



Retrospective monitoring of mercury in fish from selected European freshwater and estuary sites



Regine Nguetseng^a, Annette Fliedner^a, Burkhard Knopf^{a,*}, Benoit Lebreton^b, Markus Quack^c, Heinz Rüdell^a

^aFraunhofer Institute for Molecular Biology and Applied Ecology (Fraunhofer IME), 57392 Schmallenberg, Germany

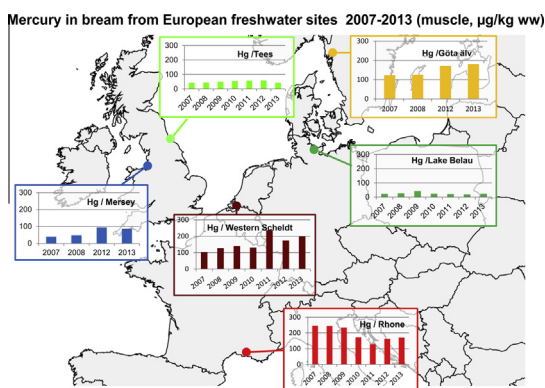
^bUMR Littoral, Environnement et Sociétés (LIENSs), CNRS – Université de La Rochelle, Institut du littoral et de l'environnement, 17000 La Rochelle, France

^cDepartment of Biogeography, Trier University, 54286 Trier, Germany

HIGHLIGHTS

- Total Hg and Methyl-Hg were analyzed in bream from five rivers and one lake in Europe.
- Between 2007 and 2013 the environmental quality standard for Hg was exceeded at all riverine sites.
- Highest Hg levels were detected in the Rhône (FR), Göta älv (SE) and Western Scheldt (NL).
- Since 2007 THg and Methyl-Hg significantly decreased in bream from the Rhône estuary.
- The Methyl-Hg fraction of THg was always >80% and comparable at all riverine sites.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 20 January 2015

Received in revised form 23 April 2015

Accepted 28 April 2015

Available online 15 May 2015

Keywords:

Trend monitoring

Biota monitoring

Environmental quality standard

Abramis brama

Mercury

Methylmercury

ABSTRACT

Levels and trends of total mercury (THg) and methylmercury (MeHg) compounds in bream (*Abramis brama*) from different European sites were compared. Bream were collected between 2007 and 2013 in the estuaries of the rivers Scheldt (Netherlands), Rhône (France), Göta älv (Sweden), Tees (United Kingdom), and Mersey (UK), and in Lake Belau (Germany). A direct mercury analyzer was used to determine THg concentrations while MeHg was measured by gas chromatography/inductively coupled plasma-mass spectrometry applying stable isotope dilution. THg and MeHg in annual pool samples of bream ranged between 15.9 and 251 $\mu\text{g kg}^{-1}$ wet weight (ww) with lowest concentrations found at the reference site Lake Belau and highest in samples from the river Rhône. The EU environmental quality standard (EQS) of 20 $\mu\text{g kg}^{-1}$ ww was exceeded at all sites and in all years except at Lake Belau in 2012. Significantly decreasing trends over time were observed only in bream from the Rhône, while THg increased in bream from the Western Scheldt. The MeHg fractions of THg were always >80% and a significant difference between sites was detected only in one case (Rhône vs. Lake Belau).

© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Mercury (Hg) is in the focus of environmental science since many years because of its ubiquitous presence in the ecosphere and its high toxicity. It exists in a large number of different

* Corresponding author.

E-mail address: burkhard.knopf@ime.fraunhofer.de (B. Knopf).

physical and chemical forms of which elemental Hg and monomethylmercury compounds (CH_3HgX , here designated as MeHg) are the most relevant. Especially MeHg is of great concern because of its combination of high toxicity and high potential for bioaccumulation and biomagnification (EQS substance data sheet, 2005; Merian et al., 2004).

A major part of the environmental Hg is of anthropogenic origin and enters aquatic ecosystems via atmospheric deposition (E-PRTR, 2014; Lepom et al., 2012; Merian et al., 2004; Pacyna et al., 2009). Emission reduction is therefore of decisive importance. With the new Minamata Convention on Mercury (UNEP, 2013) a global treaty was agreed upon to protect human health and the environment. It includes, e.g., a ban on new mercury mines, the phase-out of existing ones and control measures on air emissions.

The European Water Framework Directive (WFD, EC, 2000) sets an environmental quality standard (EQS) for Hg at $20 \mu\text{g kg}^{-1}$ wet weight (ww) in fish (EC, 2008, 2013). The EQS is intended to protect top predators against secondary poisoning and refers to total Hg (i.e., the sum of inorganic and organic Hg species). However, because of its special environmental relevance, speciation analysis is worthwhile to determine the fraction of MeHg in fish samples and study whether this fraction is constant or differs between years and geographical sites. This approach allows a risk assessment based on the actual present Hg species.

The aim of the present study is the comparison of different freshwater sites in Europe regarding levels and trends of THg and MeHg in fish. To enhance comparability, uniform methods of chemical analysis were used for all samples. Moreover, the study focused on one fish species, namely bream (*Abramis brama*) which is abundant at all selected sites.

2. Materials and methods

2.1. Sampling

In order to ensure a high degree of continuity and consistency, sampling and sample treatment were performed according to standard operating procedures developed for the German Environmental Specimen Bank (ESB, German Federal Environment Agency, 2014; Klein et al., 2012; Paulus et al., 1996; Rüdél et al., 2008). Bream (*A. brama*) was sampled between 2007 and 2013 at six European sites, i.e., in the rivers Scheldt (Netherlands), Rhône (France), Göta älv (Sweden, no sampling 2009–2011), Tees (United Kingdom, UK), and Mersey (UK, no sampling 2009–2011) and in Lake Belau (Germany). The sampling was performed in the context of a project designed to monitor changes of environmental concentrations of hexabromocyclododecane (Rüdél et al., 2012). Briefly, about 15 fish were collected per site and year after the spawning season. After sampling and dissecting the muscle tissues were directly cooled to -150°C . Pooled annual samples were prepared by a cryomilling procedure (Rüdél et al., 2008) using a Palla VM-KT with titanium milling cylinder and titanium milling rods (manufactured by KHD, Cologne) cooled to $<-150^\circ\text{C}$ with liquid nitrogen before usage. The homogenized sample material was transferred into glass vials and stored above liquid nitrogen.

2.2. Sampling sites

The river sampling sites are located near the river mouths or in the estuary area, the respective geographical coordinates are summarized in Table S1 (Supplementary material).

Scheldt: The River Scheldt flows through France, Belgium and the Netherlands before it enters the North Sea. Its catchment area is about $22,000 \text{ km}^2$ with an average population density of 352

inhabitants km^{-2} (International Scheldt Commission, 2015). The sampling site 'Western Scheldt' is located near Appenzak in the industrialized estuary area of the Scheldt downstream of Antwerp. The water quality here is poor and affected by industrial and urban waste waters. Likely Hg sources are the chloralkali industry, the nonferrous industry, PVC production and phosphate industry located around the harbor of Antwerp or the tributaries of the Scheldt (Baeyens et al., 1998). Sediment contamination is high with reported Hg levels up to $1900 \mu\text{g kg}^{-1}$ in the early 1990s (Baeyens et al., 1998; Leermakers et al., 1993). In 2012, Hg emissions in the adjoining river basin district 'Scheldt in Flanders' amounted to 308 kg (air) and 4.05 kg (water) (E-PRTR, 2014, Supplementary material, Table S2).

Rhône: The Rhône originates in Switzerland and runs through Lake Léman and southeastern France. At Arles, shortly before entering the Mediterranean Sea the river divides into two branches, the Grand Rhône and the Petit Rhône. The catchment area of the French Rhône between Lake Léman and the Mediterranean Sea encompasses about $91,000 \text{ km}^2$ with an average population density of 98 inhabitants km^{-2} (Olivier et al., 2008). The sampling site 'Rhône' is located in the Grand Rhône downstream of Arles and is influenced by industrial and urban waste waters as well as by the surrounding agriculture. In the river basin district 'Rhône and Coastal Mediterranean' atmospheric Hg emissions stem mainly from chemical and metal working industry and added up to 758 kg in 2012. The majority of direct Hg emissions are associated with urban waste water treatment plants (WWTPs, E-PRTR, 2014).

Göta älv: The Swedish river Göta älv rises in Lake Vänern in western Sweden and flows into the Kattegat at Gothenburg. Its catchment area comprises approximately $48,000 \text{ km}^2$ with an average population density of about 21 inhabitants km^{-2} (Nilsson, 2006). The sampling site 'Göta älv' is located in the city of Gothenburg shortly upstream of the harbor area. The atmospheric Hg emissions in this area are mainly associated with chemical industry and waste incineration and were relatively low in 2012 (i.e., 32.4 kg). The direct releases into the water were even lower (i.e., 4 kg in 2012) and stem from urban WWTPs and from pulp industry (E-PRTR, 2014, Supplementary material, Table S2).

Mersey: The River Mersey originates from the confluence of the rivers Tame and Goyt in North West England and enters the Irish Sea at Liverpool. Its catchment area covers approximately 4700 km^2 and is densely populated ($1070 \text{ inhabitants km}^{-2}$, website of the Mersey Basin Campaign). The adjacent city of Manchester was one of the leading industrial cities in England in the 19th and 20th century and its industrial activities had a major impact on the environment. Heavy industry and textile industry, however, have turned down in the 1960s and the large port of Manchester closed in the 1980s. Since then the water quality of the rivers Irwell and Mersey and of the Manchester Ship Canal have greatly improved but a legacy of former times can still be found in the sediments of the Mersey estuary where average Hg concentrations of 0.84 mg kg^{-1} and 3.16 mg kg^{-1} were detected in the surface sediment (0–10 cm depth) and the 10–50 cm layer, respectively, in 2000–2002. The lower Hg concentrations in the surface sediments are attributed to more stringent emissions controls and diminution in manufacturing since the 1970s (Vane et al., 2009). Nowadays atmospheric Hg emissions as well as direct releases into the water are mostly caused by chemical industry and waste management. Other contributors are mineral industries, thermal power stations, gas and oil refineries and (for direct releases) urban WWTPs. In 2012 atmospheric Hg emissions of 505 kg were reported compared to direct emissions into the water of 42 kg (E-PRTR, 2014, Supplementary material, Table S2). The sampling site 'Mersey' is located near Warrington about 20 km upstream of Runcorn where the Mersey widens into its estuary.

Tees: River Tees in the northeast of England rises in the Cross Fell and enters the North Sea near Middlesbrough. Its catchment area covers 1840 km². The population density in the Tees valley is about 840 inhabitants km⁻² (JMP, 2010). The Tees sampling site is situated near Stockton-on-Tees about 25 km upstream of the Tees mouth. In the Tees area and the river basin district Northumbria metal working- and mineral industries are responsible for most of the atmospheric Hg emissions while direct inputs into the water are mostly caused by urban WWTPs and metal working industry. Atmospheric emissions in 2012 amounted to 53 kg while releases into the water were only around 13 kg (E-PRTR, 2014, Supplementary material, Table S2).

Lake Belau: Lake Belau was selected because of its location in a rural area with little anthropogenic influence. The lake is located approximately 30 km south of Kiel and 70 km north of Hamburg and belongs to the river basin district Schlei/Trave. Atmospheric Hg emissions in Kiel are mainly related to combustion in of thermal power stations while emissions from industry and coal-burning power plants are responsible for the majority of atmospheric Hg emissions in the area around Hamburg (E-PRTR, 2014).

2.3. Stable isotope analysis for nitrogen

Cryo-milled muscle tissue samples were freeze-dried and analyzed raw. Baselines for $\delta^{15}\text{N}$ values, i.e., trophic level (TL) = 1, refer to organic matter of suspended particulate matter (SPM) in rivers (available for 1–3 sampling years) or sediment (upper 1–2 cm layer) in Lake Belau (available for 2012). Sampling of SPM and sediment was performed by M. Ricking, Institute for Hydrogeology and Environmental Geochemistry, Freie Universität Berlin, Germany, based on protocols by Schulze et al. (2007). It is assumed that the $\delta^{15}\text{N}$ values of these matrices approximate that of primary producers because their organic fractions are mostly composed of detritus. They also represent a mixture of the potential food sources available to primary consumers. TLs were computed using data of fish and SPM or sediment from overlapping sampling periods.

Analyses were realized using an elemental analyzer (Flash EA 1112, Thermo Scientific, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta V Advantage with a ConFlo IV interface, Thermo Scientific, Bremen, Germany) at LIENSs stable isotope facility of the University of La Rochelle, France. Results are expressed in the δ unit notation as deviations from a standard (N_2 in air) following the formula: $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where R is $^{15}\text{N}/^{14}\text{N}$ (McCutchan et al., 2003; Post, 2002). Reference gas was calibrated against reference materials (IAEA-N2, IAEA-NO-3, IAEA-600). The analytical precision, based on the analyses of acetanilide (Thermo Scientific) used as laboratory internal standards interspersed among the samples, was <0.15‰ for $\delta^{15}\text{N}$ values.

2.4. Hg determination

Measurement of total mercury (THg) was performed with dedicated atomic absorption spectrometry (AAS) methods applying Direct Mercury Analyzer (DMA) instruments (DMA-80 instruments for solid and liquid samples, respectively; MLS GmbH, Leutkirch, Germany). Measurements of dried solid samples were directly performed by DMA-80 without any further pretreatment. About 50 mg of the dried samples were weighted into nickel combustion vessels and decomposed thermally by controlled heating (300 °C for 30 s – drying; 850 °C for 180 s – decomposition; 60 s – purge; 12 s – heating amalgamator; 30 s – measurement). Decomposition products were carried by an oxygen flow to a catalyst and through a mercury amalgamator which collects Hg^0 . Afterwards the amalgamator was heated and Hg^0 was released

and quantified. For liquid samples mercury was reduced by tin(II)-chloride and ascorbic acid (20 g $\text{SnCl}_2 \cdot 2 \text{H}_2\text{O}$, 20 mL 30% hydrochloric acid, 1 g ascorbic acid, filled up to 1 L with ultrapure water). Hg^0 was cast out by a nitrogen flow and collected in a mercury amalgamator from which it was released after heating and quantified. The limits of quantification (LOQs) were 2.4 $\mu\text{g kg}^{-1}$ for the solid sample DMA and 0.1 $\mu\text{g L}^{-1}$ for the liquid sample DMA, respectively. The recovery of certified reference material (DORM-4, National Research Council Canada, Canada; 410 \pm 55 ng Hg g^{-1}) was 96 \pm 1% ($n = 33$) for solid samples and 115 \pm 10% ($n = 21$) for liquid samples. For the liquid sample DMA liquid certified reference material (NIST 1641d, National Institute of Standards and Technology, USA, 1.557 $\mu\text{g Hg L}^{-1}$) was measured along with the samples. The recovery of this material was 107 \pm 8% ($n = 15$).

Analysis of MeHg was performed with SID-GC/ICP-MS (stable isotope dilution-gas chromatography coupled to inductively coupled plasma-mass spectrometry). Pooled muscle tissue samples were extracted by a microwave assisted procedure with tetramethylammonium hydroxide (TMAH). After extraction, samples were derivatized with sodium tetrapropylborate. Volatile mercury species were then extracted in *n*-hexane and analyzed by SID-GC/ICP-MS. For details see Davis et al. (2007) and Monperrus et al. (2004). The respective LOQs were in the range of 0.02–0.15 $\mu\text{g kg}^{-1}$ (derived from blanks measured along with each set of samples). All data are reported on a wet weight (ww) basis. The recovery of certified reference material (DORM-4, 354 \pm 31 ng MeHg g^{-1}) was 99 \pm 1% ($n = 35$).

2.5. Data treatment

TLs were calculated according to the following formula: $\text{TL} = ((\delta^{15}\text{N}_{\text{fish}} [\text{‰}] - \delta^{15}\text{N}_{\text{food source}} [\text{‰}]) / 3.4) + 1$ (Post, 2002), where $\delta^{15}\text{N}_{\text{food source}}$ is the $\delta^{15}\text{N}$ value of the organic matter of SPM or sediment sampled at the respective fish sampling site.

Statistical analysis of the data was performed applying tools from the VassarStats website (<http://vassarstats.net/index.html>). THg and MeHg levels, MeHg/THg ratios, mean condition factors (CFs) and $\delta^{15}\text{N}$ values of fish from sites with continuous sampling were compared by the two-sided non-parametric Mann–Whitney U-test. Correlations were analyzed using Pearson's correlation coefficient.

Time series comprising at least four consecutive years were analyzed for temporal trends with the two-sided non-parametric Mann–Kendall test (Salmi et al., 2002) and a statistical program retrieved from the Arctic Monitoring and Assessment Programme homepage (Bignert, 2007).

3. Results and discussion

3.1. Comparability of fish

Biometrical data of the fish are summarized in Table S1 (Supplementary material). Mean fish size and age varied between sites and years with generally smaller and younger fish sampled in the Rhône and the Mersey. During the study period bream from the Western Scheldt and Lake Belau slightly increased in age and size (Mann–Kendall Test, $\alpha = 0.05$) whereas no such trends were observed elsewhere. Mean CFs of bream from all sites were in the range of 1.0–1.4 g cm^{-3} with low standard deviations ($\leq 0.2 \text{ g cm}^{-3}$). Differences in CFs between sites were not detected (Supplementary material, Tables S1, S5), nor were temporal trends of the CFs during the study period. Only bream from the Mersey seemed to be in a better nutritional state in 2012/2013 compared to 2007/2008, however, no trends were calculated here because sampling was omitted between 2009 and 2011. A good nutritional

state of bream corresponds to a CF of about 1.1 g cm^{-3} whereas values below 0.9 g cm^{-3} indicate suboptimal life conditions. Thus, all bream analyzed here were in a good nutritional state.

3.2. Trophic position of fish

The trophic position of bream was determined based on the concept of continuous enrichment of the stable nitrogen isotope ^{15}N between food source and consumer (McCutchan et al., 2003; Minagawa and Wada, 1984; Post, 2002; Vander Zanden et al., 1997). The $\delta^{15}\text{N}$ value can therefore be used as indicator for the trophic position in the respective ecosystem, i.e., the higher the $\delta^{15}\text{N}$ value the higher the trophic position of the organism.

Especially when dealing with biomagnifying substances like Hg and its compounds which may pose a serious risk to top predators, the trophic position of the analyzed organism is of interest.

Mean $\delta^{15}\text{N}$ values of bream are summarized in Table 1. Values ranged from 9.9‰ (Göta älv) to 18.1‰ (Western Scheldt) and differed significantly between sites ($p = 0.002$, Supplementary material, Tables S1, S3). The only exceptions were bream from the Rhône and Tees which had quite similar $\delta^{15}\text{N}$ values. Due to lack of data, Göta älv and Mersey were not included in the statistical analysis.

Calculation of trophic levels using the $\delta^{15}\text{N}$ value of the organic matter of SPM or sediment as baseline was possible only for the years 2008, 2010 and 2012. The calculated TL values differed between sites and years and ranged mainly between 2.5 and 3.3

Table 1
 $\delta^{15}\text{N}$ values (mean \pm standard deviation, range in parentheses) and trends in $\delta^{15}\text{N}$ determined in bream (*Abramis brama*) collected between 2007 and 2013 at five riverine sampling sites and one lake in Europe. Data refer to annual pooled muscle samples. Also shown are the calculated trophic levels (TL) using $\delta^{15}\text{N}$ data for suspended particulate matter or sediment as baseline (assumed TL = 1; data only for single years 2008, 2010, 2012). n: Number of pooled annual samples.

| Sampling site | Years | n | $\delta^{15}\text{N}$ (‰) | Trend | TL (2008) | TL (2010) | TL (2012) |
|-----------------|------------------------|---|----------------------------|-------|-----------|-----------|-----------|
| Western Scheldt | 2007–2013 | 7 | 18.1 ± 1.6 (16.2–20.0) | No | 3.0 | 3.9 | 3.3 |
| Rhône | 2007–2013 | 7 | 11.6 ± 0.5 (10.9–12.3) | No | 3.3 | – | 3.3 |
| Göta älv | 2007, 2008, 2012, 2013 | 4 | 9.9 ± 0.1 (9.8–10.0) | No | 3.1 | – | – |
| Tees | 2007–2013 | 7 | 11.8 ± 0.2 (11.3–12.0) | No | 2.6 | 2.7 | 3.1 |
| Mersey | 2007, 2008, 2012, 2013 | 4 | 11.6 ± 0.7 (11.0–12.6) | No | 2.5 | – | – |
| Lake Belau | 2007–2013 | 7 | 13.8 ± 0.7 (13.0–14.8) | No | – | – | 2.7 |

– No calculation possible (no $\delta^{15}\text{N}$ data available for SPM or sediment).

Table 2
Concentrations of total mercury (THg) and methylmercury compounds (MeHg) in muscle of bream (*Abramis brama*) sampled in five European rivers and one lake between 2007 and 2013. Data refer to pooled annual samples; given are means and standard deviations of replicate measurements.

| Sampling location | Sampling year | Number of fish in pool sample | THg ($\mu\text{g kg}^{-1}$ ww) | MeHg ($\mu\text{g kg}^{-1}$ ww) | MeHg fraction of THg (%) |
|-------------------|---------------|-------------------------------|---------------------------------|----------------------------------|--------------------------|
| Western Scheldt | 2007 | 7 | 103 ± 4 | n.a. | – |
| | 2008 | 15 | 127 ± 4 | 134 ± 1 | 105 |
| | 2009 | 15 | 140 ± 2 | 148 ± 1 | 105 |
| | 2010 | 15 | 131 ± 1 | 138 ± 1 | 106 |
| | 2011 | 15 | 236 ± 5 | 213 ± 5 | 90 |
| | 2012 | 15 | 173 ± 2 | 154 ± 4 | 89 |
| | 2013 | 9 | 199 ± 6 | 197 ± 22 | 99 |
| Rhône | 2007 | 15 | 246 ± 1 | 251 ± 3 | 102 |
| | 2008 | 15 | 244 ± 3 | 248 ± 3 | 102 |
| | 2009 | 15 | 234 ± 3 | 235 ± 2 | 100 |
| | 2010 | 15 | 172 ± 3 | 175 ± 6 | 102 |
| | 2011 | 15 | 129 ± 2 | 130 ± 3 | 101 |
| | 2012 | 15 | 163 ± 1 | 161 ± 2 | 99 |
| | 2013 | 15 | 171 ± 3 | 163 ± 27 | 96 |
| Göta älv | 2007 | 15 | 123 ± 1 | 126 ± 1 | 102 |
| | 2008 | 15 | 126 ± 4 | 122 ± 1 | 97 |
| | 2012 | 15 | 172 ± 2 | 171 ± 10 | 100 |
| | 2013 | 15 | 180 ± 2 | 211 ± 7 | 117 |
| Tees | 2007 | 15 | 43.0 ± 0.6 | 35.4 ± 0.6 | 82 |
| | 2008 | 15 | 44.9 ± 0.5 | 41.5 ± 0.3 | 92 |
| | 2009 | 15 | 49.5 ± 0.9 | 47.2 ± 0.5 | 95 |
| | 2010 | 15 | 55.6 ± 0.7 | 57.8 ± 4.3 | 104 |
| | 2011 | 15 | 58.2 ± 1.0 | 58.7 ± 1.6 | 101 |
| | 2012 | 14 | 59.4 ± 0.9 | 58.9 ± 2.0 | 99 |
| | 2013 | 14 | 42.9 ± 1.2 | 34.6 ± 2.5 | 81 |
| Mersey | 2007 | 11 | 38.8 ± 0.5 | 36.1 ± 0.8 | 93 |
| | 2008 | 15 | 48.5 ± 1.8 | 46.6 ± 4.7 | 96 |
| | 2012 | 11 | 94.7 ± 2.9 | 91.0 ± 10 | 96 |
| | 2013 | 10 | 86.3 ± 1.0 | 88.5 ± 5.7 | 103 |
| Lake Belau | 2007 | 15 | 22.9 ± 0.5 | 21.7 ± 0.4 | 95 |
| | 2008 | 15 | 27.3 ± 0.6 | 25.7 ± 0.2 | 94 |
| | 2009 | 15 | 42.6 ± 0.4 | 39.4 ± 0.2 | 92 |
| | 2010 | 15 | 24.3 ± 0.4 | 22.7 ± 0.3 | 94 |
| | 2011 | 15 | 21.3 ± 0.5 | 20 ± 0.3 | 94 |
| | 2012 | 15 | 18.2 ± 0.3 | 15.9 ± 0.4 | 87 |
| | 2013 | 15 | 23.5 ± 0.7 | 19.2 ± 1.0 | 81 |

n.a. – Not analyzed.

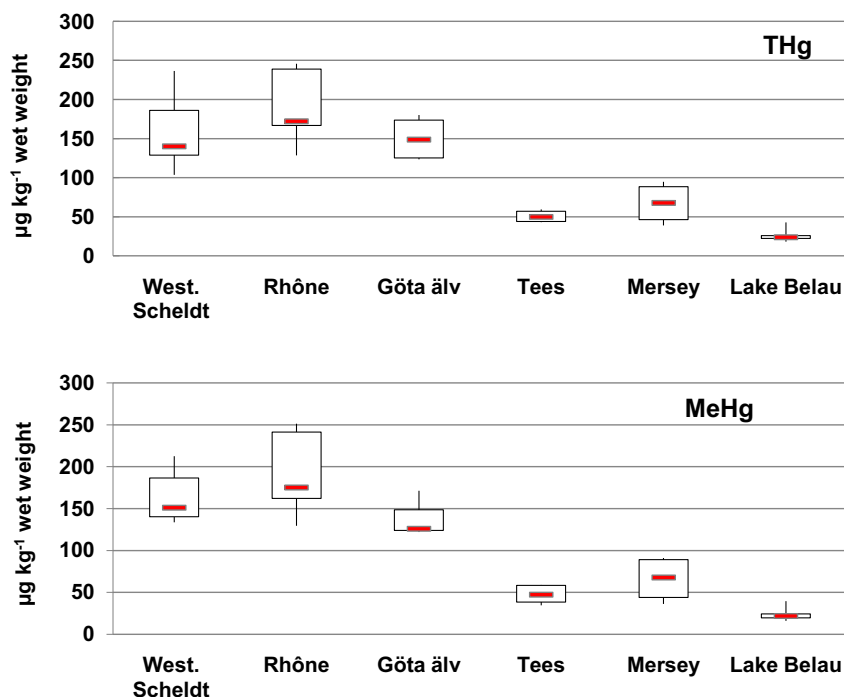


Fig. 1. Levels of total mercury (THg) and methylmercury compounds (MeHg) in muscle of bream (*Abramis brama*) sampled between 2007 and 2013 in five European rivers and one lake. Boxes defined by 1. and 3. quartile; red bar: median. Boxes of Göta älv and Mersey refer to four years only (2007, 2008, 2012, 2013). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

for bream, ranking them as primary to secondary consumers. However, bream caught in the Western Scheldt in 2010 apparently occupied a higher trophic position (TL 3.9) close to piscivorous predators like pike, pikeperch or perch (all TL ≥ 4). Based on diet studies, TL values of 3.1 ± 0.1 are reported for bream (Froese and Pauly, 2014) which is in good agreement with the majority of our findings.

3.3. THg and MeHg levels

The results of the mercury analyses are summarized in Table 2 and illustrated in Fig. S1 (Supplementary material).

Levels of THg and MeHg were highest in bream from the Rhône, the Western Scheldt, and the Göta älv. Bream from Lake Belau and the Tees were the least contaminated. At all sites concentrations of THg and MeHg were positively correlated ($p < 0.005$, Göta älv: $p = 0.04$; Supplementary material, Table S4). MeHg accounted for at least 80% of the THg concentration in all samples (Table 2, fractions above 100% reflect the combined measurement uncertainty of both analytical methods). A significant difference in MeHg/THg ratios was detected only between Lake Belau and the Rhône with a significantly lower ratio at Lake Belau ($p = 0.002$; Supplementary material, Table S5).

The difference in Hg levels between the sites is illustrated by the box plot diagram in Fig. 1 which integrates the data of the whole studied period. All sites except Western Scheldt and Rhône differed significantly ($p \leq 0.005$) with respect to the THg and MeHg contamination of bream (Supplementary material, Table S6). Göta älv and Mersey were not included in the statistical comparison because of discontinuous sampling.

The observed dissimilarity in Hg contamination between the sites probably reflects true differences in environmental Hg levels. Biases by age and trophic level are rather unlikely as no respective correlations are noticeable (Tables 1 and 2, Supplementary material, Table S1).

No comparative fish monitoring data are available for the river sampling sites under investigation. However, high Hg concentrations in Swedish freshwater fish have also been reported by others (e.g., Åkerblom et al., 2014; Miller et al., 2013; Nyberg et al., 2012) and are attributed mainly to atmospheric depositions (Meili et al., 2003). For roach (*Rutilus rutilus*) which resemble bream with respect to feeding behavior and trophic level (TL 3.0, Froese and Pauly, 2014), average Hg concentrations of $130 \mu\text{g kg}^{-1}$ ww (range $20\text{--}540 \mu\text{g kg}^{-1}$ ww) are reported in Swedish lakes around Uppsala in 1991–1993 (Sonesten, 2001). Åkerblom et al. (2014) report mean Hg levels of $270 \mu\text{g kg}^{-1}$ ww in roach sampled in Swedish lakes between 1975 and 2011. Even though these values are mostly higher than those detected in our bream (i.e., $123\text{--}180 \mu\text{g kg}^{-1}$) they nevertheless support our findings of high Hg levels at Göta älv.

Clearly lower levels are reported from the UK: Jürgens et al. (2013) detected Hg concentrations of $17\text{--}49 \mu\text{g kg}^{-1}$ ww in whole fish (roach and common bleak (*Alburnus alburnus*, TL 2.7) sampled in 4 rivers in eastern England between 2007 and 2011. These data are in good accordance to our finding at the east English site Tees ($43\text{--}59 \mu\text{g kg}^{-1}$ ww). No recent data are available for freshwater fish caught in the Mersey. However, the area is known for high historical inputs of Hg from chloralkali plants (website of the BGS). Between 1999 and 2001 Hg levels up to $374 \mu\text{g kg}^{-1}$ ww were reported for flatfish in the Mersey estuary (Marine Environment Monitoring Group, 2004), and in a survey on the state of the UK seas in 2007 highest Hg levels were detected in fish from the estuaries of Mersey and Thames (UKMMAS, 2010).

No fish monitoring data are available for the Rhône section around Arles. In 2003, eel (*Anguilla anguilla*) caught in a near-by lagoon and canal in the Camargue Nature Reserve had Hg concentrations of $160\text{--}610 \mu\text{g kg}^{-1}$ dry weight (Ribeiro et al., 2005). Assuming a conversion factor dry mass to wet mass of 0.378 for eel (Froese and Pauly, 2014) this would correspond to $60\text{--}230 \mu\text{g kg}^{-1}$ ww thus being in the same range as our bream from the Rhône ($129\text{--}246 \mu\text{g kg}^{-1}$ ww).

Table 3
Temporal changes in levels of total mercury (THg) and methylmercury compounds (MeHg) in bream (*Abramis brama*) from European freshwater sites. For sites with continuous annual sampling a trend analysis using the PIA program (Bignert, 2007) was performed.

| Sampling site | Sampling years | Temporal trends | |
|-----------------|------------------------|--|--|
| | | THg | MeHg |
| Western Scheldt | 2007–2013 | Yes, increase ($p < 0.02$) | No |
| Rhône | 2007–2013 | Yes, decrease ($p < 0.04$) | Yes, decrease ($p < 0.02$) |
| Tees | 2007–2013 | No | No |
| Lake Belau | 2007–2013 | No | No |
| | | Change ^a | |
| Mersey | 2007, 2008, 2012, 2013 | Significant increase (two-tailed t -test; $p < 0.02$) | Significant increase (two-tailed t -test; $p < 0.02$) |
| Göta älv | 2007, 2008, 2012, 2013 | Significant increase (two-tailed t -test; $p < 0.01$) | No |

^a Comparison of levels 2007 and 2008 vs. 2012 and 2013.

For the Scheldt estuary, Baeyens et al. (2003) report THg levels in roach ($n = 5$) of $71 \mu\text{g kg}^{-1}$ ww which is lower than what we observed in bream ($103\text{--}240 \mu\text{g THg kg}^{-1}$). This may be related to our findings regarding the relatively high trophic position of bream at this site which would go along with the uptake of higher contaminated food (Table 1).

For the reference site Lake Belau comparative bream data are available from the German ESB. Lepom et al. (2012) report mean THg concentrations in bream of $30.2 \mu\text{g kg}^{-1}$ ww for the years 1993–2009 which is in good agreement with our findings.

All fish except those from Lake Belau in 2012 clearly exceeded the European EQS of $20 \mu\text{g kg}^{-1}$ ww for THg by factors of up to 12. For the most recent study year (2013) the THg concentrations in bream were 1.2–9.9 times higher than the EQS with especially high values in bream from the Western Scheldt, the Göta älv and the Rhône (Table 2). Accordingly, top predators in these areas are assumed to be at risk of secondary poisoning. The requirements for fish and fishery products intended for human consumption are less stringent and allow a maximum of $500\text{--}1000 \mu\text{g kg}^{-1}$ THg ww in fishery products (EC, 2006). This value was not exceeded in any of the samples analyzed here.

3.4. Temporal trends

The results of the temporal trend analysis are summarized in Table 3, details are given in Table S7 (Supplementary material).

A decreasing temporal trend in Hg contamination was observed only for bream from the Rhône (yearly decreases: THg 8.5%, MeHg 9.3%; Supplementary material, Table S7). This may be related to decreasing atmospheric Hg emissions in the Rhône and Coastal Mediterranean area since 2008 (E-PRTR, 2014, Supplementary material, Table S2).

In contrast, THg in bream from the Western Scheldt increased during the study period (12% yearly increase; Supplementary material, Table S7) while no trend was detected for MeHg at this site. The increasing THg trend in bream cannot be explained by current emissions in the neighborhood since atmospheric emissions in this area were more or less constant between 2007 and 2012 while direct releases to the water decreased (E-PRTR, 2014, Supplementary material, Table S2). A possible explanation may be that Hg from the contaminated sediment was remobilized during dredging activities in the area around the Port of Antwerp and taken up by bream (TIDE, 2013).

In bream from the Göta älv and the Mersey THg levels increased significantly between 2007/2008 and 2012/2013 as did MeHg levels in bream from the Mersey (Table 3, Supplementary material, Fig. S1). Since sampling was discontinuous at these sites no trends were calculated. Regarding bream from the Mersey the observed increase may be related to the higher age and larger size of fish sampled in 2012/2013 (Supplementary material, Table S1).

According to the E-PRTR (2014) atmospheric Hg emissions in the area around Göta älv were higher in 2012 compared to 2007/2008 while direct releases to the water were generally lower and decreased between 2008 and 2012 (Supplementary material, Table S2). In contrast, decreasing atmospheric Hg emissions are reported for the Mersey area while direct releases were more or less constant (except for the year 2008 where emissions were about 4–5 times higher than in the other years; Supplementary material, Table S2).

No temporal trends were detected for bream from the Tees and Lake Belau. For the Tees area decreasing emissions and releases are reported between 2007 and 2011 which, however, slightly increased again in 2012 (E-PRTR, 2014, Supplementary material, Table S2).

Data on atmospheric emissions in the river basin district Schlei/Trave which includes Lake Belau are scarce but emissions appear to be low and more or less constant between 2007 and 2012. Clearly higher atmospheric emissions which seem to increase in 2012 are reported for the areas Hamburg and Schleswig–Holstein (E-PRTR, 2014, Supplementary material, Table S2). However, it is unlikely that these emissions have major influence on the Hg levels around Lake Belau. The approach to determine atmospheric Hg levels by analyzing ESB samples of spruce and beech sampled in the neighborhood of the lake gave no clear results: THg levels in spruce needles (reflecting whole year exposure) seem to decrease between 2007 and 2013 while concentrations in beech leaves (reflecting growing season exposure) varied between years (www.umweltprobenbank.de).

4. Summary and conclusions

The design of the present study, i.e., a uniform method of analysis and usage of one common fish species, allowed a direct comparison between different European freshwater and estuarine sites with respect to Hg contamination of fish. According to the results, Hg levels in fish differed between sites but always exceeded the European WFD EQS of $20 \mu\text{g kg}^{-1}$ ww except in one case (2012, Lake Belau).

During the study period 2007–2013, Hg contamination of bream seemed to increase at three of six sites (Western Scheldt, Göta älv and Mersey) while decreasing Hg concentrations were observed only in bream from the Rhône where initial contamination was highest. These findings indicate that further efforts are required to reduce Hg emissions.

The analysis of MeHg in addition to THg in fish allows a more specific risk assessment. However, in our study we found a significant correlation between MeHg and THg in fish and no differences in MeHg fractions between sites except in one case (Rhône vs. Lake Belau). Since at least 80% of THg in fish muscle was MeHg, THg is

an appropriate measure for MeHg levels. For compliance monitoring and in view of precaution the measurement of THg is therefore considered sufficient.

Acknowledgements

The authors thank the European Flame Retardant Association EFRA, the PlasticsEurope Association, and the European Extruded Polystyrene Insulation Boards Association EXIBA for the permission to use fractions of the samples collected during the project “Environmental Monitoring of HBCD in Europe” for the present study. The excellent technical assistance by Sonja Uhlig, Martin Weingärtner and Diana Wilhelm (all Fraunhofer IME) and Gaël Guillou (LIENSs) is gratefully acknowledged.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2015.04.094>.

References

- Åkerblom, S., Bignert, A., Meili, M., Sonesten, L., Sundbom, M., 2014. Half a century of changing mercury levels in Swedish freshwater fish. *Ambio* 43, 91–103.
- Baeyens, W., Meuleman, C., Muhaya, B., Leermakers, M., 1998. Behaviour and speciation of mercury in the Scheldt estuary (water, sediments and benthic organisms). *Hydrobiologia* 366, 63–79.
- Baeyens, W., Leermakers, M., Papina, T., Saprykin, A., Brion, N., Noyen, J., De Gieter, M., Elskens, M., Goeyens, L., 2003. Bioconcentration and biomagnification of mercury and methylmercury in North Sea and Scheldt estuary fish. *Arch. Environ. Contam. Toxicol.* 45, 498–508.
- BGS (British Geological Survey): Contaminants in the Mersey estuary. <<http://www.bgs.ac.uk/research/climatechange/coastpol/mersey.html>> (accessed 04.15).
- Bignert, A., 2007. PIA Statistical Application Developed for Use by the Arctic Monitoring and Assessment Programme. <www.amap.no>.
- Davis, W.C., Christopher, S.J., Pugh, R.S., Donard, O.F.X., Point, D., Horvat, M., Gibiar, D., Kljakovic, Z., Porter, B.J., Schantz, M.M., 2007. Certification of methylmercury content in two fresh-frozen reference materials: SRM 1947 Lake Michigan fish tissue and SRM 1974b organics in mussel tissue (*Mytilus edulis*). *Anal. Bioanal. Chem.* 387, 2335–2341.
- EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. OJ L 327 (22 December 2000). <http://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF> (accessed 01.15).
- EC, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. OJ L364. <<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF>> (accessed 01.15).
- EC, 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on Environmental Quality Standards in the Field of Water Policy, Amending and Subsequently Repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council OJ L 348 <<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:348:0084:0097:EN:PDF>> (accessed 01.15).
- EC, 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 Amending Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy. OJ L226. <<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:EN:PDF>> (accessed 01.15).
- E-PRTR, 2014. The European Pollution Release and Transfer Register. <<http://prtr.ec.europa.eu/PollutantReleases.aspx>> (accessed 01.15).
- EQS substance data sheet, 2005. Environmental Quality Standards (EQS) Substance Data Sheet. Priority Substance No. 21: Mercury and its Compounds. Brussels 2005. <https://circabc.europa.eu/sd/a/ff8e163c-71f6-4fc0-98ef-875a20add4c8/21_Mercury_EQSdatasheet_150105.pdf>.
- Froese, R., Pauly, D., (Eds.), 2014. FishBase. World Wide Web electronic publication. <www.fishbase.org> (accessed 01.15).
- German Federal Environment Agency, 2014. Internet Portal of the German Environmental Specimen Bank. <www.umweltprobenbank.de> (English pages available, accessed 01.15).
- International Scheldt Commission, 2015. The Scheldt at a Glance. <<http://www.isc-cie.org/the-scheldt-at-a-glance.html>> (accessed 04.15).
- JMP, 2010. Tees Valley Transport Strategy: Strategic Environmental assessment. Report. JMP Consultants Ltd. Leeds. p. 122. <<http://m.middlesbrough.gov.uk/CHttpHandler.ashx?id=2563&p=0>> (accessed 04.15).
- Jürgens, M.D., Johnson, A.C., Jones, K.C., Hughes, D., Lawlor, A.J., 2013. The presence of EU priority substances mercury, hexachlorobenzene, hexachlorobutadiene and PBDEs in wild fish from four English rivers. *Sci. Total Environ.* 461–462, 441–452.
- Klein, R., Paulus, M., Tarricone, K., Teubner, D., 2012. German Environmental Specimen Bank: Guideline for Sampling and Sample Treatment – Bream (*Abramis brama*). <http://www.umweltprobenbank.de/upb_static/fck/download/SOP_Bream.pdf> (accessed 01.15).
- Leermakers, M., Elskens, M., Panutrakul, S., Monteny, F., Baeyens, W., 1993. Geochemistry of mercury in an intertidal flat of the Scheldt estuary. *Netherland J. Aquat. Ecol.* 27, 267–277.
- Lepom, P., Irmer, U., Wellmitz, J., 2012. Mercury levels and trends (1993–2009) in bream (*Abramis brama* L.) and zebra mussels (*Dreissena polymorpha*) from German surface waters. *Chemosphere* 86, 202–211.
- Marine Environment Monitoring Group: UK National Marine Monitoring Programme – Second Report, 1999–2001. CEFA Lowestoft. <<http://jncc.defra.gov.uk/pdf/nmmp2ndreport.pdf>> (accessed 04.15).
- McCutchan Jr., J.H., Lewis Jr., W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *OIKOS* 102, 378–390.
- Meili, M., Bishop, K., Bringmark, L., Johansson, K., Munthe, J., Sverdrup, H., De Vries, W., 2003. Critical levels of atmospheric pollution: criteria and concepts for operational modelling of mercury in forest and lake ecosystems. *Sci. Total Environ.* 304, 83–106.
- Merian, E., Anke, M., Ihnat, M., Stoeppel, M., 2004. Elements and their compounds in the environment. *Metals and their Compounds*, vol. 2. Wiley-VCH, pp. 932–934.
- Mersey basin Campaign. <http://www.merseybasin.org.uk/archive/assets/5/original/MERSEY_6_MINUTE_EXPERT.pdf> (accessed 04.15).
- Miller, A., Bignert, A., Porvari, P., Danielsson, S., Verta, M., 2013. Mercury in perch (*Perca fluviatilis*) from Sweden and Finland. *Water Air Soil Pollut.* 224, 1472–1484.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and relation between $\delta^{15}\text{N}$ and animal age. *Geochim. Cosmochim. Acta* 48, 1135–1140.
- Monperrus, M., Krupp, E., Amouroux, D., Donard, O.F.X., Rodriguez Martin-Doimeadios, R.C., 2004. Potential and limits of speciated isotope-dilution analysis for metrology and assessing environmental reactivity. *Trends Anal. Chem.* 23, 267–272.
- Nilsson, S., 2006. International river basins in the Baltic Sea region. Fact sheet. rreg IIIB project TRABANT. <<http://www.baltex-research.eu/material/downloads/riverbasins.pdf>>.
- Nyberg, E., Faxälv, S., Danielsson, S., Bignert, A., Eriksson, U., Holm, K., Borg, H., Berger, U., Haglund, P., 2012. The National Swedish Contaminant Monitoring Programme for Freshwater Biota, 2012. Swedish Museum of Natural History. Report No. 13. p. 214.
- Olivier, J.-M., Carrel, G., Lamouroux, N., Dole-Olivier, M.J., Malard, F., Bravard, J.-P., Amoros, C., 2008. The Rhône River Basin. In: Tockner, K., Uehlinger, U., Robinson, C.T. (Eds.), *Rivers of Europe*. Academic press, pp. 247–295.
- Pacyna, J.M., Pacyna, E.G., Aas, W., 2009. Changes of emissions and atmospheric deposition of mercury, lead, and cadmium. *Atmos. Environ.* 43, 117–127.
- Paulus, M., Klein, R., Wagner, G., Müller, P., 1996. Biomonitoring and environmental specimen banking. *Environ. Sci. Pollut. Res.* 3, 169–177.
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703–718.
- Ribeiro, C.A.O., Voltaire, Y., Sanchez-Chardi, A., Roche, H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. *Aquat. Toxicol.* 74, 53–69.
- Rüdel, H., Uhlig, S., Weingärtner, M., 2008. German Environmental Specimen Bank: Guidelines for Sampling and Sample Processing: Pulverization and Homogenization of Environmental samples by Cryomilling. <http://www.umweltprobenbank.de/upb_static/fck/download/IME_SOP_preparation_De22008_V200.pdf> (accessed 04.15).
- Rüdel, H., Müller, J., Quack, M., Klein, R., 2012. Monitoring of hexabromocyclododecane diastereomers in fish from European freshwaters and estuaries. *Environ. Sci. Pollut. Res. Int.* 19, 772–783.
- Salmi, T., Määttä, A., Anttila, P., Ruoho-Airola, T., Amnell, T., 2002. Detecting trends of annual values of atmospheric pollutants by the Mann-Kendall test and Sen's slope estimates – The EXCEL template application MAKESSENS. Report code FMI-AQ-31. Finnish Meteorological Institute, Helsinki, Finland.
- Schulze, T., Ricking, M., Schröter-Kermani, C., Körner, A., Denner, H.-D., Weinfurter, K., Winkler, A., Pekdeger, A., 2007. The German Environmental Specimen Bank. Sampling, processing, and archiving sediment and suspended particulate matter. *J. Soils Sediments* 7, 361–367.
- Sonesten, L., 2001. Mercury content in roach (*Rutilus rutilus* L.) in circumneutral lakes – effects of catchment area and water chemistry. *Environ. Pollut.* 112, 471–481.
- TIDE, 2013. Tidal River development. Work package 5 “Measures”. Dredging and Disposal Strategies. Scheldt estuary – MOW. Final version – March 2013. p. 18.
- UKMMAS, 2010. United Kingdom Marine Monitoring & Assessment Strategy. Charting Progress 2. The State of the UK seas. Department for Environment, Food and rural Affairs (Defra). <<http://chartingprogress.defra.gov.uk>> (accessed 04.15).
- UNEP, 2013. Minamata Convention on Mercury. United Nations Environment Programme. Geneva, Switzerland: Text agreed upon in UNEP (DTIE)/Hg/INC.5/

- 3; January 13–19, 2013. <www.mercuryconvention.org/Portals/11/documents/conventionText/MinamataConventiononMercury_e.pdf> (accessed 01.15).
- Vander Zanden, M.J., Cabana, G., Rasmussen, J.B., 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios $\delta^{15}\text{N}$ and literature dietary data. *Can. J. Fish Aquat. Sci.* 54, 1142–1158.
- Vane, C.H., Jones, D.G., Lister, T.R., 2009. Mercury contamination in surface sediments and sediment cores of the Mersey estuary, UK. *Mar. Pollut. Bull.* 58, 940–946.