# Mercury accumulation in muscle and liver tissue and human health risk assessment of two resident freshwater fish species in Flanders (Belgium) : a multilocation approach

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**Abstract**

Detrimental effects of chemical pollution - primarily caused by human activities - on aquatic ecosystems, have increasingly gained attention. Because of its hydrophobic qualities, mercury is prone to easily bioaccumulate and biomagnify through the food chain, decreasing biodiversity and eventually also affecting humans. In the present study, accumulated mercury concentrations were measured in muscle and liver tissue of perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*) collected at 26 sampling locations in Flemish (Belgian) waterbodies, allowing a comparison of these species within a variety of environmental situations. Furthermore, effects of size and weight have been assessed, expected to influence accumulation and storage of pollutants. Mercury concentrations in perch ranged up to 1.7 µg $g^{-1}$ dw (median: 0.29 µg $g^{-1}$ dw) in muscle and from 0.02 to 0.77 µg $g^{-1}$ dw (median: 0.11 µg $g^{-1}$ dw) in liver tissue. For eel, these concentrations were between 0.07 and 1.3 µg $g^{-1}$ dw (median: 0.39 µg $g^{-1}$ dw) and between 0.08 and 1.4 µg $g^{-1}$ dw (median: 0.55 µg $g^{-1}$ dw) respectively. We found a correlation of accumulated mercury with length in perch, independent of location. Furthermore, a significant difference in accumulated mercury concentrations between the targeted species was measured, with the highest mean concentrations per dry weight in eel liver and muscle tissue. In perch, higher concentrations were found in muscle compared to liver tissue, while in eel, liver tissue showed the highest concentrations. These findings were further considered with concentrations corrected for lipid content, excluding the fat compartment, which is known to a hold negligible portion of the total and methyl mercury concentrations. This confirmed our previous conclusions, except for mercury concentrations in eel. Here there was no longer a significant difference between muscle and liver concentrations. Finally, health risk analyses revealed that only frequent consumption of local eel (> 71 g $day^{-1}$) could pose risks to humans.

**Keywords:** European perch, European eel, Hg, pollution, internal distribution, biomonitoring

## 1. Introduction

Mercury (Hg) is a naturally occurring element, but is widely applied on a global scale in industry (i.e. production of car components), gold mining, households (i.e. batteries) and agriculture (i.e. pesticides). These activities cause it to be introduced in aquatic ecosystems through among others erosion and both industrial and domestic discharges (Selin, 2009; Kidd and Batchelar, 2012; Horowitz et al., 2014). The largest portion, however, originates from atmospheric deposition as a result of fuel combustion, causing long-range transport (Pacyna et al., 2010; Pirrone et al., 2010; Horowitz et al., 2014). Elevated Hg concentrations have previously been found in aquatic environments of remote areas (Durnford et al., 2010; Fitzgerald and Mason, 1998). Due to its highly persistent character, Hg remains present in the environment.

Mercury can be present in the aquatic environment in dissolved or particle bound state. For piscivorous or omnivorous fish, dietary intake is the main exposure route (Hall et al., 1997; Régine et al., 2006; Bradley et al., 2017). Methylmercury is readily bioavailable and therefore causes a strong biomagnification through the food chain. Because of its high affinity for sulfur-based amino-acids and thiol groups in proteins it will easily pass through the gut and can be transported via blood to different organs (Ribeiro et al., 1999; Amlund et al., 2007; Bradley et al., 2017). The muscle tissue is known to be the major sink for MeHg. The gut, however, shows a poor absorption for Hg (II). Therefore, the highest inorganic Hg concentrations are often found in the intestine (Peng et al., 2016). Since the liver acts as a demethylation and re-distribution organ, this might also be a target tissue for both organic and inorganic mercury (Régine et al., 2006; Havelková et al., 2008). A secondary pathway is uptake of dissolved mercury (mainly in its inorganic Hg (II) form) through the gills. In general, the majority (80 – 100%) of mercury in fish muscle tissue consists of MeHg (Chvojke et al., 1990; Bloom, 1992; Kannan et al., 1998; Nguetseng et al., 2015; Golzadeh et al., 2020). High concentrations of MeHg are further due to the low elimination rates and higher accumulation efficiency compared to inorganic Hg (II) (Wang and Wong, 2003; Peng et al., 2016; Bradley et al., 2017; Wang and Wang, 2018).

Mercury can act as a potent neurotoxicant, especially in its organic, methylated form (i.e. methylmercury - MeHg), and will interfere both with perceptive systems (i.e. vision, hearing) and movements (i.e. immobility, uncontrollable movements), even at low concentrations in humans (Clarkson, 1992; Karagas et al., 2012). Exposed fish can experience deleterious effects on their growth, development, and reproduction (Beckvar et al., 2005; Scheuhammer et al., 2007). Furthermore, high accumulated mercury concentrations have been found in piscivorous predators due to biomagnification, ultimately leading to mortality. These observations were made, among others, in European otter (*Lutra lutra),* mink (*Mustela vison*) and Eurasian bittern (*Botaurus stellaris*) (Macdonald

and Mason, 1994; Newton et al., 1994; Wiener et al., 2003; Yates et al., 2005; Scheuhammer et al., 2007).

*Perca fluviatilis* (European perch), and *Anguilla anguilla* (European eel) in its "yellow" eel stage, are frequently used for monitoring purposes (de Boer and Brinkman, 1994; Ion et al., 1997; Belpaire and Goemans, 2007b; Havelková et al., 2008; Batchelar et al., 2013; Van Ael et al., 2014), despite the IUCN status of eel as "critically endangered" (Pike et al., 2020). These are very common and widespread species in Europe, allowing for a straightforward comparison of accumulated concentrations between different countries (Belpaire and Goemans, 2004; Belpaire and Goemans, 2007a; Bignert et al., 2011). Furthermore, they are resident, creating a reliable image of a relatively restricted area and they are relatively tolerant to pollution (Järv, 2000; Belpaire et al., 2008). Because of their high trophic levels, they may accumulate high concentrations of pollutants, inducing possible toxic effects to their predators (Wiener et al., 2003; Belpaire and Goemans, 2007b).

In general, older individuals, having experienced a longer exposure time, tend to have accumulated higher mercury concentrations (Park and Curtis, 1997; Cizdziel et al., 2002; Szefer et al., 2003; Durrieu et al., 2005; Weis and Ashley, 2007; Gewurtz et al., 2011; Batchelar et al., 2013). Larger fish also tend to eat larger prey, containing higher mercury concentrations. Furthermore, size related biokinetics might play a role. A reduced growth efficiency in larger individuals, for example, diminishes the effect of somatic growth dilution, resulting in higher concentrations (Dang and Wang, 2012). Finally, it has been shown that MeHg is eliminated slower in older fish (Lescord et al., 2018). Although the increase of Hg accumulation with size has been researched elaborately, there is a lack of studies regarding a multiple population approach.

Within the present study we selected a broad variation of locations with different environmental backgrounds, in order to study general trends of Hg accumulation in multiple populations throughout Flanders (Belgium), instead of investigating a specific local study situation. Internal distribution of mercury over liver and muscle tissue and comparison between two important freshwater monitoring species, *Perca fluviatilis* and *Anguilla anguilla* was the main focus of this study. The impact of species, fish length, weight, and sample site (background) on internal Hg levels was assessed, using generalized linear mixed models. Furthermore, analyses were repeated with a correction of the mercury concentration, excluding seasonality in lipid content. Finally, a human health risk assessment was performed based on consumption of muscle tissue of both perch and eel.

In general we hypothesize that (1) higher mercury concentrations are to be expected in fish with higher weight and size, independent of location; (2) eel accumulates higher mercury concentrations, as it is a

bottom dwelling species exposed to mercury contamination in sediment particles; (3) higher accumulated total Hg concentrations are to be found in muscle compared to liver tissue.

## 2. Material and methods

### 2.1 Study area and sample selection

A total of 26 sampling locations were selected in Flanders (Belgium). Sites were selected in order to fulfil the reporting requirements for the Water Framework Directive as coordinated by the Flemish Environmental Agency. Although some locations were situated in the same water body, we interpreted them as independent because of local point sources or different surroundings (i.e. industry and agriculture). Typology of sampling sites included canals, rivers and streams, brooks and polder water courses. Perch and eel where caught by the Institute for Nature and Forest Research between 2013 and 2016 and euthanized with MS-222 (Acros Organics, Geel, Belgium). Eels were collected in their juvenile, yellow eel stage and a length class of 45-55 cm was targeted, while for perch the largest sizes were targeted. Fish were sampled using electrofishing and/or fykes, depending on the depth and characteristics of the water body. However, we did not manage to collect both species at all sites. Sampling locations are indicated in Figure 1 and Table 1, as well as the total number of fish collected per site.

### 2.2 Fish sampling

A total of 300 perch and 100 eel were caught and individually analysed (Table 1). Before dissection, 3 different length (± 0.1 cm) measures and the weight (± 0.1 g) of individual fish were determined. Length measures included total length, fork length (tip of the snout to posterior end of the middle caudal rays) and standard length (tip of the snout to the midlateral posterial edge of the hypural plate). For eel, however, only total length was recorded. Fish were dissected, muscle (N=397) and liver tissue (N=308) isolated, weighed (Mettler AT261 DeltaRange, Mettler-Toledo) and frozen at -20°C until further processing. Muscle samples were taken in the dorsal part, behind the dorsal fin for perch and in the dorsal part, opposite to the anus for eel. Aliquots for all muscle and liver samples were taken to perform total mercury and lipid analyses.

### 2.3 Mercury analysis

In the present study total Hg (tHg) was measured and used as a proxy for MeHg, being the predominant form in fish muscle (80-100%) (Golzadeh et al., 2020; Nguetseng et al., 2015). Samples were freeze-dried (Heto PowerDry LL 3000, Thermo Scientific) and dry/wet weight ratios were determined before digestion in a 1:3 (v/v) mixture of HNO3 (69%) and HCl (37%) (“Aqua Regia”). Digestion was conducted in a pressurized microwave digestion system, Discover SP-D (CEM Corporation, Matthews, NC 28106,

USA). Analysis was performed using a high-resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS; Element XR, Thermo Scientific, Bremen, Germany). Procedural blanks were incorporated. Reference material used was freeze-dried mussel tissue (NIST-2976; National Institute of Standards and Technology, USA; certified concentration: 61.0 ± 3.6 µg/kg dw). Recoveries ranged from 70 to 136 %. Concentrations in batches with recoveries below 90% or above 110 % were corrected for this error by dividing the measured concentration by the proportion of the recovery. The method quantification limit for mercury was 0.005 µg/L. Mercury concentrations below LOQ (0.01 µg/g dw) were set at ½ of the LOQ (Bervoets et al., 2004; Custer et al., 2000). Overall, concentrations below LOQ were only detected in muscle tissue of perch (2% of samples).

Most studies report accumulated mercury concentrations on a wet weight basis. There is, however, a large variation in the dry weight/wet weight ratio for European eel (0.22 to 0.74 in the present study), leading to more variable datasets and interpretations, based on the water content in and on the fish tissue. Therefore, we suggest a more robust method using dry weight based mercury concentrations.

### 2.4 Lipid determination and lipid based correction

Total lipid percentage was determined in muscle (N=230) and liver (N=91) tissue of perch and in muscle (N=63) and liver (N=62) tissue of eel. Lipid extraction from lyophilized samples was based on the Bligh and Dyer method (Bligh and Dyer, 1959). A chloroform/methanol/water 5:5:2 (v/v/v) mixture was used. After centrifugation, lipids were isolated in the chloroform fraction. Addition of sulphuric acid (95%) induced a colour change when samples were heated to 200°C, for 15 minutes. The optical density was measured using a spectrophotometer ($EL_X$ 808 IU Ultra Microplate Reader, Bio-Tek Instrumenst Inc.) at 405 nm. Samples were analysed in duplicate. Calculations were performed using a calibration curve with a stock solution of glycerol tripalmitate (98%) dissolved in chloroform (99%).

Seasonal variation in lipid content, due to for example food availability and reproduction, may have a strong effect on measured Hg concentrations in different fish tissues and species. A higher lipid content will have an effect on the total weight of the tissue or individual and therefor affect the Hg concentration per weight unit. However, mercury is known to almost exclusively accumulate in proteins (Amlund et al., 2007). Therefore, we chose to correct for lipid percentage, excluding this portion from the total tissue weight, based on the approach of Kahilainen et al. (2016). This correction was performed using the following formula:

$$tHg_{corr} = Hg\ tot\ /\ (1 - lipid_{prop})$$

where $tHg_{corr}$ is the lipid-corrected total mercury concentration (µg $g^{-1}$ dw), Hg tot is the measured total mercury concentration (µg $g^{-1}$ dw) and $lipid_{prop}$ is the lipid content proportional to the tissue weight.

## 2.5 Human health risk assessment

Muscle tissue of fish, being the commonly eaten part, is often considered in human health risk analysis. Several international organizations have estimated safe methylmercury concentrations on a wet weight basis and defined them as the Minimum Risk Level (MRL; ATSDR, 2018), the US EPA Reference Dose (RfD; UNEP, 2008) and the Provisional Tolerable Weekly Intake (PTWI; FAO/WHO, 2010), respectively 0.3 µg $kg^{-1}$ body weight $day^{-1}$, 0.1 µg $kg^{-1}$ body weight $day^{-1}$ and 1.6 µg $kg^{-1}$ body weight $week^{-1}$. The maximum tolerable amount of both fish to be eaten per day, taking into account all of the above reference values, without potential human health risk was calculated for an average adult of 70 kg. The previous values, although set for methylmercury, can be used for total mercury as well. As mentioned before, in fish over 90% of total mercury is assumed to be in its methylated form (Chvojke et al., 1990; Bloom, 1992; Kannan et al., 1998). All of these calculations were performed on both species for each location separately (SI 8) and on the entire dataset (Table 2).

Another widely used tool to determine human health risks is calculation of the Hazard Quotient (HQ; USEPA, 1989). The HQ is usually defined as the ratio of the estimated daily intake (EDI), in relation to the tolerable daily intake (TDI) of a pollutant. If the HQ exceeds one, this suggests potential health effects. In order to determine the EDI, median accumulated mercury concentration per location were multiplied by the known median consumption of the local adult population. In Flanders, mainly recreational fishermen are exposed to contaminated fish through consumption of their catch. Therefore, results based on an interview of fishermen were included, with a mean consumption of 2.7 g of perch $day^{-1}$ and 18 g of eel $day^{-1}$ (ANB-VF/2015/4). As for TDI, converted concentrations for an average adult of 70 kg were used for MRL, RfD and PTWI. Bilau et al. (2007) performed a risk analysis for the worst-case scenario, based on the amount of eel that was taken home by recreational fishermen. It was calculated that people always taking home the eel caught, would consume an average of 71.14 g $day^{-1}$ if they ate everything themselves. This worst-case scenario was also included in the present analysis.

For all calculations concerning human health, concentrations were recalculated on a wet weight base (µg $g^{-1}$ ww) using the dry/wet weight ratio measured in both species (SI 4, 5).

## 2.6 Statistical analysis

Statistical analyses were performed using the software package R (R version 3.3.2; R Core Team, 2016). A Spearman correlation test was conducted between weight and length measures, between dry and wet weight based concentrations and between concentrations in liver and muscle tissue. To investigate different accumulated mercury concentrations in respectively species, tissues (muscle/liver) and age indicators (length/weight) linear mixed models were composed for each of these variables with

location as random intercept. For these analyses an F-test with Kenward-Roger Degrees of Freedom Approximation was used to determine the significance of the variables, comparing the full model (including one explanatory variable) to the reduced model (without explanatory variable). Furthermore, these analyses were repeated with Hg concentrations corrected for the lipid content. Finally, a prediction equation to extrapolate Hg concentrations between both fish species, was created using a linear regression model. Significant outliers were removed using the Grubb test in Graphpad. Overall significance levels were considered at a p-value <0.05.

## 3. Results

### 3.1 Total Hg accumulation and internal distribution

Individual mercury concentrations in perch were found to range from <0.01 to 1.7 µg $g^{-1}$ dw (median: 0.29 µg $g^{-1}$ dw; <0.001-0.35 µg $g^{-1}$ ww) and from 0.02 to 0.77 µg $g^{-1}$ dw (median: 0.11 µg $g^{-1}$ dw; 0.003-0.19 µg $g^{-1}$ ww), respectively in muscle and liver tissue (SI 6, 8). For eel, the Hg concentrations in muscle tissue were between 0.07 and 1.3 µg $g^{-1}$ dw (median: 0.39 µg $g^{-1}$ dw; 0.03-0.43 µg $g^{-1}$ ww), while there was a concentration range from 0.08 to 1.4 µg $g^{-1}$ dw (median: 0.55 µg $g^{-1}$ dw; 0.02-0.29 µg $g^{-1}$ ww) measured in liver tissue (SI 6, 8). A significant difference in accumulated concentrations was found between both species in muscle tissue ($F=20.26$; $p<0.001$), as well as in liver tissue ($F=336.54$; $p<0.001$), with the highest concentrations in eel for both tissues (Figure 2). For perch, a significant difference between liver and muscle accumulated concentrations was found ($F=127.25$; $p<0.001$). Accumulated mercury concentrations in muscle were higher than those in liver. Also for eel, a significant difference in mercury concentrations between tissues was found ($F=6.30$; $p<0.05$), however this time with highest concentrations in the liver tissue (Figure 2). In all of the above analyses, the effect of location was taken into account, using linear mixed models. A correlation of Hg concentrations in liver and muscle tissue was detected for both species ($r\geq0.56$; $p<0.001$) (SI 2, 3).

The median accumulated Hg concentrations per location in eel and perch in both liver and muscle tissue were compared in order to identify extrapolation possibilities between the species. A significant correlation was found for wet weight concentrations in muscle tissue ($r=0.66$; $p<0.01$). Linear regression resulted in the following equation ($R^2=0.44$):

$$[Hg_{perch\ (ww)}] = 0.36 * [Hg_{eel\ (ww)}] + 0.01 \quad (1)$$

### 3.2 Effects of fish size

All length measures were highly correlated ($r\geq0.99$; $p<0.001$), as well as dry weight and wet weight based concentrations ($r\geq0.92$; $p<0.001$) (SI 1). For all analyses hereafter, total length (TL) was used as

length measure. Fish weight (W) showed an exponential increase with increasing length for both eel (X= $9.4205e^{0.0062TL}$; $R^2$=0.94) and perch ($W = 0.8374e^{0.0252TL}$; $R^2$=0.96).

Mercury accumulation increased with increasing length for perch in both muscle (F=184.61; p<0.001) and liver tissue (F=72.44; p<0.001) (Figure 3, SI 10). For eel, no significant effect of length could be found for Hg accumulation in muscle tissue (F=3.67; p=0.06) or liver tissue (F=0.30; p=0.59) (Figure 3, SI 10). For these analyses as well, influences of locations were included (mixed model). Furthermore, fat content in muscle tissue of eel increased significantly with both length (F=13.24; p<0.001) and weight (F=19.10; p<0.001). Increasing length (F=32.71; p<0.001) and weight (F=16.99; p<0.001) of perch, however, resulted in a slight but significantly decreasing fat content. Liver fat content in perch was affected by neither length (F= 0.66: p=0.42) nor weight (F=1.88; p=0.17). The total lipid content in liver of eel significantly increased with increasing length (F=4.55; p<0.05) and weight (F=13.90; p<0.001).

3.4 Correction for lipid content

Total lipid content in perch ranged from 0.50 to 2.5 % (median: 0.92%) in muscle tissue and from 1.6 to 4.2 % (median: 2.4%) in liver tissue (SI 4, 5). In eel, lipid percentages ranged from 1.6 to 28 % (median: 7.6%) in muscle tissue and from 1.8 to 10 % (median: 2.7%) in liver tissue (SI 4, 5). The results demonstrated that muscle tissue of eel contained a significant higher lipid concentration than muscle tissue of perch (F=356.8; p<0.001; SI 4, 5). The same was true for liver tissue (F=7.3; p<0.01).

Additionally, mercury concentrations were corrected for lipid content (µg $g^{-1}$ dw), excluding the lipid proportion from the total tissue weight. This caused concentrations to lay between <0.01 and 1.3 µg $g^{-1}$ dw (median: 0.26 µg $g^{-1}$ dw) in muscle tissue of perch. For liver tissue in this species, concentrations were calculated to be between 0.02 and 0.79 µg $g^{-1}$ dw (median: 0.17 µg $g^{-1}$ dw). For eel concentrations were between 0.09 and 1.4 µg $g^{-1}$ dw (median: 0.48 µg $g^{-1}$ dw) and between 0.08 and 1.4 µg $g^{-1}$ dw (median: 0.56 µg $g^{-1}$ dw) for muscle tissue and liver tissue respectively.

Concentrations between both species still differed significantly for both muscle (F=53.30; p<0.001) and liver tissue (F=132.64; p<0.001). The highest concentrations were still measured in eel, both for muscle and liver tissue (SI 7). The difference in accumulated concentrations between muscle and liver was only significant for perch. In perch the highest concentrations were measured in muscle tissue (F=44.40; p<0.001), whereas in eel there was no significant difference between liver and muscle concentrations (F=2.28; p=0.13) (SI 7).

Finally, after correction for lipid content a significant increase of accumulated mercury with size could still be detected in muscle ($F=114.71$; $p<0.001$) and liver tissue ($F=13.14$; $p<0.001$) of perch. For both muscle ($F=0.86$; $p=0.41$) and liver tissue ($F=0.89$, $p=0.35$) in eel this effect was once again absent.

### 3.5 Human health risk assessment

Mercury concentrations (µg $g^{-1}$ ww) in the muscle tissue were used to perform a human health risk analysis. None of the sampled fish showed an exceedance of the WHO guideline for human consumption of mercury, namely 0.5 µg $g^{-1}$ ww for perch and 1 µg $g^{-1}$ ww for eel (EC 2006; EC 2008). Table 2 contains the maximum amount of fish (g) to be eaten per day, for an adult weighing 70 kg, without posing health risks (MADC). This value was determined on the pooled dataset of all sample locations, using both median and 95% percentile values of perch and eel. The MRL, RfD and PTWI concentrations were interpreted. Clearly, the recommended amount of eel is considerably lower than that of perch. Furthermore, the hazard quotient (HQ) was determined, based on the annual consumption of caught fish by fishermen. Maximum tolerated daily intake of fish (TDI) was far above the estimated daily intake dose (EDI), resulting in an HQ <1 for perch. For eel on the other hand, MADC's were very low. A low EDI, however, still resulted in HQ <1. The highest concentration measured in eel (0.43 µg $g^{-1}$ ww in 'Kanaal Gent-Oostende III') gave an HQ of 1.12 for RfD, possibly posing a health risk through consumption. For the results of the above for the mean mercury concentrations on each location, we refer to SI 8.

In the worst case scenario for eel in Flanders the average amount consumed was 71.14 g $day^{-1}$ (Bilau et al., 2007). This is almost 4 times higher than the average estimated daily intake dose (EDI) for eel. Therefore, with this consumption rate, this would result in a HQ>1 for RfD on the median Hg concentration in eel of this study. For the $95^{th}$ percentile concentrations this leads to HQ values of 1.05, 3.1 and 1.4 respectively for MRL, RfD and PTWI, by dividing 71.14 g $day^{-1}$ by the MADC. Consequently, this would mean that for MRL, RfD and PTWI values respectively 8%, 68% and 24% of the locations on average resulted in a HQ>1, posing a possible threat to human health (SI 9).

Additionally, the European Biota Quality Standard ($EQS_{biota}$), a threshold concentration for protection of the integrity of aquatic ecosystems under the Water Framework Directive, namely 0.02 µg $g^{-1}$ ww for Hg (EC, 2013), was exceeded in every sampled location, indicating potential health risks to the food web, mainly on top predators.

## 4. Discussion

### 4.1 Total Hg accumulation and internal distribution

In the present study, measured concentrations in muscle tissue ranged from <0.01 to 1.7 µg $g^{-1}$ dw (<0.001-0.35 µg $g^{-1}$ ww) in perch and from 0.07 to 1.3 µg $g^{-1}$ dw (0.03-0.43 µg $g^{-1}$ ww) in eel. These results fall within ranges reported in other studies on Flemish waterbodies. Bervoets et al. (*subm.*) measured Hg concentrations between 0.58 and 1.1 µg $g^{-1}$ dw in perch and between 0.28 and 0.94 µg $g^{-1}$ dw in eel from the Winterbeek (Flanders, Belgium). Furthermore, a preliminary monitoring study published results ranging from 0.04 to 0.93 µg $g^{-1}$ ww in perch and from 0.05 to 0.32 µg $g^{-1}$ ww in eel from 16 different water bodies in Flanders (De Jonge et al., 2014). Other studies, based on data from the Eel Pollution Network of Flanders, reported concentrations between 0.005 and 1.2 µg $g^{-1}$ ww in eel muscle tissue (Maes et al., 2005; Belpaire and Goemans, 2007b; Maes et al., 2008). Comparable results were found in other European studies, with concentrations in muscle tissue ranging from 0.03 to 1.4 µg $g^{-1}$ ww for perch (Svobodová et al., 1999; Szefer et al., 2003; Dusek et al., 2005; Petkovšek et al., 2012; Noël et al., 2013; Jirsa et al., 2014; Foekema et al., 2016; Łuczyńska et al., 2016) and from 0.001 to 0.79 µg $g^{-1}$ ww for eel (Downs et al., 1999; Edwards et al., 1999; Eira et al., 2009; Has-Schön et al., 2006; Has-Schön et al., 2008; Noël et al., 2013; Genç and Yilmaz, 2017). A very elaborate Canadian study reported median Hg concentrations of 0.32 and 0.14 µg $g^{-1}$ ww in American eel (*Anguilla rostrata*) and yellow perch (*Perca flavescens*) respectively (Depew et al., 2013).

Furthermore, in the present study, liver concentrations were found to be between 0.02 and 0.77 µg $g^{-1}$ dw (0.003-0.19 µg $g^{-1}$ ww) for perch and between 0.08 and 1.38 µg $g^{-1}$ dw (0.02-0.29 µg $g^{-1}$ ww) for eel. European studies on freshwater systems reported liver concentrations between 0.03 and 1.03 µg $g^{-1}$ ww in perch (Svobodová et al., 1999; Petkovšek et al., 2012; Jirsa et al., 2014) and between 0.007 and 2.23 µg $g^{-1}$ ww in eel (Downs et al., 1999; Has-Schön et al., 2006; Has-Schön et al., 2008; Eira et al., 2009; Genç and Yilmaz, 2017).

Overall, in the present study, higher median Hg concentrations were accumulated in eel compared to perch. This is in line with observations from other Flemish studies (Teunen et al., 2020; Bervoets et al. *subm.*). A considerable amount of the ingested mercury is diet or particle bound, available in its organic form and will be transported to the muscle tissue (Hall et al., 1997; Régine et al., 2006; Bradley et al., 2017). Nonetheless, it is noteworthy that in the present study most perch were rather small, 59% was smaller than 12 cm. This might have contributed to the lower mercury concentrations in perch. Since perches reach adulthood at a mean length of around 11 cm (fishbase.se), most of the sampled fish are considered juveniles. Besides a shorter exposure time, their diet consist mostly of zooplankton, in contrast to macro-invertebrates and small fish for adult perch (Lappalainen et al., 2001). Smaller fish have been shown to contain a lower relative MeHg percentage due to somatic growth dilution caused by a higher growth rate of these smaller fish, a more readily absorption of the aqueous (dissolved) Hg(II) and a faster excretion of MeHg (Lescord et al., 2018). Therefore, we should take into account

that the proportional contribution of Hg(II) to the total Hg concentrations might be higher in small than in larger individuals. Since the total Hg concentration in muscle tissue is largely still comprised of MeHg, we consider this a good proxy for MeHg. In liver tissue, however, inorganic mercury might have a larger contribution to total mercury concentrations, due to demethylation processes. On the other hand, higher total mercury concentrations in eel compared to perch might be due to the specific bottom-dwelling habitat of this species and therefore bioaccumulation of sediment-bound mercury (Edwards et al., 1999). Bacteria that live in the sediment are known to take part in the methylation process, creating a more direct exposure of this bioavailable compound (Macalady et al., 2000).

In perch, higher concentrations were found in the muscle tissue compared to liver tissue, even after correcting for lipid content. This is in line with existing literature on perch (Svobodová et al., 1999; Voigt, 2007; Jirsa et al., 2014). These results confirm the high affinity of (methyl)mercury for muscle tissue. In eel, however, higher accumulated concentrations were detected in the liver compared to muscle tissue. This is in line with the results found by Genç and Yilmaz (2017). Eira et al. (2009) and Has-Schön et al. (2006; 2008), on the other hand, found higher accumulated Hg concentrations in muscle than in liver tissue of eel. After correction for lipid content in the present study, however, there was no longer a significant difference between both tissues for eel. The accumulation and (acute) detoxification role of the liver might result into high concentrations stored in liver tissue (Scheuhammer et al., 2007; Havelková et al., 2008; Kružíková et al., 2013). Higher muscle concentrations, on the other hand, reflect that deposited mercury is slowly evacuated over a long-term period, due to overall lower accumulated mercury concentrations in lightly- or non-contaminated sites. This effect might explain individual cases with higher concentrations accumulated in liver tissue than in muscle tissue (SI 10). However, the influence of other factors needs to be taken into account (e.g. environmental factors, diet, bioavailability). In the present study, using linear mixed models, site-related effects were incorporated.

The larger variation in eel concentrations could be explained by the variable fat content of these fish as well. Monitoring studies in Flanders reported fat percentages between 1.3 and 32% in eel, compared to between 0.18 and 1.02% in perch (Teunen et al., 2017, 2018). Lower lipid concentrations might indicate lower food availability and quality. Because of the high biomagnification potential of (Me)Hg, larger and more numerous prey might lead to higher accumulated concentrations (Dang and Wang, 2012). Furthermore, difference in internal distribution between both species might point out a different inter-organ transport and toxicological response. Processes of internal distribution and biokinetics (elimination and assimilation) can be species-dependent (Ribeiro et al., 1999; Peng et al., 2016), resulting in different concentrations and distribution patterns.The results in the present study

underline the importance of taking into account the large variation caused by environmental and species-specific life history traits.

Finally, we found a possible extrapolation of wet weight muscle concentrations in perch to wet weight muscle concentrations of eel (Eq. 1). This implies that, for monitoring purposes, it might no longer be needed to catch both species. Instead, analysis of one species can be used to predict concentrations in the other. This relationship is even strengthened through the resident nature of these species. However, also other criteria determine to a large extent the suitability of a species for monitoring purposes (occurrence, distribution, habitat choices, density, catchability, size) (Belpaire and Goemans, 2007a).

### 4.2 Effects of length

For perch, an increase in accumulated Hg concentrations, in both muscle and liver tissue, was found with increasing length, independent of the background. This effect was still present after correction on lipid content. An increased accumulation as effect of age (i.e. weight or length) is often detected in perch (Driscoll et al., 1995; Svobodová et al., 1999; Szefer et al., 2003; Łuczyńska, 2005; Voigt, 2007; Järv et al., 2013; Łuczyńska et al., 2016;). This confirms the hypothesis of bioaccumulation with older individuals, who were exposed for a longer period of time, showing higher concentrations of mercury. Furthermore, larger individuals usually occupy a higher trophic level, being exposed to higher concentrations through diet, because of the biomagnification effect of mercury (Olsson et al., 2000).

Seasonal variation in accumulated concentrations has been reported, caused by differences in food availability, summer growth (diminishing Hg concentrations), reproduction (lipid-rich egg production) and changes in trophic position (Braaten et al., 2014). Nevertheless, there was no significant effect of length or weight on accumulated Hg in muscle or liver tissue of eel, even after correction for lipid content. This is according to the results for muscle tissue found by Noël et al. (2013) and Genç and Yilmaz (2017). However, other studies did report a positive correlation of accumulated mercury with length and weight parameters in muscle tissue of eel (Downs et al., 1999; Edwards et al., 1999). It is therefore important to note that we selected on a specific life stage (i.e. yellow eel) and targeted a limited size range. Finally, the relation between Hg accumulation and length showed to be location (background) dependent.

### 4.4 Human health risk assessment

Although the Hg concentration in muscle tissue of both perch and eel did not exceed the WHO guideline for human consumption, the maximum recommended consumption (g) per day is rather low, especially for eel (often less than 100 g of eel per day). Nonetheless, due to a very low consumption

rate, even for fishermen, the HQ was lower than one for both species at all locations. Polak-Juszczak and Nermer (2016) likewise reported that the largest eels might pose a health risk for Hg.

Including the worst case scenario for eel, however, it became clear that these few fishermen consuming a higher amount of eel have a high chance of experiencing detrimental effects of accumulated mercury concentration. Due to the variation of MeHg contribution to tHg, however, using total Hg as a proxy for MeHg, might lead to an overestimation of the risks. It was reported that large fish consumers in Flanders showed higher Hg levels in hair and blood (Croes et al., 2014). Moreover, based on high PCB (Polychlorinated biphenyl) concentrations measured in fish from Flemish water bodies, the recommendation already exists not to consume fish caught in Belgium (Maes et al., 2008).

In every sample location an exceedance of the $EQS_{biota}$ was observed for both fish species. A widespread exceedance of this standard was already found in monitoring reports of perch and eel in the Flemish water bodies (Teunen et al., 2017, 2018; De Jonge et al., 2014). On a global scale, this is a reoccurring finding as well, even on isolated locations (Durrieu et al., 2005; Wyn et al., 2010; Noël et al., 2013; Guhl et al., 2014; Van Ael et al., 2014; Mataba et al., 2016; Verhaert et al., 2019). It should however be noted that this is a highly conservative threshold, which is well be below calculated critical body residues for risk of MeHg toxicity (Depew et al., 2012; Fuchsman et al., 2016). There have been indications that the current $EQS_{biota}$ might be too strict and not ecologically relevant (Teunen et al., 2018, 2017). Van Ael et al. (2014) demonstrated that, even with high accumulated Hg concentrations in eel, a good ecological quality could still be observed in aquatic ecosystems.

## 5. Conclusion and implications for management purposes

The results observed in the present study confirmed the suitability of both European ‘yellow’ eel and European perch as monitoring species, as they accumulated high concentrations of mercury. Furthermore, these species are rather easy to collect and provide sufficient tissue to perform the analyses needed. However, it is important to note that a large portion of the perch collected where very small, potentially skewing the results.

A significant difference in accumulated mercury concentrations between targeted species was found, with the highest concentrations in eel. In perch, higher concentrations were found in muscle compared to liver tissue. For eel, the opposite was found. The correlation of accumulated mercury with length in perch confirmed the fact that mercury concentrations increase with fish size.

Based on the findings, some implications for management purposes can be made. First, for perch, muscle tissue seems to be the most relevant tissue to evaluate both the environmental exposure risk and the risk for human health through consumption of fish contaminated with mercury. For eel, on the

other hand, higher concentrations were found in liver tissue. The difference between both tissues, however, is small and even disappeared when correcting for lipid content. In order to be able to compare both species and include human health risk, it is more relevant to continue monitoring in muscle tissue. Accumulated concentrations, although relatively high, did not pose any direct threat to human health through average fish consumption. However, it is not recommended to consume over 100g a day of fish caught in Flemish water bodies based on accumulated Hg concentrations.

The fact that mercury accumulation increases with size, should be taken into account during selection of appropriate monitoring species and sizes of the individuals (i.e. biota-monitoring). Therefore, it is important to select fish of a certain length or weight range. Alternatively, a correction for size can be implemented.

We would like to stress the importance of using linear mixed models when including fish from different sampling sites in order to more correctly take into account site-specific environmental effects of fish inhabiting a specific location.

**Declarations**

Ethics approval and consent to participate

At the moment procedures for ethical approval at INBO are being set up, but are not yet available. INBO collaborators (C. Belpaire and technical crew) have attestations of being experienced with handling animals for experiments (Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu- ref.213913/13_23/2013). All procedures were followed in order to minimize suffering.

Consent for publication

Not Applicable

Availability of data and material

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

LT performed the sample preparation, dissections, analysis of fish samples and contributed to the original draft. CB participated in sample collection. CB, GDB, RB and LB contributed to the review of the original draft. The final manuscript was read and approved by all authors.

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779 **Appendix**

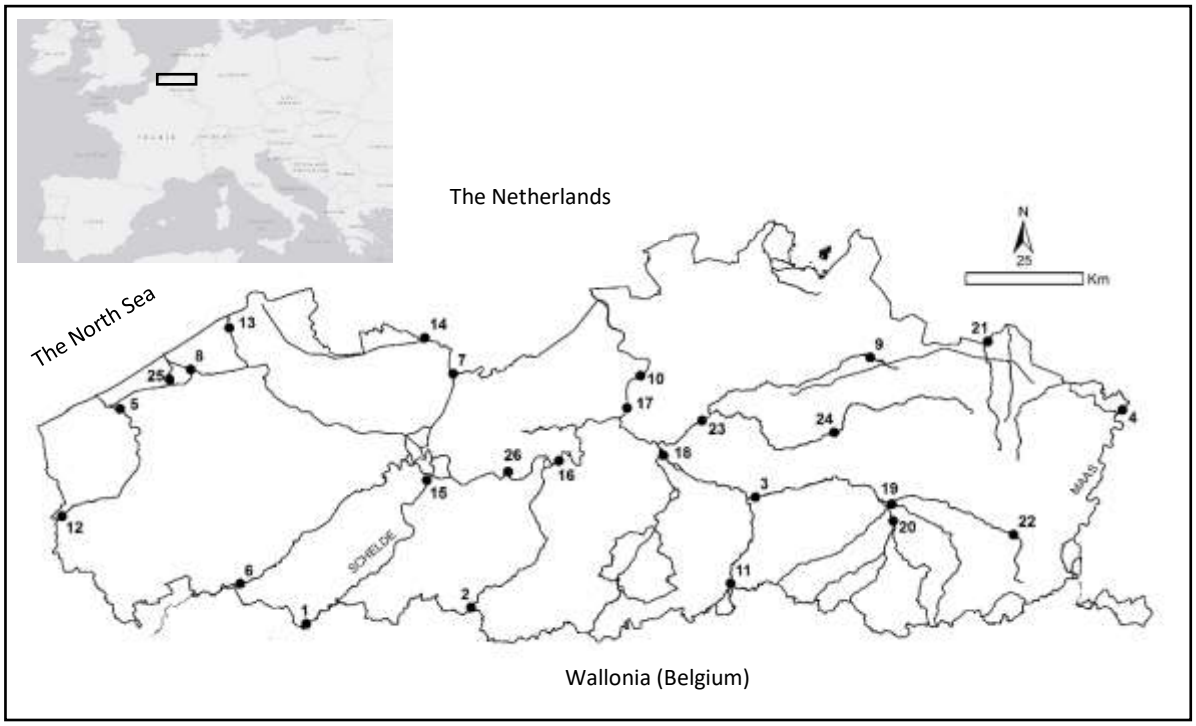


*Figure 1. Sampling locations in Flanders (Belgium).*

780 *Table 1. European perch (N=300) and European yellow eel (N=100) were collected in 26 sampling locations between 2013*
781 *and 2016. This study was carried out in Flanders (Belgium).*

| Nr | Sampling Site | Water body | Lambert X coordinate | Lambert Y coordinate | Sampling year | N | |
|---|---|---|---|---|---|---|---|
| | | | | | | Eel | Perch |
| **1** | Pecq | Boven-Schelde I | 79181 | 157135 | 2015 | 3 | 20 |
| **2** | Geraardsbergen | Dender I | 114132 | 160631 | 2015 | 3 | 20 |
| **3** | Werchter | Demer VII | 174581 | 184472 | 2015 | 3 | 9 |
| **4** | Kinrooi | Maas I+II+III | 252525 | 203301 | 2015 | 4 | 21 |
| **5** | Nieuwpoort | IJzer III | 39617 | 203488 | 2015 | 3 | 19 |
| **6** | Wevelgem | Leie I | 65139 | 165773 | 2015 | 3 | 14 |
| **7** | Zelzate | Kanaal Gent-Terneuzen | 110399 | 211142 | 2015 | 0 | 20 |
| **8** | Oostende | Kanaal Gent-Oostende III | 54608 | 212041 | 2015 | 3 | 20 |
| **9** | Retie | Kleine Nete I | 198974 | 214563 | 2015 | 3 | 17 |
| **10** | Antwerpen | Zeeschelde IV | 150151 | 210616 | 2015 | 11 | 0 |
| **11** | Sint-Joris-Weert | Dijle I | 169300 | 165850 | 2015 | 3 | 0 |
| **12** | Poperinge | IJzer I | 27250 | 180320 | 2016 | 1 | 20 |
| **13** | Blankenberge | Blankenbergse vaart | 62799 | 220991 | 2016 | 3 | 6 |
| **14** | Oostburg | Leopoldkanaal I | 104330 | 218850 | 2016 | 3 | 20 |
| **15** | Gent | Boven-Schelde IV | 104745 | 188127 | 2016 | 3 | 18 |
| **16** | Dendermonde | Zeeschelde II | 132788 | 192322 | 2016 | 4 | 3 |
| **17** | Hemiksem | Zeeschelde III + Rupel | 147328 | 203675 | 2016 | 3 | 0 |
| **18** | Mechelen | Getijdedijle-Getijdezenne | 155010 | 193500 | 2016 | 3 | 4 |
| **19** | Herk-de-Stad | Herk + Kleine Herk | 203500 | 182930 | 2016 | 2 | 0 |
| **20** | Herk-de-Stad | Melsterbeek I+II | 203850 | 179330 | 2016 | 2 | 0 |
| **21** | Neerpelt | Dommel | 223950 | 218080 | 2016 | 2 | 15 |
| **22** | Bilzen | Demer I | 229423 | 176366 | 2016 | 1 | 4 |
| **23** | Lier | Polder van Lier | 163177 | 201016 | 2015 | 5 | 25 |
| **24** | Westerlo | Laakdal | 191289 | 198420 | 2015 | 4 | 25 |
| **25** | Camerlinckxgeleed | Camerlinckxgeleed | 50720 | 209853 | 2015 | 5 | 0 |
| **26** | Bergenmeersen | Boven-Schelde | 122024 | 189948 | 2013 | 20 | 0 |

782 N: sample size.

783 *Table 2. Determination of human health risk through consumption of contaminated fish in Flanders. Maximum amount (g) of*
784 *contaminated fish muscle a 70 kg person can consume per day without posing health risks (MADC) were calculated for the*
785 *median and 95$^{th}$ percentile of the observed mercury concentrations in fish muscle tissue in Flemish water bodies, based on*
786 *MRL (ATSDR, 2018), RfD (UNEP, 2008) and PTWI (FAO/WHO, 2010). The Hazard Quotient (HQ) was determined by dividing*
787 *the estimated daily intake (EDI) for perch (2.7 g/day) and eel (18 g/day) with the MADC.*

| | **Hg concentration in muscle tissue (µg/g ww)** | | **MADC (g/day/70 kg adult) and HQ** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | MRL | | RfD | | PTWI | |
| | **50$^{th}$** | **95$^{th}$** | **50$^{th}$** | **95$^{th}$** | **50$^{th}$** | **95$^{th}$** | **50$^{th}$** | **95$^{th}$** |
| *Perca fluviatilis* | 0.06 | 0.21 | 357 (0.01) | 100 (0.03) | 119 (0.02) | 33 (0.08) | 274 (0.01) | 76 (0.04) |
| *Anguilla anguilla* | 0.11 | 0.31 | 185 (0.10) | 68 (0.27) | 62 (0.30) | 23 (0.81) | 142 (0.13) | 52 (0.35) |

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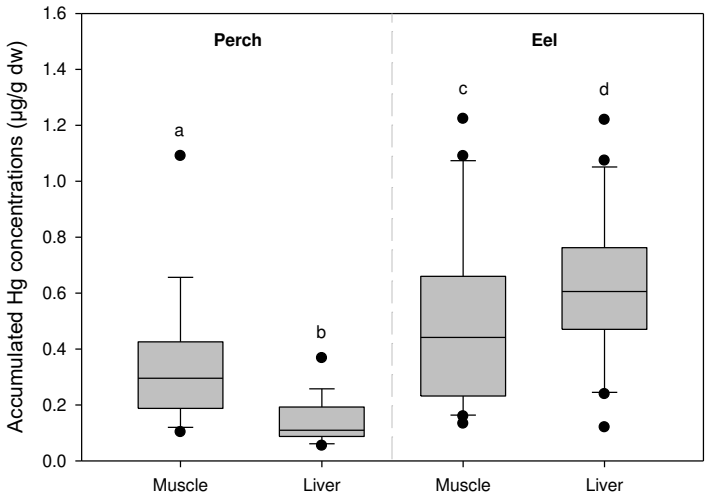


*Figure 2. Boxplots accumulated mercury concentration in perch and eel, muscle and liver tissue. Median concentrations per location were used. Different letters stand for a significant difference (p<0.05).*

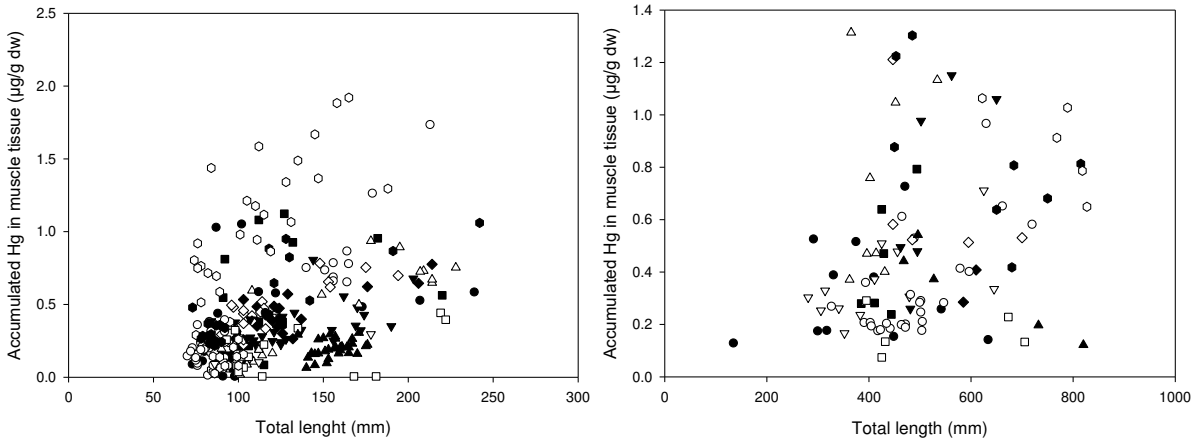


*Figure 3. Regression between total length of the individual and the accumulated mercury concentration in muscle for perch (LEFT; F=184.61, p<0.001) and for eel (RIGHT; F=3.67, p=0.06). Every symbol refers to a different location.*