Mercury accumulation in two freshwater fish species in Flanders (Belgium). Internal distribution and effects of length, weight and sex.

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Abstract Detrimental effects of chemical pollution - primarily caused by human activities - on surface waters and aquatic ecosystems, have increasingly gained attention. This pollution causes destruction of habitat, leading to a decrease in biodiversity. Mercury is the only metal incorporated in the EU list of priority compounds recommended to be measured in biota, preferably in tissue of prey species (Directive 2013/39/EG). Because of its hydrophobic qualities, mercury is prone to easily bio-accumulate and magnify through the food chain, which will eventually also affect humans. In the present study, accumulated levels of mercury are compared in both muscle and liver tissue of perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*) collected at 15 sampling locations in Flemish (Belgian) waterbodies. These results will create a better insight in respect to which concentrations are accumulated in fish species with different backgrounds as well as the internal distribution within the organism. Furthermore, effects of size, weight and sex are taken into account, since both age and reproduction are expected to have an influence on accumulation and storage of pollutants. The results show a correlation of accumulated mercury with indicators of age and/or condition (i.e. length, weight. No difference between sexes could be found. Furthermore, a significant difference in accumulated mercury levels between targeted species could be found, with the highest concentrations in eel. In perch, higher concentrations could be found in muscle compared to liver tissue.

Keywords: mercury, accumulation, fish, internal distribution.

1. Introduction

The global threat of chemical pollution of surface waters to the aquatic environment has increasingly gained attention during the past decades. This contamination can have acute and chronic toxic effects on aquatic organisms. Furthermore, pollutants accumulate in the ecosystem, leading to a loss of habitats and biodiversity, and a possible threat to human health (EC 2013). Most chemical compounds, such as metals, will enter the environment due to anthropogenic activities.

Mercury has a wide applicability, such as in industry (i.e. production of car components), mining, households (i.e. batteries), agriculture (i.e. pesticides) and will enter aquatic ecosystems through among others erosion and both industrial and domestic discharges (Kidd & Batchelar 2012; Selin 2009). The largest portion, however originates from atmospheric deposition as a result of fuel combustion, causing long-range transport. Due to its very persistent characteristics, mercury will remain present in the environment for a considerable amount of time.

Due to its hydrophobic and lipophilic characteristics, mercury has a very low solubility in water (Kidd & Batchelar 2012). It binds to small organic particles and sediment. In this way, it may bio-accumulate in biota and biomagnify through the food chain (Lavoie *et al*. 2013; Wiener *et al*. 2003). Once ingested, mercury will become bio-available to the individual through enzymatic and bacterial processes inside the digestive system (Kidd & Batchelar 2012). Subsequently, mercury is transformed into its organic, methylated form (i.e. methylmercury) and translocated to different body parts or organs. In general, in fish the largest portion (>90%) of mercury consists of methylmercury (Bloom 1992; Kannan *et al*. 1998; Chvojka *et al*. 1990).

Internal distribution of mercury depends mainly on the exposure route (Régine *et al*. 2006). Ingestion of particle bound mercury, mostly in its organic form will be transported to the muscle. On the other hand, absorption through the gills, mainly in its inorganic form, will be accumulated in the organs, with the liver being a main target tissue for inorganic mercury. For piscivorous or omnivorous fish, the main exposure route will consist of diet- or particle bound (methyl)mercury (Régine *et al*. 2006).

Mercury can act as a potent neurotoxicant, especially in its organic form, and therefore, will interfere with both perceptive systems (i.e. vision, hearing) and movements (i.e. inability, spasms) (Clarkson 1992; Kidd & Batchelar 2012). Exposed fish can experience deleterious effects on

their growth, development, and reproduction. Through the effect of biomagnication, mercury present in the environment can also affect top predators and mammals, including humans.

Both fish species are frequently used for monitoring purposes (Ion *et al*. 1997; Belpaire & Goemans 2007). These are very common species, which allows for a comparison of accumulated levels between different countries. Furthermore they are residents, creating a reliable image of a relatively restricted area and they are relatively tolerant to pollution (Ion *et al*. 1997). Furthermore, because of their high trophic level, they accumulate higher levels of pollutants, due to biomagnification (Belpaire & Goemans 2007). This facilitates the detection of the problem (high accumulation levels) in the food chain.

Both size and weight are considered indicators of age (Ion *et al*. 1997). Older individuals have been exposed to polluted areas for a longer amount of time and are likely to have accumulated higher levels (Weis & Ashley 2007; Szefer *et al*. 2003; Batchelar *et al*. 2013; Durrieu *et al*. 2005). Furthermore, sex can also play a role in the accumulated mercury levels. As females produce a large amount of lipid-rich eggs, it is considered that fat-soluble contaminants, such as mercury, are eliminated during spring spawning.

Within the present study we hypothesize that (1) higher mercury levels are to be expected in fish with higher weight and size; (2) no significant difference in mercury content is to be found between sexes, since sampling took place during autumn; (3) higher concentrations are expected in the most fat containing fish species, eel. (4) higher accumulated Hg concentrations are expected to be found in muscle compared to liver tissue.

2. Methods

A total of 15 sampling locations were selected in Flanders (Belgium). Perch and/or eel where caught by the Institute for Nature and Forest Research (INBO) between 2012 and 2015 as part of their Fish Monitoring Project. Fish were sampled using electrofishing/fykes, depending on the depth of the water body.

2.1. Sample preparation

A total of 178 perch and 73 eel were caught. Before dissection, length and weight of individual fish were determined. During dissection, sex of the individual perch was visually determined (N=160), for eel this was not possible. Fish were dissected, muscle (N=277) and liver tissue (N=196) isolated, and frozen at -20°C until analysis.

2.2. Mercury analysis

After freeze drying, the digestion of the samples was performed in a mixture of HNO_3 and HCl (1:3), in order to keep the stability of mercury in solution, using 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) (Thermo scientific Finnigan element 2, Altham, MA, USA), in cold plasma mode. Reference material included freeze dried muscle tissue (no. 2976) from NIST (National Institute of Standards and Technology-USA).

2.3. Statistical analysis

Statistical analyses were performed using the software package R. A Pearson correlation was determined between weight and length measures, between dry and wet weight and between liver and muscle tissue. A linear regression was performed to investigate the relation between accumulated levels and length/weight. To investigate different accumulated mercury levels in respectively species, sex and tissues (muscle of liver), linear mixed models were composed for each of these variables with location as fixed effect. For these analyses a 'backward stepwise variable' selection was used. Furthermore, a log-likelihood ration test (χ^2 test) was performed to determine the significance of the variables. Significance levels were set at a p-value <0.05.

3. Results and discussion

All length measures correlated very well ($r \geq 0.99$), as well as dry weight and wet weight ($r \geq 87$), and Hg levels in liver and muscle ($r \geq 62$). For further analysis, mercury concentrations in dry weight are used to be able to perform a more standardized comparison between species and tissues. Weight showed and exponential increase with increasing length for both eel ($R^2=0.86$) and perch ($R^2=0.88$).

Mercury accumulation increased with increasing length for perch in both muscle ($F=63.37$; $p<0.001$; $R^2=0.30$) and liver tissue ($F=47.28$; $p<0.001$; $R^2=0.23$) (Figure 1). For eel, this was true for muscle ($F=14.65$; $p<0.001$; $R^2=0.17$) but not for liver tissue ($F=0.15$; $p=0.70$; $R^2=0.004$).

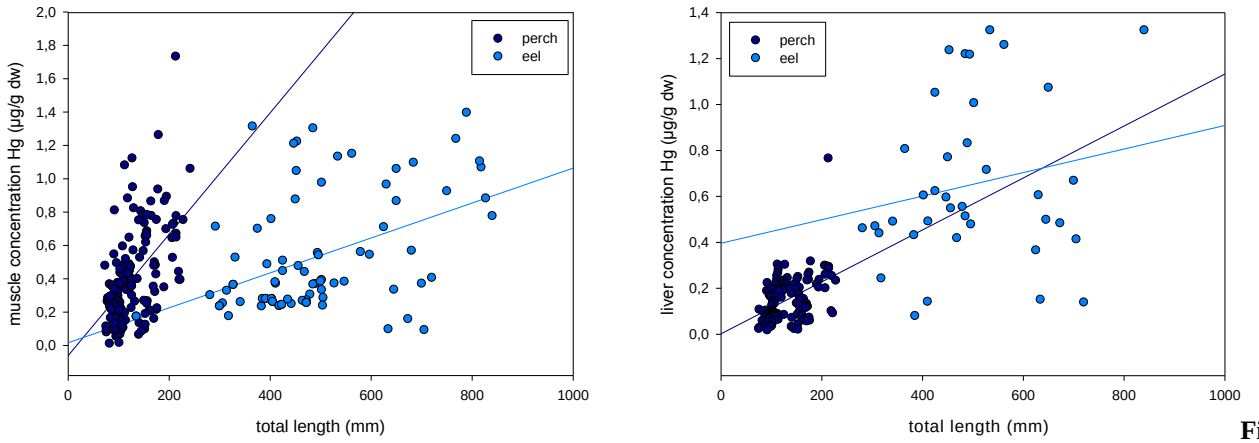

Figure 1. Relation between total length of the individual and the accumulated mercury concentration in muscle (LEFT) and liver tissue (RIGHT), for both perch and eel, mean values with standard error.

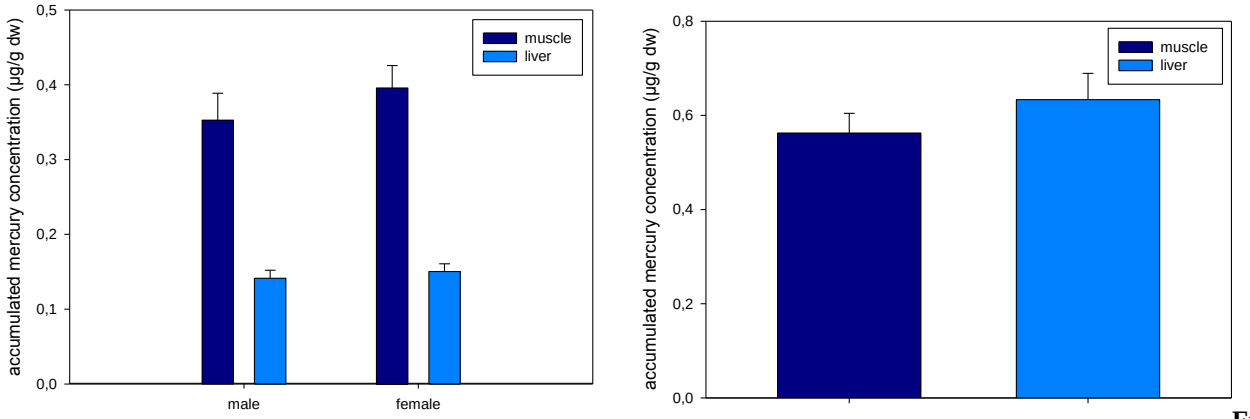

Figure 1: Accumulated mercury level in perch (LEFT) and eel (RIGHT), mean values with standard error. For perch both sexes and tissues are displayed. For eel only tissues are shown.

No significant difference in accumulated mercury levels was found between sexes in perch in muscle tissue (df=1; χ^2= 1.16; p=0.28), neither in liver tissue (df=1; χ^2=0.34; p=0.56) (Figure 2). This could be explained by the fact that sampling took place during autumn, outside spawning season. During this time period, females don't shed lipid-rich eggs, possibly containing mercury.

A significant difference in accumulated levels was found between both species in muscle tissue (df=1; χ^2=32.98; p>0.001) as well as in liver tissue (df=1; χ^2=163.55; p<0.001), with higher levels in eel than in perch for both tissues. For perch a significant difference between liver and muscle accumulated levels could be found (df=1; χ^2=75.94; p<0.001). Accumulated mercury levels in muscle were higher than those in liver. 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. For eel, no significant difference in mercury levels between tissues was found (df=1; χ^2=0.95; p=0.33). Higher concentrations in eel could be explained by the high fat content of these fish. The hydrophilic properties of mercury cause it to easily accumulate in fat tissue. A higher fat content, therefore, can lead to a higher mercury level.

4. Conclusion

The correlation of accumulated mercury with indicators of age and/or condition (i.e. length, weight), confirms the fact that mercury is bio-accumulated inside these animals. No difference between sexes could be found probably due to sampling outside the breeding season. A significant difference in accumulated mercury levels between targeted species could be found, with the highest concentrations in eel. In perch, higher concentrations could be found in muscle compared to liver tissue. These findings should be taken into account during selection of appropriate monitoring species and sizes of the individuals (i.e. biota-monitoring).

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