Södertörn University | School of Natural Sciences, Technology and Environmental Studies

Bachelor Thesis 15 ECTS | Environmental Science | Spring 2013

Mercury concentration in Baltic herring, *Clupea harengus*, coupled to abiotic environmental factors

– A study using monitoring data

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Abstract

Earlier studies on the relationship between mercury concentration in fish – or alternatively bioavailability of mercury in aquatic systems – and different abiotic oceanographic factors such as water temperature, pH and salinity, have shown that such associations often exist. The results are not always consistent between studies, however. The aim of this study is to investigate if this type of relationships can be established in Baltic herring, *Clupea harengus*, with the use of temporal monitoring data from the northern Bothnian Bay (northern Sweden), compiled within Swedish monitoring programmes. The abiotic factors being assessed in this study are water temperature, salinity, dissolved oxygen, pH and measurements of nutrient content in the water; phosphate, total phosphorus and total nitrogen. Relationships between these parameters and mercury concentration in herring were investigated using multiple linear regression. Generally however, relationships were absent, and only one significant association was found, a positive relationship between total phosphorus in water and herring mercury load. Difficulty to reveal relationships in the presence of confounding factors, which cannot be controlled for when using monitoring data from the field, is suggested as one reason for the absence of such relationships. Furthermore, the somewhat incomplete monitoring data of abiotic factors likely made it hard to detect any relationships.

Keywords: methylation, bioaccumulation, oceanographic data, Bothnian Bay

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Introduction

Background and aim of the study

Mercury (Hg) is a metal that occurs naturally in minerals in the Earth's crust, and also in fossil fuels. Although mercury is released through natural processes, e.g. weathering and volcanic eruptions, today anthropogenic activity accounts for a major part of the releases of bioavailable mercury into the environment. Mining and combustion of fossil fuels, especially coal, is the major source of anthropogenic emissions, but mercury is also emitted in various industrial processes, and associated with the disposal of consumer products containing mercury, e.g. thermometers, compact fluorescent lamps and batteries (UNEP 2013).

Mercury is a cause for concern for human health because of its adverse effects on the nervous system, the risk being highest for infants and *in utero* children. Also the human immune system is affected by mercury (Sterner 2010). Consumption of fish containing mercury accounts for the major part of mercury uptake in humans (UNEP 2013), and based on these facts, the Swedish National Food Agency recommends women to limit their consumption of mercury-containing fish species to 2-3 times per year during pregnancy and breast-feeding (Swedish National Food Agency 2013).

Mercury is transported in the air bound to particulate matter or as gaseous elemental mercury, which is deposited to soil and water through oxidation (UNEP 2013). Historically, mercury was also emitted directly into water from factories, e.g. metal processing plants and pulp and paper plants, but this practice has now largely ceased. Also, emissions into the air and subsequent deposition to land and water have decreased in Europe since the 1970s as a result of improved industrial cleaning techniques and stricter regulation (Bernes 2005).

Despite these reductions in anthropogenic mercury emissions, concentrations in aquatic environments can be elevated for long time periods, for example because of mercury that is deposited in sediments and later re-suspended (Bernes 2005). The Baltic Sea has levels of contaminant burdens, including mercury, which are typically higher than in the world's oceans in general. This is due to a number of geographical characteristics, one of them being a large catchment area in relation to the total sea volume. Because the catchment area is also densely populated, the inflow of contaminants into the sea is high. Furthermore, water exchange with the adjacent ocean is limited, leading to a long turn-over time for water in the Baltic Sea. Consequently, contaminants stay in the sea for a long time (Bernes 2005).

A wide array of contaminants, among them mercury, is monitored in a number of different marine species at several locations along the coast of Sweden through the Swedish National

Marine Monitoring Programme. The programme is financed by the Swedish Environmental Protection Agency and co-ordinated by the Department of Environmental Research and Monitoring (former Department of Contaminant Research) at the Swedish Museum of Natural History (Bignert et al. 2011). It gives a possibility to study the state of the environment concerning contaminants in marine biota, e.g. spatial variation and time trends. The monitoring programme includes food fish such as herring, *Clupea harengus,* which is relevant in relation to the risks for human health associated with mercury and other contaminants (Bignert et al. 2011). Another monitoring programme in Swedish marine areas, co-ordinated by the Swedish Meteorological and Hydrological Institute (SMHI), measures different oceanographic parameters over time at predefined locations off the Swedish coast. The measurements include water temperature, salinity, dissolved oxygen, water pH and nutrient content; total nitrogen, total phosphorus and phosphate (SMHI 2013).

Using these monitoring data in the present study, it will be investigated whether any relationships exist between abiotic factors, i.e. physical and chemical parameters measured in water, and total mercury concentration in herring over time. This question has previously been investigated mainly in experimental settings (e.g. Dutton & Fisher 2011; Wang et al. 2011), and in the field primarily on fish in freshwater environments (e.g. Doetzel 2007; Bodaly et al. 1993). Levels of abiotic parameters have then either been controlled experimentally, or alternatively, the question has been studied on a spatial rather than temporal scale. That is, relationships have been studied using e.g. lakes with different characteristics rather than on the same place over time.

Given this lack of temporally oriented studies, it could be of interest to investigate if relationships can be detected using long-term temporal monitoring data. Due to its importance as a food fish, herring is a suitable study matrix. Certainly, causal effects cannot be established with the use of monitoring data from the field, but nevertheless, this study could give an indication on possible effects of variation in abiotic factors on mercury bioaccumulation in herring. Knowledge about this could be valuable in relation to the human health risks associated with mercury. Furthermore, information on relationships in the recent past between herring mercury load and environmental parameters could form a useful basis if the effects of anthropogenic climate change on mercury load in fish are to be estimated. In conclusion, the aim of this study is to investigate whether there are relationships between abiotic environmental parameters in water and total mercury concentration in Baltic herring over time, using monitoring data.

Theory

In aquatic systems, divalent inorganic mercury (Hg(II)) can be transformed into highly toxic methylmercury (MeHg) through methylation, which can be abiotic but is mainly performed by bacteria (biomethylation) (UNEP 2013). This process takes place in the water column, in sediments – which are thought to be the main site of methylation in coastal regions – as well as in wetlands. MeHg has a large potential to bioaccumulate and biomagnify to great concentrations in aquatic food chains. Both uptake in plankton and absorption through the intestinal wall in animals at higher trophic levels is much more efficient for MeHg than is the case with Hg(II) (UNEP 2013). Therefore, MeHg comprises the major part of the total mercury load found in fish (Swedish National Food Agency 2007). Except for uptake through ingestion of food, MeHg can also be taken up by fish directly from the water through transport over the gills, although this pathway only accounts for a minor part of the total MeHg accumulation in fish (Ullrich et al. 2001).

In the following sections, effects of abiotic factors on fish mercury concentrations that theoretically could be expected are reviewed briefly. However, as Ullrich et al. state, discussing factors affecting methylation; "…it should be borne in mind … that they [parameters affecting methylation] cannot be viewed independently from each other, as they often interact, forming a complex system of synergistic and antagonistic effects." (Ullrich et al. 2001, p. 258)

Temperature

There is a general understanding that rising water temperatures lead to elevated concentrations of mercury in aquatic organisms (Dijkstra et al. 2013). This is probably linked to an increased activity of methylating bacteria at higher temperatures. At the same time, while methylation seems to be favoured at higher temperatures, demethylation, which is also primarily mediated by bacteria, seems to increase at lower temperatures (Ullrich et al. 2001). Hence, increasing temperature makes more MeHg available to enter the aquatic food chain.

Bodaly et al. (1993) suggested that higher water temperature can increase mercury concentrations in fish because of higher metabolic rates, leading to a greater food intake and more water being passed over the gills. Increased food consumption due to higher temperatures was confirmed by Arrhenius & Hansson (1994), studying juvenile Baltic herring. The theory of Bodaly et al. (1993) was later investigated further, when differences in fish mercury loads between two lakes with different temperatures was assessed. The positive temperature-

mercury correlation was then established empirically, but found to be of minor importance compared to the influence of variation in mercury content of the prey (Harris & Bodaly 1998).

In a field and laboratory study, Dijkstra et al. (2013) showed that the zooplanktivorous estuarine killifish, *Fundulus heteroclitus,* accumulate higher mercury loads at higher temperatures. Using a bioenergetics model to evaluate the results, they conclude that this might be due to a higher respiration rate and consequently lower growth rate at higher temperatures. Thus, at higher temperatures a smaller share of the ingested food is redistributed to growth, resulting in increasing mercury loads per unit weight. This being said, it must be noted that Dijkstra et al. (2013) conducted their investigation under considerably higher temperatures (15-27 °C) than is used in the present study, and the same is true for the Harris & Bodaly (1998) study.

Salinity

While the opinion about the effects of temperature seems to be quite unambiguous, the effect of salinity on mercury accumulation in fish is harder to interpret. As far as the effect of salinity on methylation is concerned, methylation seems to diminish at higher salinities. This is explained by the fact that in freshwater and at low salinities, Hg(II) mainly forms uncharged complexes with hydroxide and chloride ions, whereas in seawater with full salinity, Hg(II) is mainly forming negatively charged complexes with chloride ions (Ullrich et al. 2001). Because uncharged molecules are more easily transported over biological membranes, bioavailability of mercury to methylating bacteria is increasing with decreasing salinity (Ullrich et al. 2001). The sea salt anions sulphate and bicarbonate have also been shown to reduce methylation rates (Compeau & Bartha 1983). However, what complicates things is the fact that at low salinities, more Hg(II) can be complex-bound to large organic molecules such as humic acid, than at higher salinities (Leermakers et al. 1995), which could be hypothesised to decrease mercury availability for methylation with decreasing salinity.

For the small part of mercury, both MeHg and Hg(II), that is accumulated in fish directly from the water through the gills, Dutton & Fisher (2011) have shown a more rapid uptake under higher salinities in the abovementioned killifish during a short-time (Hg(II) 72 h, MeHg 48 h) experiment. For MeHg, however, the concentrations had equalled out at the end of the experiment so that concentrations were as high in fish held under low salinity compared to fish from high salinity water. The same was not true for Hg(II), which was more accumulated at higher salinities throughout the whole 72 h time period (Dutton & Fisher 2011). The explanation that is offered to the more rapid accumulation at higher salinities is that chloro-

complexation is favoured over complexation with hydroxide ions. Uncharged chloro-complexed Hg(II) and MeHg have higher octanol-water partition coefficients (K_{ow}) than their hydroxide-complexed counterparts, leading to a greater ability to cross biological membranes, i.e. the gills (Dutton & Fisher 2011).

Studies on aquatic invertebrates have at least in some cases come to other conclusions, however. A study on the shore crab *Carcinus maenas*, found an inverse relationship between Hg(II) and salinity, whereas MeHg didn't show any particular pattern (Laporte et al. 1997). The same negative relationship was observed for both mercury species in the green mussel *Perna viridis* (Pan & Wang 2004). It should be noted that all three studies referred to above used a wider span and generally higher salinities than is the case in this investigation.

Dissolved oxygen

It is an often noted fact that anoxic conditions in sediment and water favour methylation in general (Ullrich et al. 2001). In oxic water, MeHg is formed mainly through the slower process of abiotic methylation, while demethylation seems to be microbially mediated (Matilainen & Verta 1995), and generally enhanced under oxic conditions (Ullrich et al. 2001). However, these facts cannot be investigated with the monitoring data in this study, because dissolved oxygen data are in this case well above the limit for hypoxic water; 2 ml/l (Bernes 2005).

Nevertheless, dissolved oxygen in the water column at normoxic concentrations has been included as a parameter in some studies concerning effects of environmental factors on mercury load in fish (e.g. Dijkstra et al. 2013; Doetzel 2007; Sonesten 2003), but these studies have not been able to detect any effect of dissolved oxygen. On the other hand, laboratory studies on carp, *Cyprinus carpio* (Yediler & Jacobs 1995), and Nile tilapia, O*reochromis niloticus* (Wang et al. 2011), have shown increasing mercury accumulation with decreasing dissolved oxygen levels, possibly due to increased ventilation over the gills at lower oxygen levels.

Nutrients

Higher densities of freshwater phytoplankton and zooplankton have been shown to correlate negatively with mercury concentrations in the same plankton groups, as well as in zooplank-tivorous fish (Chen & Folt 2005). This could likely be coupled to the fact that increased nutrient content in water can support a higher primary productivity, and consequently a larger biomass up the food chain (Bernes 2005), leading to a mercury dilution effect in biota (Chen

& Folt 2005). A negative correlation has also been shown between mercury concentration in Swedish freshwater perch and increasing lake nutrient content (Sonesten 2003). In a conceptual model concerning the effects of nutrients on mercury concentration in fish, Driscoll et al. (2012) argues that the same kind of relationship should apply also in marine – and especially coastal water – settings. Although the model is focused on nitrogen, the authors state that it is valid for phosphorus as well. They refer to the effect of biodilution as well as to increased production of organic matter – which in turn should lead to increased complexation with mercury and settling to sediments – as reasons for a negative relationship. Increased decomposition near the bottom, if leading to depletion of oxygen, is hypothesised to reduce bioturbation and thereby limit re-suspension of mercury, further augmenting the negative relationship (Driscoll et al. 2012).

pH

Water pH is one of the chief factors thought to affect accumulation of mercury in fish, with lower pH levels raising mercury concentrations. At higher pH, a greater proportion of mercury is methylated to the dimethylmercury form which is volatile and easily evaporates into the atmosphere. At lower pH, methylation into monomethylmercury, which is less volatile, is favoured (Sterner 2010). Furthermore, at higher pH, Hg(II) is to a greater extent reduced to elemental mercury, leaving less Hg(II) to be methylated (Watras et al. 1995). It has also been hypothesised (e.g. Miskimmin et al. 1992) that at lower pH, Hg(II) is more readily disassociated from dissolved organic carbon (DOC) and particulate matter in the water, because of increased competition for negative binding sites with hydrogen ions. This makes more mercury available for methylation. Thus, lower pH should lead to greater MeHg concentrations in the water, and following this, burdens of total mercury in fish should increase.

Method

Data compilation

To investigate whether there is an association between mercury concentration in herring and abiotic water parameters, data from the area around Harufjärden in the northern Bothnian Bay are used in this study. Monitoring data showing total mercury concentration over time in 3-4 years old herring, caught at Harufjärden (65° 35'N, 22° 53'E within a radius of 3', see figure 1), were obtained from the Swedish Museum of Natural History. Data were in the form of

geometric means, and the mercury concentration is measured in herring muscle tissue expressed on a wet weight basis. The number of individuals on which the mean values are estimated varies from 12 to 20. The time series from Harufjärden starts in 1980 and ends in 2010, and no measurements were made in 1989 (table 1). Sampling has consistently taken place in week 38-42, i.e. second half of September to first half of October (Bignert et al. 2011).

Figure 1. The green marker in the map shows the location at Harufjärden where the herring analysed for mercury are caught. The blue marker denotes SMHI's sampling station for oceanographic parameters, F3A5. The distance between the two locations is 49 km. The red rectangle in the small map (upper right corner) shows where the area depicted in the large scale map is situated in the northernmost Bothnian Bay, between Sweden and Finland. Maps from Google Maps (large scale map) and www.ne.se (small scale map).

Monitoring data for chemical and physical parameters in sea water (table 1) were obtained from SMHI's database SHARK (Svenskt HavsARKiv) (SMHI 2013). The data have been generated within the Swedish coordinated environmental monitoring programme by the Swedish Agency for Marine and Water Management. Several sampling stations for the monitoring of environmental parameters were available in the area around Harufjärden. However, the station with the most complete record was the one called F3A5 (65° 10'N, 23° 14'E, see figure 1), and thus the environmental parameters data were taken from that station. The distance between the sampling site for herring at Harufjärden and station F3A5 is 49 km.

Even at station F3A5, measurements were quite unevenly sampled within and between years. In order to be comparable between years, it was desirable that the time series for different parameters would consist of measurements from the same season throughout the investigated time period. Time series that were long enough were put together from measurements made in spring (May 4-June 30) and autumn (November 4-December 9), respectively. It was supposed that by using measurements from two seasons of each abiotic parameter, the likelihood of detecting any relationships could be increased.

Table 1. A record of the parameters included in the analysis of associations between mercury concentration in herring (dependent variable) and different abiotic water parameters (explanatory variables). Mercury concentration is measured in early autumn (week 38-42), whereas the abiotic water parameters are measured in late autumn (Nov 4-Dec 9) and (except for pH) in spring (May 4- June 30), respectively. In the "Years with measurements" column, details about which years sampling has been made are given for each parameter. The "n" column denotes the total number of years with measurements for each parameter, spring and autumn.

Parameter (unit)	Season	Years with measurements	n
Mercury, geom. mean conc. (ng/g wet weight)	-	1980-88, 90-2010	30
Temperature (°C)	Spring	1980-81, 83, 86-87, 89-96, 98-2010	26
	Autumn	1980-83, 85-86, 88-2010	29
Salinity (psu)	Spring	1980-81, 83, 86-87, 89-96, 98-2010	26
	Autumn	1980-83, 85-86, 88-2010	29
Dissolved oxygen (ml/l)	Spring	1980-81, 83, 86-87, 89-96, 98-99, 2001-10	25
	Autumn	1980-83, 85-86, 88-95, 97-2010	28
Phosphate (µmol/l)	Spring	1980-81, 83, 86-87, 89-90, 92-96, 98-99	14
	Autumn	1980-83, 85-86, 88-2003, 06-10	27
Total phosphorus (µmol/l)	Spring	1991, 93-96, 98-2010	18
	Autumn	1991, 93-2010	19
Total nitrogen (µmol/l)	Spring	1991, 93-96, 98-2010	18
	Autumn	1991, 93-2010	19
pH	*Autumn only*	1994-2001, 03, 06-10	14

Measurements of all parameters except for dissolved oxygen and pH were available from the surface down to approximately 90 m depth, with fixed intervals. Because herring resides primarily at greater depths (Vilt- och fiskeriforskningsinstitutet 2007), and also to avoid short-time fluctuations under the influence of atmospheric conditions, a mean between measurements from 40, 60 and 80 m was calculated for those parameters for every year. In 2001, measurements were sampled at slightly different depths, differing 0.2-2.3 m from the above-mentioned 40, 60 and 80 m depths. Measurements of dissolved oxygen were available from 80 m depth only, and pH was only measured near the surface. Therefore, dissolved oxygen values are based on measurements from the 80 m depth, and measurements of pH are from a depth of 4-5 m (mean depth 4.58 m). In a number of years, more than one measurement were sampled during the spring and/or the autumn period. In such cases, a mean value for each parameter was calculated. Hence, there is only one value representing a parameter a given season each year. The data set in its entirety is given in appendix A.

Statistical treatment

All statistical computations were made using the statistical package R, version 2.15.2 (R Core Team 2013). To find any relationships between mercury concentration in herring and the various parameters measured in water, multiple linear regressions were made. The limitations caused by the incomplete data set and the relatively small number of observations made it impossible to include all explanatory (abiotic) variables in the same multiple regression. Instead, several different multiple regressions were made, starting with different combinations of explanatory variables. Spring and autumn measurements of the different variables were consistently held apart in separate regressions. Using the autumn measurements, it was possible to include all explanatory variables in the same model, whereas the spring measurements did not allow for that, and hence were separated in three multiple regressions with different combinations of variables (table 2). Except for these regressions, the variables with the most complete records (i.e. temperature, salinity, oxygen and in autumn also phosphate) were analysed separately as well, in both spring and autumn (table 2). Against the background of results from previous studies, indicating a clear relationship between mercury bioaccumulation or bioavailability and pH and/or temperature, yet another multiple regression was made with these two abiotic parameters – measured in autumn – included (table 2).

Table 2. A record of the multiple linear regressions that were made to examine relationships between abiotic parameters and mercury concentration in herring. Regression models with explanatory variables measured in spring are listed in the left column, and the right column shows regression models with explanatory variables measured in autumn. **Hg** = geometric mean concentration of total mercury in herring, **Temp** = water temperature, **Sal** = salinity, **Oxy** = dissolved oxygen, **PO4** = phosphate, **TotP** = total phosphorus, **TotN** = total nitrogen, **pH** = water pH.

Spring measurements	Autumn measurements
Hg ~ Temp+Sal+Oxy+PO4	Hg ~ Temp+Sal+Oxy+PO4+TotP+TotN+pH
Hg ~ Temp+Sal+Oxy+TotP+TotN	Hg ~ Temp+Sal+Oxy+PO4
Hg ~ PO4+TotP+TotN	Hg ~ Temp+pH
Hg ~ Temp+Sal+Oxy	

Starting from these initial combinations of explanatory variables, the multiple regressions were simplified using manual backwards elimination. With this approach, significant relationships between one or several of the parameters measured at F3A5 and mercury concentration in herring were searched for. The principle of backwards elimination is to start with a linear model including several explanatory variables. By removing the most insignificant explanatory variables one by one, one searches for the model which in the simplest way explains as much of the variation in the response variable as possible (Crawley 2005). Due to the limited number of observations, only main effects were included in the analyses, i.e. no interactions between two or more explanatory variables were assessed.

Results

Generally, for those parameters where both spring and autumn records were available, the mean values are quite similar between seasons, except for the somewhat higher mean temperature in autumn compared to spring (table 3). Some parameters, e.g. pH, only exhibit small fluctuations from the mean as shown by the standard deviation, whereas others, e.g. mercury concentration and phosphate are quite variable (table 3). As already shown by Bignert et al. (2011), there is no significant linear time trend for mercury concentration in herring caught at Harufjärden. Simple linear regressions with year as the explanatory variable were made to get a picture of the time trends for each abiotic parameter. Significantly negative time trends existed for salinity and phosphate load, in both seasons. For salinity, the time trend is quite

strong, as indicated by the high R^2 values (table 3). The other environmental parameters did not exhibit any significant time trends.

Table 3. Mean values ± standard deviation for all parameters included in the analysis. S denotes the spring measurement of the respective parameter, and A denotes the autumn measurement. Significant negative time trends exist for salinity and phosphate, and significance level and coefficient of determination (R^2) are shown for these parameters.

Parameter (unit)	Season	Mean ± SD	Time trend (if significant)
Hg, geom. mean conc. (ng/g w.w.)	-	39.639 ± 10.451	
Temperature (°C)	S	1.25 ± 0.64	
	A	3.92 ± 0.99	
Salinity (psu)	S	3.470 ± 0.255	Negative ($p<0.001$, $R^2=0.8443$)
	A	3.426 ± 0.262	Negative ($p<0.001$, $R^2=0.7162$)
Dissolved oxygen (ml/l)	S	8.46 ± 0.63	
	A	8.17 ± 0.40	
Phosphate (µmol/l)	S	0.07 ± 0.02	Negative ($p<0.05$, $R^2=0.3441$)*
	A	0.06 ± 0.03	Negative ($p<0.01$, $R^2=0.3332$)
Total phosphorus (µmol/l)	S	0.14 ± 0.03	
	A	0.17 ± 0.03	
Total nitrogen (µmol/l)	S	19.96 ± 0.74	
	A	19.90 ± 1.01	
pH	A	7.76 ± 0.13	

* Phosphate values in spring transformed using $\log_{10}$.

The use of multiple linear regression and backwards elimination to assess any relationships between mercury concentration in herring and physical and chemical water parameters only rendered one significant relationship with one explanatory variable (i.e., the multiple linear regression was simplified into a simple ditto). The variable in question was total phosphorus in autumn, and the relationship was weakly positive ($p<0.05$, $R^2=0.2309$, see figure 2). However, there is no indication of a significant relationship between mercury concentration in herring and water total phosphorus content during spring.

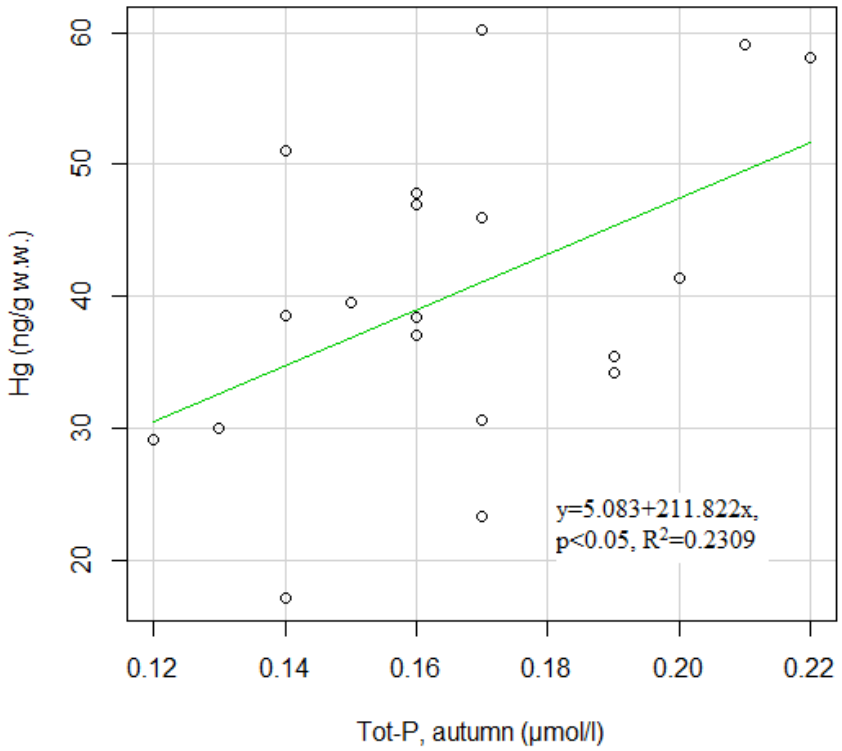

Figure 2. The relationship between water total phosphorus (Tot-P) content in autumn and geometric mean concentration of mercury (Hg) in herring. The regression equation as well as the R^2 is given in the figure.

Indeed, the multiple regression with temperature and pH resulted in what first appeared to be yet another significant relationship. It is a significant negative relationship between water temperature in autumn and mercury concentration, when pH is still included in the general linear model (p-value for temperature<0.05, p-value for pH>0.05, adjusted R^2=0.3506, df=11). There is no relationship between mercury and temperature in autumn alone, so one could have concluded that it is the inclusion of pH in the model that renders the temperature-mercury relationship significant. However, when more closely examined, it turns out that the subset of temperature values for the years when pH is measured, i.e. from 1994 and onwards, are significantly negatively related to mercury concentration even without the inclusion of pH in the model. This seems to be mainly because of two influential data points with high temperature values and low values for mercury concentration. These observations do not influence the slope of the regression line as much when the whole range of temperature values, from 1980 and onwards, are examined.

Therefore, in conclusion, no relationship between temperature and mercury can be proved to exist, neither under the influence of pH, nor when included in any other multiple regression in combination with other explanatory variables. As already stated, the same is true for all other environmental parameters except for total phosphorus in autumn.

Discussion

This study has shown that very few relationships between the studied environmental parameters and mercury load in herring at Harufjärden can be established, using existing long-term temporal data sets from Swedish monitoring programmes. The only exception is the weak positive relationship between mercury load in herring and water total phosphorus in autumn. At least two circumstances make it hard to believe that this relationship indicates a direct causal effect of total phosphorus content in water on the concentration of mercury in herring. Firstly, if the relationship really was causal, it could be reasoned that there should have been at least an indication of a similar relationship using the spring phosphorus values, which there isn't. Certainly, on the other hand, the outcome of the regression analyses greatly depends on measurements sampled at one single or a few occasions in spring and autumn, respectively. Thus, a similar relationship between mercury concentration and total phosphorus during both seasons cannot be taken for granted, because single measurements can reflect short-time fluctuations rather than long-term time trends. A yearly mean between the spring and autumn measurements for the environmental parameters may have been preferential and more logical to use, but that on the other hand, would have meant a substantial loss of observations ("n's") for some parameters.

A second reason to believe that the mercury-phosphorus relationship is not causal is the results from theory and earlier studies, which indicate that nutrients should have an inverse effect on mercury load in fish (Chen & Folt 2005; Sonesten 2003; Driscoll et al. 2012). The total-phosphorus value is the sum of both phosphate, which is a nutrient easily available for primary producers, as well as phosphorus bound to organic material in the water, and hence not directly accessible to phytoplankton (Swedish Agency for Marine and Water Management 2013). Therefore, total phosphorus cannot be seen as a direct representation of nutrient availability in the water. Nevertheless, because a higher total phosphorus value means a higher organic content in the water *per se,* the conceptual model by Driscoll et al. (2012), which hypothesises an increased removal of bioavailable mercury with more organic content in the water column, may still be valid.

Organic matter in the water is on the other hand a factor known to affect methylation, although it is contentious whether it leads to increased or decreased methylation rates (Ullrich et al. 2001). Therefore, it could be hypothesised that the significant mercury-phosphorus relationship found in this study is caused not by the direct effect of phosphorus, but rather by a positive effect caused by organic matter in the water, which is something that the measured

total phosphorus values might reflect. Mechanisms causing a positive effect of organic matter on methylation include increased activity of methylating bacteria, as well as increased abiotic methylation mediated by organic substances. However, other studies have shown an inhibiting effect, e.g. due to increased complexation between mercury and organic matter, leading to decreased bioavailability for methylation (Ullrich et al. 2001). Therefore, this reasoning should be viewed as a mere hypothesis.

Why then, could so few relationships be found between bioaccumulated mercury in herring and the different abiotic parameters, despite the more or less well-established effects that have been observed in other studies? One reason is probably the generally small variation between years within each abiotic parameter. Studies evaluating the effects of e.g. pH and salinity have generally used a wider range under which mercury accumulation has been assessed. So, for example, Miskimmin et al. (1992) studied the effect of changing pH when pH ranged from 5 to 7 as compared to the stable pH values in this investigation. Dutton & Fisher (2011) used salinities ranging from 0 to 25 psu, and in the mentioned studies on aquatic invertebrates, the examined salinities were closer to full-strength sea water (Laporte et al. 1997; Pan & Wang 2004). When it comes to temperature, on the other hand, Dijkstra et al. (2013) and Harris & Bodaly (1998) coped with ranges in temperature more similar to at least the range seen in autumn in this investigation (1.84-6.18 °C). However, temperatures were considerably higher in these cases. Generally, and maybe particularly in field studies where it is hard to control for confounding factors, a greater variation in the investigated parameters likely increases the probability to detect relationships. Confounding factors in this case could be e.g. changing influx of mercury over time to the area through atmospheric deposition and runoff, as well as measurements of water organic content, which, as mentioned above, is known to affect methylation.

The incomplete monitoring data from SMHI is probably another aspect that has made it hard to detect any relationships between environmental parameters and herring mercury load. Parameter measurements of pH and the different nutrients were available from a limited number of years, in the case of pH and phosphate in spring just 14, making a detection of significant relationships less likely in these cases. In the case of pH, this parameter was measured at the surface, which is of less relevance compared to if it had been measured closer to the bottom, given the reported predominance of methylation at the sediment-water interface (UNEP 2013).

Generally, more measurements throughout the year for all parameters would of course have been favourable to determine a value for each parameter with greater confidence. As it is

now, the parameter values are, in many years, based on one single measurement. This should mean a decreased probability that such values really are good representations of the abiotic conditions that the herring population in the area face. However, the number of measurements at station F3A5 has increased in frequency from approximately two times a year in the 1980s, to ten times a year after 2000 (SMHI 2013). This could increase the applicability of the monitoring data in any future studies similar to this, given that the increased monitoring efforts are sustained.

One could also question the approach in this study to only compare mercury concentrations in herring caught a given year with abiotic parameters measured in the same year. Mercury is highly accumulated in fish and only very slowly eliminated (Doetzel 2007). Therefore, it may have been more logical to calculate a mean value of each environmental parameter three or four years back in time from every respective year when mercury was analysed in herring. In this way, the given values would better represent the abiotic factors that the herring face throughout their whole lifetimes. However, with such an approach the variability in the data set would have decreased even more, and it would also have resulted in more years without measurements.

Does this discussion imply that monitoring data should not be used in this kind of investigation at all, then? Not necessarily! As mentioned above, the increased monitoring efforts by SMHI in the recent past may make the collected data more useful in the future. In general, it seems to be hard to detect more than occasional significant variables using field collected data, as in this study. So, for example, Doetzel (2007) only found one out of nine physical and chemical water parameters (total mercury in water) to be significantly correlated with mercury concentration in lake trout, *Salvelinus namaycush,* from Canadian lakes (although she found quite a few significant correlations with different biological and geographical variables). Dijkstra et al. (2013) could only establish a significant relationship with temperature, whereas the four other investigated parameters didn't relate to mercury load in killifish. Still, if the monitoring data are of good quality, it could be money/time-saving to use already existing data to investigate research questions like the one in this study.

Herring might not be the best species to use if conducting an investigation using monitoring data, though. This is due to the fact that herring undertake migrations within the Baltic Sea between their spawning periods (Vilt- och fiskeriforskningsinstitutet 2007), and thus the abiotic conditions closest to where they are caught might be an inadequate estimate of the conditions facing the herring. Therefore, organisms that are sessile as adults, such as the blue mussel, *Mytilus edulis*, might be more appropriate to use in monitoring data studies. In such

case, one knows that the matrices have lived at the same place throughout the adult stage of their lifecycle, and also from which depth they are sampled. If sampled close to one of SMHI's oceanographic stations, it would therefore be more logical to couple blue mussel concentrations of mercury or other contaminants with the monitored abiotic conditions at the location in question.

Indeed, blue mussels are not spread as far north as to the northern Bothnian Bay (Bernes 2005), and thus it would not have been an alternative matrix to use in this investigation. However, the species is sampled and analysed for e.g. mercury within the Swedish National Marine Monitoring Programme at three locations along the Swedish west coast, and at one location in the Baltic Proper (Bignert et al. 2011). Therefore, such a study could be a proposal as to how further advancement might be achieved in the understanding of how mercury concentrations in aquatic biota are coupled to abiotic environmental factors, using monitoring data from Swedish coastal waters.

Conclusion

In this study, a significant positive relationship over time was established between mercury load in Baltic herring and total phosphorus concentration measured in autumn in the area around Harufjärden, northern Bothnian Bay. However, no similar relationship was found with spring-measured total phosphorus values, and there seems to be limited support for such a relationship being causal. Therefore, the result should be interpreted cautiously, and cannot be extrapolated to circumstances beyond this particular investigation. For the other investigated abiotic environmental factors, no relationships with herring mercury concentration were found, despite the often noted effects of at least pH and temperature. Limitations in the monitoring data being used might have decreased the chances to detect any relationships. Furthermore, herring as a mobile species might not have been the best study matrix in a study using site-specific monitoring data.

Acknowledgements

Firstly, I would like to thank Anders Bignert at the Department of Environmental Research and Monitoring, Swedish Museum of Natural History, for giving me the opportunity to conduct this study with assistance from staff at the department. Thank you to Aroha Miller for coming up with the idea to this investigation, to Elisabeth Nyberg for making mercury data available to me, and to both of you for valuable guidance and comments. Lastly, I would like to thank my supervisor at Södertörn University, Patrik Dinnétz, for guidance on the statistics part of this investigation, and for comments on the text prior to the completion of the thesis.

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Appendix A

The data set used in the analyses is given below. An **S** in the end of a variable name = variable sampled in spring. An **A** in the end of a variable name = variable sampled in autumn. **Year** = sampling year, **Hg** = geometric mean concentration of total mercury in herring (ng/g wet weight), **Temp** = water temperature (°C), **Sal** = salinity (psu), **Oxy** = dissolved oxygen (ml/l), **PO4** = phosphate (µmol/l), **TotP** = total phosphorus (µmol/l), **TotN** = total nitrogen (µmol/l), **pH** = water pH.

Year	Hg	TempS	TempA	SalS	SalA	OxyS	OxyA	PO4S	PO4A	TotPS	TotPA	TotNS	TotNA	pHA
1980	40.517	1.51	3.51	3.939	3.953	7.69	7.51	0.07	0.09					
1981	31.267	0.41	3.11	3.922	3.949	8.31	8.00	0.08	0.07					
1982	55.913		5.13		3.791		8.45		0.06					
1983	32.761	1.76	5.63	3.800	3.668	8.21	8.10	0.08	0.07					
1984	37.386													
1985	32.253		4.26		3.762		7.99		0.13					
1986	34.991	1.60	5.31	3.928	3.670	7.35	8.25	0.08	0.11					
1987	45.087	1.37		3.822		7.55		0.13						
1988	31.588		4.06		3.563		8.75		0.11					
1989		-0.09	2.43	3.711	3.654	9.22	8.49	0.07	0.07					
1990	36.556	0.36	4.03	3.464	3.551	8.55	7.80	0.08	0.14					
1991	46.002	1.81	3.86	3.620	3.547	8.24	7.95		0.05	0.19	0.17	19.20	21.07	
1992	47.055	0.74	3.61	3.465	3.463	9.67	8.23	0.05	0.03					
1993	35.448	0.35	1.84	3.648	3.177	9.57	9.69	0.06	0.07	0.14	0.19	20.10	18.60	
1994	58.138	1.68	3.67	3.498	3.359	8.40	8.49	0.04	0.10	0.14	0.22	19.69	21.34	8.03
1995	60.177	0.49	3.47	3.303	3.412	9.77	8.34	0.04	0.05	0.09	0.17	19.34	18.46	7.59
1996	41.328	2.09	3.83	3.394	3.338	8.41		0.07	0.04	0.18	0.20	20.06	19.98	7.79
1997	30.609		3.02		3.474		8.04		0.03		0.17		19.75	7.62
1998	38.374	1.98	3.42	3.521	3.479	7.95	7.95	0.06	0.07	0.18	0.16	19.34	20.68	7.64
1999	46.927	1.17	3.37	3.445	3.405	8.16	8.27	0.05	0.04	0.17	0.16	19.87	20.46	7.77
2000	17.158	0.70	5.45	3.272	3.045		7.69		0.05	0.12	0.14	19.79	18.87	7.71
2001	47.772	2.31	4.18	3.342	3.128	7.93	8.21		0.03	0.17	0.16	21.12	20.29	7.76
2002	30.046	0.90	3.11	3.182	3.195	8.76	8.31		0.03	0.15	0.13	21.07	19.93	
2003	38.530	1.12	3.26	3.220	3.395	8.29	7.89		0.04	0.13	0.14	20.21	18.39	7.53
2004	29.165	1.02	3.01	3.303	3.398	8.30	8.19			0.11	0.12	18.94	19.18	
2005	50.984	0.94	6.18	3.139	2.925	9.01	7.89			0.12	0.14	18.94	18.49	
2006	39.524	1.65	4.16	3.246	3.368	8.31	7.89		0.04	0.10	0.15	19.00	20.41	7.87
2007	23.296	1.82	5.34	3.232	3.033	8.27	8.08		0.04	0.14	0.17	20.72	20.02	7.81
2008	37.025	2.07	3.98	3.248	3.191	8.80	8.29		0.06	0.15	0.16	20.77	19.96	7.78
2009	59.047	1.31	3.68	3.207	3.240	8.88	7.90		0.05	0.16	0.21	20.45	21.85	7.83
2010	34.258	1.49	3.66	3.270	3.233	7.98	8.02		0.04	0.14	0.19	20.77	20.42	7.85