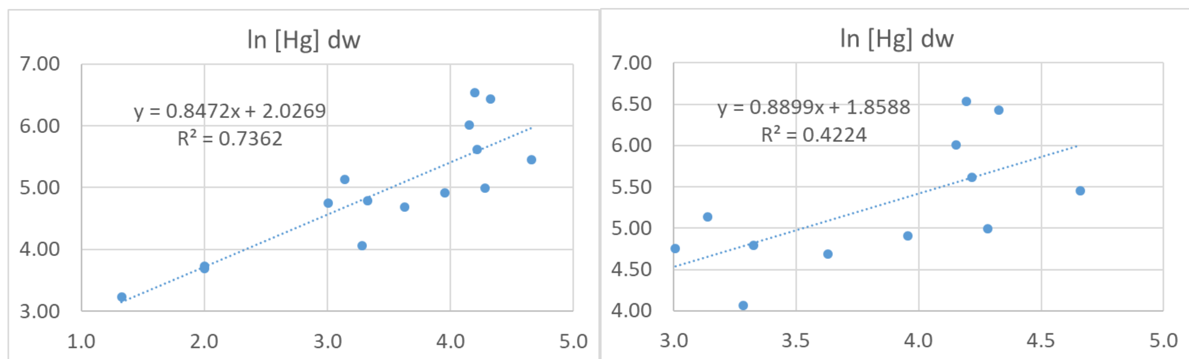

Source: own data. The TMF is 3.2 (95 % CI: 2.2 - 5.3; p<0.001) for all food web items (left diagram) and 2.1 (95 % CI: 0.8 - 5.4; p=0.122 not significant) for the fish only food web (right diagram); TMF literature data range 1.0 - 19.6, n = 20 (Franklin 2016).

**Figure 16: TMF evaluation for PFDoA: the logarithmic dry weight concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



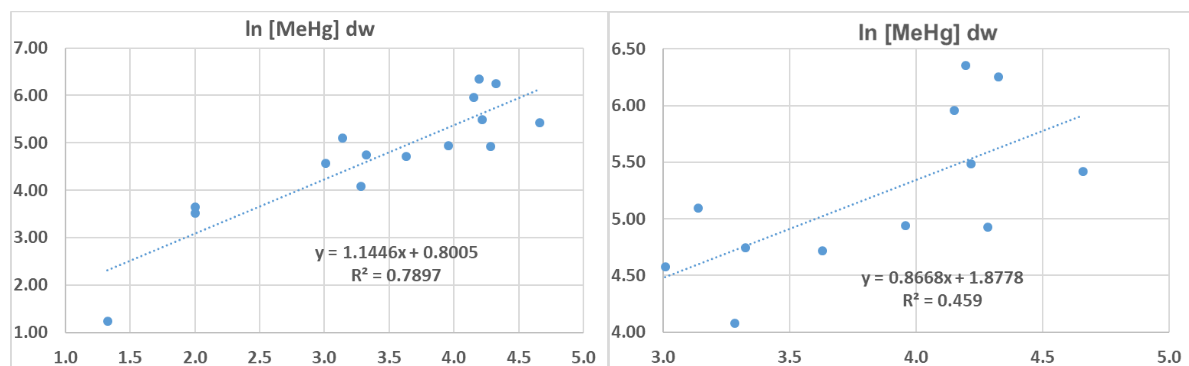

Source: own data. The TMF is 2.0 (95 % CI: 1.4 - 3.0; p=0.002) for all food web items (left diagram) and 1.7 (95 % CI: 0.7 - 4.2; p=0.195 not significant) for the fish only food web (right diagram); TMF literature data range 0.6 - 5.2, n = 7 (Franklin 2016).

**Figure 17: TMF evaluation for mercury: the logarithmic dry weight concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**



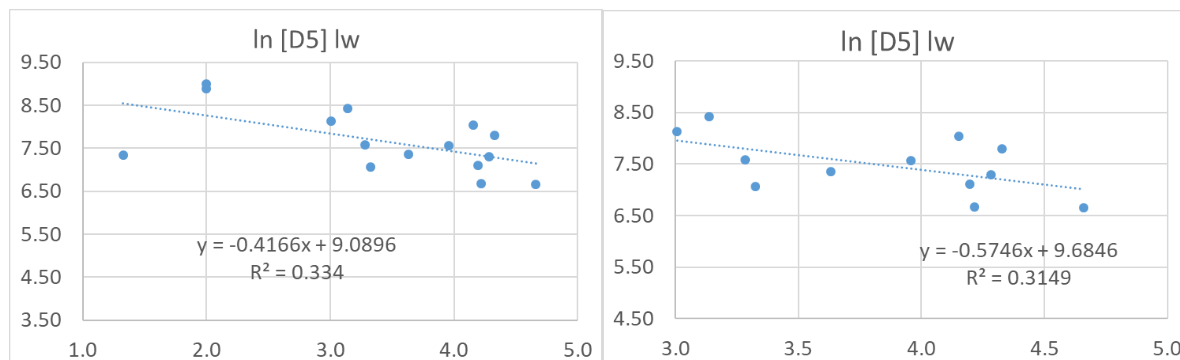

Source: own data. The TMF is 2.3 (95 % CI: 1.7 - 3.2; p<0.001) for all food web items (left diagram) and 2.4 (95 % CI: 1.1 - 5.1; p=0.025) for the fish only food web (right diagram); TMF literature data: 4.3 ± 4.8 (Lavoie et al. 2013), 1.3 - 2.5 with a $\delta^{15}N$ increment per TP of 2.0 ‰ (Jardine et al. 2013).

**Figure 18: TMF evaluation for methylmercury: the logarithmic dry weight concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**




Source: own data. The TMF is 3.2 (95 % CI: 2.2 - 4.5; p<0.001) for all food web items (left diagram) and 2.4 (95 % CI: 1.2 - 4.7; p=0.018) for the fish only food web (right diagram); TMF literature data: 8.3 ± 7.5 (Lavoie et al. 2013), 2.8 - 6.0 with a $\delta^{15}N$ increment per TP of 2.0 ‰ (Jardine et al. 2013).

**Figure 19: TMF evaluation for D5 (decamethylcyclopentasiloxane): the logarithmic lipid weight concentrations are plotted vs. the trophic levels of the food web items; left diagram: all food web items; right diagram: fish-only food web**




Source: own data. The TMF is 0.7 (95 % CI: 0.5 - 0.9; p=0.025) for all food web items (left diagram) and 0.6 (95 % CI: 0.3 - 1.0; p=0.061 not significant) for the fish only food web (right diagram); TMF literature data: in freshwaters range 0.2 - 3.2 (Gobas et al. 2015).

The following Table 12 and Table 13 give an overview over the TMFs derived for the tested benchmark compounds. TMFs were derived for the whole set of samples (n = 15), the set of samples without the plankton fraction (n = 14), and the set of samples without the plankton fraction and the two zebra mussel samples (n = 12, only fish). For this evaluation, only data sets were considered where at maximum two samples had levels below the LOQs (mainly plankton or zebra mussels). In these cases, concentrations below the LOQs were substituted by a concentration of 0.5 * LOQ.

Table 12 lists the data for the lipophilic compounds (dioxin-like and non-dioxin-like PCB congeners, PBDE congeners, organochlorine pesticides, chlorinated furans). Most TMFs are in a quite narrow range of about 1.5 - 2.5. The highest TMF was found for DDT for the fish only evaluation (about 10). For the investigated cVMS only D5 was present in all biota samples. Interestingly, a significant TMF < 1 was found (see also Figure 19). Literature reports different TMFs (< 1 / > 1) with data for (shallow) lakes tending to show also low TMFs (Gobas et al. 2015).

The TMFs derived for non-lipophilic benchmark compounds are given in Table 13 (mercury/ methylmercury, PFAS).

**Table 12: TMFs derived for lipophilic benchmark compounds: for the whole set of samples, the set of samples without the plankton fraction, and the set of samples without the plankton fraction and the two zebra mussel samples (only fish)**

| compound | TMF from all biota (n = 15) | 95% CI of TMF from all biota | significance of TMF from all biota | TMF from fish only (n = 12) | 95% CI of TMF from fish only | significance of TMF from fish only | TMF without plankton (n = 14) | 95% CI of TMF without plankton | significance of TMF without plankton |
|---|---|---|---|---|---|---|---|---|---|
| PCB 28[#$] | **1.8** | 1.4-2.3 | p=0.0003 | **1.9** | 1.1-3.5 | p=0.0317 | **1.7** | 1.2-2.4 | p=0.0038 |
| PCB 52[#$] | **2.0** | 1.5-2.6 | p<0.0001 | *1.8* | *0.95-3.4* | *p=0.0691* | **2.0** | 1.4-2.9 | p=0.0010 |
| PCB 77[§] | **1.8** | 1.5-2.3 | p<0.0001 | **1.9** | 1.1-3.3 | p=0.0187 | **1.8** | 1.4-2.4 | p=0.0005 |
| PCB 81[§&] | **2.4** | 1.6-3.7 | p=0.0007 | *1.7* | *0.7-4.0* | *p=0.2015* | **1.7** | 1.1-2.7 | p=0.0166 |
| PCB 101[#] | **2.1** | 1.5-2.9 | p=0.0004 | *2.1* | *0.97-4.7* | *p=0.0573* | **1.9** | 1.3-2.9 | p=0.0057 |
| PCB 105[§] | **2.2** | 1.6-2.9 | p<0.0001 | **2.3** | 1.1-4.6 | p=0.0278 | **2.0** | 1.4-2.9 | p=0.0019 |
| PCB 114[§] | **2.3** | 1.6-3.2 | p=0.0002 | **2.6** | 1.2-5.5 | p=0.0211 | **2.0** | 1.3-3.1 | p=0.0034 |
| PCB 118[§] | **2.2** | 1.6-3.0 | p=0.0002 | **2.2** | 1.0-4.8 | p=0.0425 | **2.0** | 1.3-3.0 | p=0.0031 |
| PCB 123[§] | **2.6** | 1.7-3.8 | p=0.0001 | **2.9** | 1.3-6.6 | p=0.0160 | **2.1** | 1.4-3.4 | p=0.0035 |
| PCB 126[§] | **2.3** | 1.7-3.2 | p=0.0001 | **2.5** | 1.1-5.7 | p=0.0282 | **2.3** | 1.5-3.6 | p=0.0012 |
| PCB 138[#] | **2.2** | 1.6-3.1 | p=0.0002 | **2.4** | 1.1-5.0 | p=0.0295 | **1.9** | 1.3-2.9 | p=0.0041 |
| PCB 153[#] | **2.3** | 1.6-3.2 | p=0.0003 | **2.4** | 1.8-5.5 | p=0.0383 | **2.1** | 1.3-3.2 | p=0.0043 |
| PCB 156[§] | **2.1** | 1.4-3.1 | p=0.0010 | *2.2* | *0.93-5.4* | *p=0.0679* | **1.9** | 1.2-3.0 | p=0.0154 |
| PCB 157[§] | **2.1** | 1.4-3.1 | p=0.0010 | *2.3* | *0.98-5.4* | *p=0.0558* | **1.8** | 1.1-2.9 | p=0.0169 |
| PCB 167[§] | **2.2** | 1.4-3.7 | p=0.0036 | *3.0* | *0.93-9.4* | *p=0.0643* | **2.2** | 1.2-4.1 | p=0.0203 |
| PCB 180[#] | **2.1** | 1.4-3.1 | p=0.0022 | *2.2* | *0.85-5.5* | *p=0.0942* | **1.8** | 1.1-2.9 | p=0.0305 |
| PCB 189[§] | **2.0** | 1.2-3.2 | p=0.0089 | *2.3* | *0.8-6.7* | *p=0.1164* | *1.7* | *0.92-3.0* | *p=0.0847* |
| BDE 49 | **1.7** | 1.4-2.2 | p=0.0003 | *1.6* | *0.93-2.8* | *p=0.0844* | **1.6** | 1.2-2.1 | p=0.0062 |
| BDE 100 | **1.8** | 1.2-2.6 | p=0.0075 | *1.9* | *0.7-4.9* | *p=0.1643* | **1.7** | 1.0-2.9 | p=0.0384 |
| 4,4'-DDT | *0.6* | *0.2-1.8* | *p=0.3654* | **9.5** | 3.1-29 | p=0.0011 | *0.9* | *0.2-3.1* | *p=0.8114* |
| 4,4'-DDD | **1.4** | 1.1-1.9 | p=0.0067 | *1.4* | *0.8-2.6* | *p=0.1848* | *1.4* | *1.0-1.9* | *p=0.0548* |
| 4,4'-DDE | **2.0** | 1.4-2.7 | p=0.0004 | *2.0* | *0.93-4.2* | *p=0.0709* | **2.1** | 1.4-3.1 | p=0.0022 |
| HCB[$] | **1.4** | 1.2-1.7 | p=0.0006 | *1.2* | *0.89-1.8* | *p=0.2876* | **1.3** | 1.1-1.6 | p=0.0121 |
| β-HCH[$] | **1.5** | 1.1-2.1 | p=0.0119 | *0.9* | *0.7-1.1* | *p=0.1520* | **1.8** | 1.3-2.6 | p=0.0033 |
| 2,3,4,7,8-PentaCDF[$] | **1.5** | 1.1-2.2 | p=0.0286 | **2.5** | 1.2-5.3 | p=0.0214 | **1.7** | 1.1-2.6 | p=0.0252 |

| compound | TMF from all biota (n = 15) | 95% CI of TMF from all biota | signifi-cance of TMF from all biota | TMF from fish only (n = 12) | 95% CI of TMF from fish only | signifi-cance of TMF from fish only | TMF without plankton (n = 14) | 95% CI of TMF without plankton | signifi-cance of TMF without plankton |
|---|---|---|---|---|---|---|---|---|---|
| 2,3,7,8-TetraCDF | **1.7** | 1.4-2.0 | p<0.0001 | **1.9** | 1.2-2.9 | p=0.0120 | **1.7** | 1.3-2.2 | p=0.0005 |
| D5 | **0.7** | 0.5-0.9 | p=0.0245 | *0.6* | *0.3-1.0* | *p=0.0606* | **0.5** | 0.4-0.7 | p=0.0005 |

TMFs were derived from lipid weight concentration data. Significance: given for the two-tailed test. # non-dioxin-like PCB; & one outlier removed; $ concentration below LOQ in mussels, substituted by 0.5 * LOQ concentration; § dioxin-like PCB. Significant TMFs are printed bold; TMF not significantly > 1 (< 1 in case of D5) are printed in italics.

**Table 13: TMFs derived for non-lipophilic benchmark compounds: for the whole set of samples, the set of samples without the plankton fraction, and the set of samples without the plankton fraction and the two zebra mussel samples (only fish)**

| compound | TMF from all biota (n = 15) | 95% CI of TMF from all biota | signifi-cance of TMF from all biota | TMF from fish only (n = 12) | 95% CI of TMF from fish only | signifi-cance of TMF from fish only | TMF without plankton (n = 14) | 95% CI of TMF without plankton | signifi-cance of TMF without plankton |
|---|---|---|---|---|---|---|---|---|---|
| Hg | **2.3** | 1.7-3.2 | p<0.0001 | **2.4** | 1.1-5.1 | p=0.0252 | **2.4** | 1.6-3.6 | p=0.0005 |
| MeHg | **3.2** | 2.2-4.5 | p<0.0001 | **2.4** | 1.2-4.7 | p=0.0179 | **2.4** | 1.7-3.5 | p=0.0002 |
| PFOS | **3.2** | 1.9-5.3 | p=0.0003 | *2.1* | *0.8-5.4* | *p=0.1226* | **4.1** | 2.3-7.6 | p=0.0003 |
| PFDA$ | **3.0** | 1.8-4.9 | p=0.0003 | *1.6* | *0.7-4.0* | *p=0.2416* | **3.6** | 2.0-6.5 | p=0.0006 |
| PFDoA | **2.0** | 1.4-3.0 | p=0.0015 | *1.7* | *0.7-4.2* | *p=0.1949* | **1.8** | 1.1-2.8 | p=0.0224 |

TMFs were derived from dry weight concentration data. Significance: given for a two-tailed test. $ concentration below LOQ in one mussel sample, substituted by 0.5 * LOQ concentration. Significant TMFs are printed bold; TMF not significantly > 1 are printed in italics.

Concentrations for diclofenac, other pharmaceuticals and pyrethroids were below LOQ in most samples so that no TMF could be determined. Analyses for chlorinated paraffins are delayed due the COVID-19 pandemic and will be reported separately by a publication in a peer-reviewed journal.

The TMF data are discussed in detail against literature values in the publication ‘Food web on ice: A pragmatic approach to investigate the trophic magnification of chemicals of concern' by Kosfeld et al. (the draft manuscript was provided to the German Environment Agency prior to submission). The abstract of the paper is shown in the following textbox.

**Abstract of the draft article: 'Food web on ice: A pragmatic approach to investigate the trophic magnification of chemicals of concern' by V. Kosfeld, H. Ruedel, C. Schlechtriem, C. Rauert, J. Koschorreck; submitted as open access publication to a peer-reviewed journal**

**Background:** The trophic magnification factor (TMF) is a metric that describes the average trophic magnification of a chemical through a food web under realistic environmental conditions. TMFs may be used for the risk assessment of chemicals, although TMFs for single compounds can vary considerably between studies despite thorough guidance is available in the literature to eliminate potential sources of error. The practical realization of a TMF investigation is quite complex while often only a few chemicals can be investigated due to low sample amounts. This study evaluated whether a more pragmatic approach that is based on practices of the German Environmental Specimen Bank (ESB) is feasible to obtain food web samples which can be sufficiently characterized to investigate the bioaccumulation behavior of chemicals with diverse properties. 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. 15 composite biota samples were 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 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 could show that 32 out of 35 compounds analyzed had TMFs significantly above 1. In the remaining two cases, an enrichment was also seen, but they were not statistically relevant.

**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 here provided benchmarking patterns.

# 12 Final Project workshop

The 'Food web on ice' project was introduced comprehensively to the scientific community by researchers from Fraunhofer IME, Umweltbundesamt and project partners during a two-day online workshop on March 16/17, 2021. Examples from other studies on trophic magnification presented by scientists from Italy and France provided a broader picture. An invited keynote presentation on TMF research built the basis for the scientific exchange. About 75 scientists from regulatory agencies, research institutes and industry participated in the workshop. Participants came from 12 countries including Canada and the USA.

An overview of the workshop program is shown in Table 14. The full workshop documentation is provided as separate digital Attachment 10 (zip-archive file).

**Table 14: Program of the 'Food web on ice' project workshop in March 2021 (online event).**

| Title of presentation | Speaker | Affiliation |
|---|---|---|
| **Day 1 - Session 'Planning, conduction and plausibility testing of the Lake Templin TMF study approach'** | | |
| Welcome address | Susanne Walter-Rohde | Umweltbundesamt, Dessau-Rosslau (DE) |
| Motivation for the UBA project 'Food web on ice' | Jan Koschorreck | Umweltbundesamt, Dessau-Rosslau (DE) |
| Study considerations, planning and organization | Heinz Rüdel | Fraunhofer IME, Schmallenberg (DE) |
| Biota sampling for the 'Food web on Ice' | Verena Kosfeld | Fraunhofer IME, Schmallenberg (DE) |
| Sample characterization and stable isotope data | Heinz Rüdel | Fraunhofer IME, Schmallenberg (DE) |
| Plausibility check of the system - TMF data for POPs | Verena Kosfeld | Fraunhofer IME, Schmallenberg (DE) |
| Accumulation of chlorinated paraffins in the Lake Templin food webs | Bo Yuan | ACES, Department of Environmental Science, Stockholm University (SE) |
| Target, suspect and non-target screening of polar substances in Lake Templin food web samples | Qiuguo Fu | EAWAG, Dübendorf (CH) |
| Passive sampling of Lake Templin water and suspect screening of pollutants | Mara Grodtke | UFZ, Leipzig (DE) |
| Lessons learnt from the Lake Templin TMF study | General discussion | - |
| **Day 2 - Session 'Use of TMF studies for the risk assessment of chemicals'** | | |
| Summary of Lake Templin TMF study and discussions of Day 1 | Heinz Rüdel | Fraunhofer IME, Schmallenberg (DE) |
| Use of biomagnification data in chemicals' risk assessment | Caren Rauert | Umweltbundesamt, Dessau-Rosslau (DE) |

| Title of presentation | Speaker | Affiliation |
|---|---|---|
| Keynote presentation: Introduction to the TMF concept | Katrine Borgå | University of Oslo (NO) |
| Determination of TMFs for PFAS in French rivers and estuaries | Hélène Budzinski | University of Bordeaux (FR) |
| Trophic Magnification of Legacy (PCB, DDT, Hg) and Emerging Pollutants (PFAS) in the Fish Community of a Small Protected Southern Alpine Lake | Stefano Polesello | Water Research Institute (IRSA) - CNR, Brugherio (IT) |
| Prospects and limits of TMFs in support of chemicals risk assessment | General discussion | - |

During the general discussion on day 1 of the workshop, the following issues were covered:

- Criteria for the selection of baseline organisms for the calculation of trophic positions (e.g., primary consumers as applied in this report);
- Possible influence of gut clearance of mussels on contaminant levels (possible disadvantage of using tap water as applied in this study);
- Possible benefits of using amino acid-specific stable isotope patterns for trophic position determinations ($\delta^{15}N$, $\delta^{13}C$);
- Possible use of passive sampling data (from Chemcatchers or PDMS sheets) to support the TMF evaluations (by allowing the estimation of dissolved water concentrations to be used for BAF calculations for comparisons/plausibility checks);
- Possible trophic magnification of polar compounds (bioconcentration by gill uptake vs. biomagnification by diet uptake).

On day 2, the discussion focused on the following topics:

- Use of TMFs in regulatory context: for the EU Water Framework Directive often BAF derived from field studies are used for quality standards deviations instead of a combination of BCFs derived from laboratory tests and TMFs;
- BAFs and BMFs are often derived from TMF studies; thus the guidance for TMF study design should cover also BAF/BMF determinations;
- It was emphasized that TMFs for higher tier assessment (e.g., to support the ‘B’ classification) can only to be applied for chemicals already in use (retrospective assessment);
- The lipid content of target biota may show a seasonal variation, so that the sampling period should be chosen carefully also considering this aspect;
- Biotransformation of PFAS-precursors may have influence on the observed TMFs of PFAS;
- Potential application of future TMF studies to investigate the accumulation of plastics in food webs (not possible with the food web samples derived in this study since the gastrointestinal tract where microplastics in fish are expected was removed before sample preparation; see section 13.2.2).

# 13 Support of the project by a scientific advisory board

## 13.1 Scientific advisory board members

In order to achieve a national and international exchange of knowledge and experiences networking with experts from various disciplines was initiated. Especially a scientific advisory board was built by inviting renowned international scientists with expertise in bioaccumulation research (Table 15).

**Table 15: Members of the scientific advisory board**

| Name | Affiliation | Comment |
|---|---|---|
| Dr. Marc Babut | IRSTEA - National Research Institute of Science and Technology for Environment and Agriculture Freshwater ecosystems, Ecology, Pollutions Research Unit, 69625 Villeurbanne, France | |
| Prof. Dr. Katrine Borgå | University of Oslo, Section for Aquatic Biology and Toxicology, Blindern, 0316 Oslo, Norway | |
| Dr. Eric Bruns | Bayer AG, Research & Development, Crop Science, Team Aquatic Organisms, 40789 Monheim, Germany | since April 2019 |
| Sara Danielsson | Swedish Museum of Natural History, Department of Environmental Research & Monitoring, 10405 Stockholm, Sweden | |
| Prof. Dr. Michael McLachlan | ACES - Department of Environmental Science and Analytical Chemistry, Stockholm University, 11418 Stockholm, Sweden | |
| Derek Muir, Ph.D. | Environment and Climate Change Canada, Aquatic Contaminants Research Division, Burlington, ON L7S1A1, Canada | |
| Eric Verbruggen | National Institute for Public Health and the Environment (RIVM), Centre for Safety of Substances and Products, 3720 BA Bilthoven, The Netherlands | |
| Kent B. Woodburn, Ph.D. | The Dow Chemical Company, Environmental Toxicology, Midland, MI 48674, USA | until retirement in December 2018 |

During the project period, several meetings were organized with members of the international advisory board. Most meetings took place as satellite events parallel to the SETAC Europe Conferences in Rome 2018, Helsinki 2019 and Dublin 2020 (the latter only virtually due to the COVID-19 pandemic). Additional meetings were organized as virtual web conferences.

During the meetings, the project team shared the status of the project and invited comments from the advisory board members. The kind support of the members of the scientific advisory board is gratefully acknowledged.

## 13.2 Suggestions of the advisory board taken into account

### 13.2.1 Passive sampling

In an advisory board meeting during the planning phase, the inclusion of passive sampling to determine water concentrations of pollutants was recommended.

The recommendation was considered. In cooperation with UFZ Leipzig research center, a passive sampling campaign was conducted. End of June 2018, three cages equipped with different sorbent materials (silicone sheets, Chemcatcher) were placed at three sites for different time periods (see section 4.2). By this means the recommended checks for concentrations of legacy contaminants and steady state conditions during the sampling campaign can be performed (example shown in section 8).

### 13.2.2 Removal of the gastrointestinal tract (GIT)

In an advisory board meeting it was recommended to remove the GIT of the individually sampled fish to prevent a potential bias of pollutant burdens by sediment taken up by the fish.

The recommendation was considered. During the dissection of fish, the GIT of each fish (for individuals > 20 cm) was removed, as suggested by members of the advisory board. In this step, the composition of the stomach content was also checked and the fish's last meal roughly classified as well as possible. Stomach content and GIT were stored at cryogenic temperatures for additional investigations.

Following the discussion with the advisory board during the meeting in Helsinki in 2019, GIT contents were freeze-dried and some samples were subjected to stable isotope analysis (see section 9.2).

The information derived from the GIT investigations supported the selection of samples for the final set of food web items.

### 13.2.3 Field blanks

Advisory board members suggested to include field blanks into the sampling campaign. Field blanks are commonly used to investigate whether measured contaminants concentrations are deriving from sampling artifacts. The recommendation was considered.

Plankton field blanks were collected in an uncontaminated water body near Lake Templin. The passive sampling campaign involved exposure of sorbent material to air during sampling activities to exclude that airborne contaminants are part of the total contaminants determined. Commercially available fillet of Alaska pollock (*Gadus chalcogrammus*) obtained from a local supermarket was used as field blank for fish. Likewise, commercially available seafood was used as mussel field blank (however, these samples could not be analyzed routinely due to a limited budget and serve only as back-up samples for future investigations). These field blanks of marine origin are expected to have only low levels of organic contaminants and thus should help to identify possible contaminations during sampling or processing.

The pollock sample was investigated parallel to all measurement series of organic compounds. As expected, found traces of compounds were either below LOQ or negligible in comparison to the concentrations of the target substances in the food web samples (except for mercury as ubiquitous contaminant).

# 14 Acknowledgments

The authors acknowledge the support of the project by numerous persons and institutions and thank especially

- the members of the advisory board:
  Dr. Marc Babut, Prof. Dr. Katrine Borgå, Dr. Eric Bruns, Sara Danielsson, Prof. Dr. Michael McLachlan, Derek Muir Ph.D., Eric Verbruggen, Kent B. Woodburn Ph.D.

- the persons and institutions involved in the sampling campaign:
  Steffen Zahn and colleagues, Institut für Binnenfischerei Potsdam-Sacrow;
  Dr. Wolfgang Arp (LimPlan) and Bernd Koppelmeyer (enviteam);
  Dr. Albrecht Paschke and Mara Grodtke (UFZ);
  the boat team at Wasser-und Schifffahrtsamt Potsdam.

- the members of the ESB lab team involved in the sample treatment at Fraunhofer IME:
  Martin Weingärtner, Sonja Uhlig.

- the persons and institutions involved in the analysis of the samples:
  Dr. Nina Lohmann and team, Eurofins GfA Lab Service GmbH;
  the team of agroisolab GmbH; Bo Yuan, University of Stockholm, Sweden;
  Georg Radermacher, Kathrin Schneider (Fraunhofer IME);
  Dr. Bernd Göckener and team (Fraunhofer IME);
  Dr. Burkhard Knopf, Dirk Hansknecht, Annette König (Fraunhofer IME);
  Dr. Heidrun Windisch, Dana Esser (Fraunhofer IME).

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