Cadmium, lead, and mercury concentrations in whole-fish, liver, and muscle of herring (*Clupea harengus*) and perch (*Perca fluviatilis*)

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Summary 3
Background 3
Research Design and Methods 4
  Study Species 4
  Sampling and preparation 4
  Statistical analyses 5
Results 5
  Distribution of metals in fish tissue 5
  Relationship between metal concentrations in whole-fish, liver, and muscle 5
  Species-dependent differences in distribution between tissues and organs 5
Discussion 8
References 10

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Summary

Monitoring of metal concentrations in fish is commonly conducted using samples of fish liver or muscle. This becomes problematic when these values are used for environmental status evaluations regarding chemical pollution, as many of the thresholds evaluating environmental status are designed to evaluate concentrations in whole-fish.

In this study, concentrations of cadmium, lead, and mercury in herring and perch are compared between liver, muscle, and whole-fish. The aim is to create conversion factors that can be used to convert metal concentrations between tissues and organs.

Mercury and cadmium were detected in all analyzed organs and tissues, whereas lead concentrations were below the level of quantification for most muscle and liver samples. In general, there was a strong relationship between liver and muscle concentrations and concentrations in whole-fish, indicating that creation of conversion factors between tissues and organs is suitable.

The resulting conversion factor between whole-fish and liver concentrations for cadmium was 0.1 and 0.16 for herring and perch, respectively. The conversion factor between whole-fish and liver concentrations for mercury was 0.52 and 1.66 for herring and perch, respectively, while for whole-fish and muscle concentrations for mercury, the conversion factor was 0.86 and 0.74 for herring and perch. As there was no significant relationship between liver and whole-fish concentrations for lead, a conversion is not appropriate. Conversion factors levels differed significantly between herring and perch, indicating that species-specific conversion factors are necessary.

Background

The Marine Strategy Framework Directive 2008/56/EC (MSFD) set the goal of achieving Good Environmental Status (GES) for chemical pollutants in the marine environment. GES is determined by using quality assessments based on target levels that represent a threshold that should not be exceeded. Estimation of GES for fish is often problematic, as target levels provided for many metals are set for concentrations in whole-fish, although most metal concentrations are analysed using liver or muscle tissue.

Heavy metals e.g., cadmium, lead, and mercury, bioaccumulate and biomagnify in aquatic organisms (Huber 1998, Dietz et al. 2000). This accumulation can result in a negative impact on fish metabolism, physiology, behavior and ecology (Gbem et al. 2001). Metals are known to accumulate at varying concentrations in different tissues and organs of fish (Ciardullo et al. 2008, Vieira et al 2011). The patterns of bioaccumulation and biomagnification differ between tissue, organs (Honda et al. 1983, Gbem et al. 2001, Rashed 2001, Ciardullo et al. 2008), and species (Kljaković Gašpić et al. 2002, Viera et al. 2011).

To date, monitoring of metal concentrations in fish within the OSPAR and HELCOM regions are conducted using samples of fish liver or muscle. Heavy metals analysed within the Swedish National Monitoring Programme are measured in liver, or, for mercury, in muscle tissue. Some of the Environmental Quality Standards (EQS) and Environmental Assessment Criterias (EAC) for metals are set to protect against secondary poisoning in top predators, and are designed to evaluate concentrations in whole fish. In order to evaluate GES, metal concentrations measured in fish liver and muscle need to be converted to a concentration representative of the whole-fish body. If a relationship can be established between muscle, liver and whole-fish for metal concentrations, conversions between tissues can then easily be calculated using a conversion factor. However, information on the relationship of metal concentrations between different tissues within fish, and therefore conversion of concentrations, is limited within the scientific literature (however, see Bevelhimer et al. (1997) and Strandmark et al. (2008) for further information).

The main objectives here are to 1) compare cadmium, lead, and mercury concentrations between liver, muscle, and whole-fish tissue to establish if any relationship exists in concentrations between the different tissues, and 2) using these relationships, create conversion factors that can be used to convert concentrations from muscle or liver tissue into whole-fish body concentrations. Such conversion factors will be of use in establishing more robust target levels for better environmental and health protection.

Research Design and Methods

Study Species

Baltic herring (*Clupea harengus*) and perch (*Perca fluviatilis*) were used as they represent species feeding at different trophic levels (adult herring mainly feed on zooplankton, while perch more than 13cm are piscivorous). These are also fish species commonly used in monitoring programmes within the HELCOM monitoring region.

Sampling and preparation

In August 2011, 20 herring and 20 perch were collected from Öviksfjärden, located south of Umeå in the northern part of the Bothnian Sea. Gill nets were used and fish were frozen as soon as possible after collection. Following procedures described in Olsson and Zetterberg (1982), sample preparation was undertaken at the Museum of Natural History, Stockholm (NRM). For each individual, body weight, total length, body length, sex, reproductive stage, liver weight, and sample weight were recorded. Otoliths were removed and sent for age determination at the Department of Aquatic Resources, Institute of Coastal Research, Swedish University of Agricultural Sciences, Uppsala.

Muscle samples were taken from the middle dorsal muscle layer. The skin and subcutaneous fat were removed. Samples of ~2 g muscle tissue were prepared for metal analysis. The entire liver was removed and weighed. The remainder of the fish body was homogenized with stainless steel blades, following guidelines modified from the US Environmental Protection Agency (USEPA 2000). Unhomogenized pieces of fish were removed from the sample, as these can bias metal analyses. Analyses of metal concentrations were conducted at ALS Scandinavia (Sweden) using accredited methods.

Statistical analyses

Metal concentrations below detection limit were estimated by dividing the reported value below detection limit with the square root of two. Data was analysed using linear regression in Statistica (v 11).

Whole-fish concentration (C_{wf}) was calculated as:

$C_{wf} = (C_c*W_c + C_l*W_l + C_m*W_m)/(W_c+W_l+W_m)$

Where Cc, Cl, and Cm are the metal concentration (mg/kg dry weight) in the carcass, liver, and muscle, respectively. $W_{c,}$ W_l, and W_m are the weight (g) of the carcass, liver, and muscle, respectively.

Results

Distribution of metals in fish tissue

Concentrations of mercury were reported in all tissue samples from both species. Cadmium was reported in all liver samples, and in 65% and 100% of the carcass homogenate samples of perch and herring, respectively. In all muscle samples, cadmium was reported as below quantification limit. Lead was reported most often in the carcass homogenate samples, with between 85% and 100% detection rates for herring and perch, respectively. In liver tissue, only one of the herring samples had concentrations of lead high enough to be reported. Lead concentration was below quantification limits for all samples of perch liver and muscle, and herring muscle. Together with calculations of whole-fish concentrations, the results for cadmium, lead, and mercury concentrations in calculated whole-fish, liver, and muscle tissues are presented in appendix 1 (Table 1).

Relationship between metal concentrations in whole-fish, liver, and muscle

For both herring and perch, calculations of whole-fish concentrations of cadmium and mercury show a significant positive relationship to liver concentrations (Fig. 1, tab. 1). In herring, muscle concentrations were also show a significant positive relationship to whole-fish concentrations. For lead, no significant relationship was seen between calculated whole-fish and liver, and neither for perch or herring (Fig. 1, tab. 1). There was no significant relationship between any of the investigated biological factors (body length, weight, and age) and calculations of whole-fish concentrations of cadmium, mercury, or lead.

Species-dependent differences in distribution between tissues and organs

The ratios between calculated whole-fish concentrations and liver or muscle concentrations differed between species, suggesting that conversion factors need to be species-specific. For all three metals, the ratios between calculated whole-fish and liver concentrations were significantly higher in perch than herring (Fig. 2). The opposite pattern was observed for mercury, where the ratio between calculated whole-fish and muscle concentrations were significantly higher in herring than in perch (Fig. 2).

The conversion factor between whole-fish and liver concentrations for cadmium is 0.1 and 0.16 for herring and perch, respectively. The conversion factor between whole-fish and liver concentrations for mercury is 0.52 and 1.66 for herring and perch, respectively. The conversion factor between whole-fish and muscle concentrations for mercury is 0.86 and 0.74 for herring and perch, respectively. As there was no significant relationship between liver and whole-fish concentrations for lead, the conversion factor does not explain the distribution between the organs (Tab. 2)

Table 1. Correlations between liver or muscle concentrations and calculated whole-fish concentrations in herring and perch.
*** marked correlations are significant at $p < 0.05$.**

Regression liver to whole-fish				
	Herring		Perch	
	N	**r**	**N**	**r**
Cd	20	0,76*	20	0,82*
Hg	20	0,93*	20	0,88*
Pb	20	0,14*	20	0,21
Regression muscle to whole-fish				
	Herring		Perch	
Hg	20	0,9*	20	0,98*

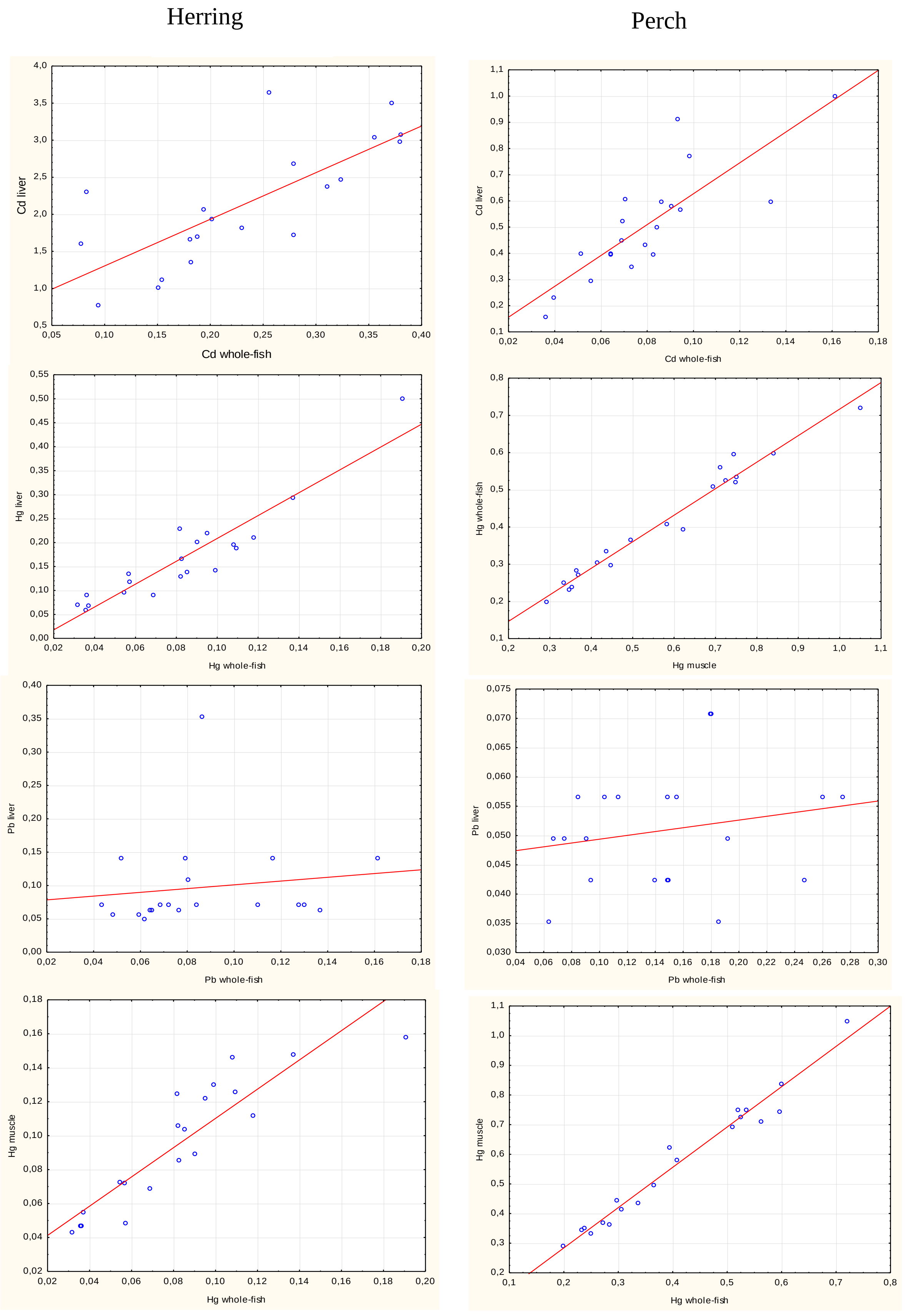

Figure 1. Linear regressions indicating the relationship between whole-fish concentrations and liver and concentrations for cadmium, lead, and mercury, and for muscle in mercury, in herring (left) and perch (right).

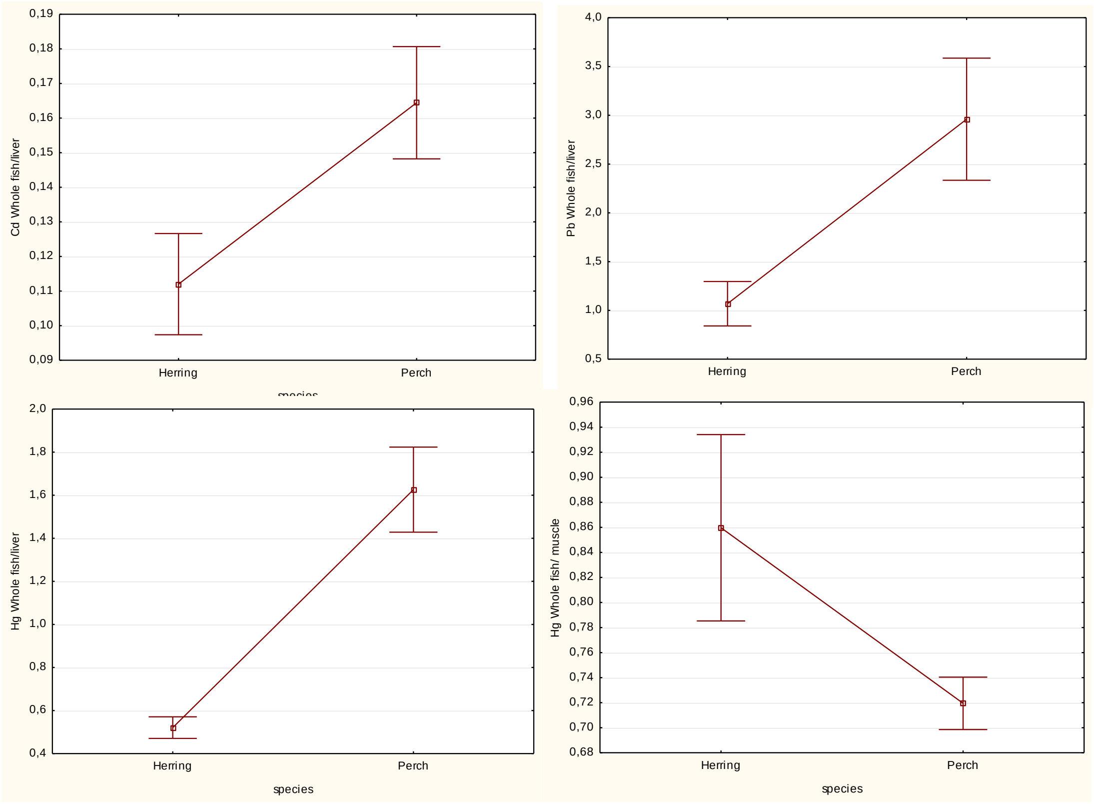

Figure 2. Mean plots of ratios between calculated whole-fish concentrations and liver or muscle concentrations of cadmium, lead, and mercury in herring and perch. Concentration means are expressed with a 95% confidence interval.

Table 2. Conversion factors between liver or muscle and whole-fish concentrations of cadmium, mercury, and lead for perch and herring.

Conversion factors from liver or muscle to whole-fish concentrations				
Whole fish/liver				**Whole fish/muscle**
Species	Cadmium	Lead	Mercury	Mercury
Herring	0.10	1.07*	0.52	0.86
Perch	0.16	2.86*	1.66	0.74
** not appropriate to use*				

Discussion

Conversion factors are needed to transform liver to whole fish concentrations for cadmium, lead, and mercury, and, for mercury, from muscle to whole fish concentrations, for evaluating Good Environmental Status (GES) based on quality standards such as Environmental Quality Standards (EQS) and Environmental Assessment Criteria (EAC) according to the Marine Strategy Framework Directive (MSFD). Here, we presented species-specific conversion factors, enabling conversion of cadmium and mercury concentrations in liver, and mercury concentrations in muscle, to whole-fish concentrations.

Mercury was detected in quantifiable levels in all tissues and organs, but it was found to mainly accumulate in muscle tissue. In line with previous research, we found no cadmium accumulated in muscle tissue (Szefer and Falandysz 1985, Andres et al. 2000, Szefer et al. 2002). The conversion factor between whole-fish:liver reveals that liver concentrations of cadmium are 10 - 16 times higher compared to whole fish concentrations. Accumulated lead is distributed to other tissues and organs such as bones, teeth and skeleton, and the kidneys (Odžak and Zvonaric 1995, Nordberg et al. 2007). Here, a weak relationship between estimates of whole-fish concentrations and liver showed that a conversion factor between these organs is not appropriate. This weak relationship can mainly be explained by a poor detection frequency. In this study, analysed herring livers were small, and sometimes weighed as little as 0.17 g. Larger livers i.e., larger fish, combined with a lower quantification level, would allow detection of lower concentrations (Bignert et al. 2011). Further studies are required to investigate the relationship between lead concentrations in whole-fish and liver.

The interspecific differences in cadmium, lead, and mercury concentrations highlight the need for species-specific estimates of the relationship between different tissues and organs (e.g., species-specific conversion factors). A previous study by Andres et al. (1999) concludes that interspecific differences in bioaccumulation of metals can be explained by trophic feeding level.

In conclusion, this report demonstrates that by converting the muscle concentrations commonly used within monitoring programmes into whole-fish concentrations, the accuracy of the GES evaluation would be improved considerably. However as this was a pilot study, further testing is required, for example, using a lower level of quantification and including a larger range of fish species, tissues, and locations.

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