Temporal, seasonal and spatial variation in dioxins and dioxin-like PCBs from Baltic herring (*Clupea harengus*) in the Baltic Sea.

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Summary

TEMPORAL

Four sites were investigated for changes in dioxins (PCDD/Fs) and dioxin-like PCBs (DLPCB) over time since the start of measurements, until 2009 - Harufjärden in the Bothnian Bay since 1990, Ångskärsklubb in the southern Bothnian Sea since 1979, Utlängan in the southern Baltic Proper since 1988, and Fladen in the Kattegatt since 1990.

PCDDs showed the greatest number of significant decreases over time, while PCDFs and DLPCBs showed fewer significant decreases. A slowing in decreases is apparent. Decreases varied depending on whether results were presented on a lipid or wet weight basis; therefore, decreases are inconsistent across sites.

Biological factors appeared to influence dioxin concentrations. Herring from Ångskärsklubb (mean 5.0±0.9 years old) were older than at the other three sites and generally had higher concentrations, although these have decreased to be closer to concentrations observed at Harufjärden and Utlängan. Lipid content at Harufjärden and Utlängan significantly decreased over time, and at Harufjärden, fish age increased although size (length/weight) did not change over time, indicating slower growth rates of herring at this site. By contrast, lipid content significantly increased over time at Fladen, age decreased but body size did not change, indicating growth dilution. While decreases were seen from herring at Fladen on a lipid weight basis, no significant decreases were seen on a wet weight basis.

Diet is another factor that may have influenced the observed dioxin concentrations. Stable isotope analysis indicated there may have been an upward shift and/or an extra level added e.g., through the introduction of a species, in trophic level in autumn-caught herring diet at Ångskärsklubb, which would result in greater bioaccumulation even though dioxin emissions are decreasing, and may explain the slowing in dioxin concentration reductions observed in herring from this site. Various ecological theories exist to explain why such a shift may have occurred. However, a lack of baseline data for the SIA means these results are indicative only.

A number of chemical, biological and environmental factors are at play; however, the contribution of each factor was not quantified here. It is apparent that herring biology and Baltic Sea ecological dynamics can and do play a part in observed temporal trends in dioxin concentrations in Baltic herring.

SEASONAL

Seasonal fluctuations in dioxin concentrations in herring from the Bothnian Sea were observed on a lipid weight basis, but on a wet weight basis were not so apparent. There are a number of biological, chemical and ecological factors that could contribute to seasonal variation, but as lipid content was the biological parameter most strongly associated with dioxin concentration, it seems likely that factors affecting lipid content are the drivers of observed seasonal changes in dioxin concentrations. Thus, seasonal dioxin changes are most likely due to the re-distribution of dioxins to
other tissues when lipid content decreases, and on a lipid weight basis, lipid normalisation of concentrations, rather than an actual loss of dioxins from the fish.

**SPATIAL**

Here, spatial differences between coastal sites sampled within the Bothnian Sea were seen, but only on a wet weight basis. A single difference was seen between coastal and offshore sites, also on a wet weight basis using age-adjusted data (TEQ$_{PCDD/F}$), but no differences were seen on a lipid weight basis. Differences in herring diet may offer some explanation for the differences seen, as indicated by SIA results. A link could not be established here between sediment and herring dioxin concentrations. The overall lack of differences seen between coastal and offshore herring can probably be attributed to the migratory nature of herring populations within the Bothnian Sea.
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Background

Dioxins refer to polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofuran (PCDF) compounds. Seventeen (10 furans, 7 dioxins) of the 210 possible congeners, substituted in the positions 2,3,7,8, are considered to be of toxicological importance. Twelve polychlorinated biphenyls (PCBs) are called dioxin-like PCBs (DL-PCBs) because they have a structure similar to that of dioxins and have dioxin-like effects. PCDD/Fs are characterised by low water solubility and low vapour pressure. In the environment, they can undergo photolysis; however, they are generally very resistant to chemical and biological degradation. Due to their persistent and hydrophobic properties, dioxins and DL-PCBs accumulate in sediments and organisms in the aquatic environment.

PCDD/Fs are not produced intentionally. They are formed as by-products in several industrial processes and from most combustion processes, such as municipal waste incineration and small-scale burning under poorly controlled conditions. They can also be produced from natural processes, such as volcanoes and forest fires (Baars et al. 2004). They are minor impurities in several chlorinated chemical products (e.g., PCBs, chlorophenols, hexachlorophene etc.). Formerly, pulp bleaching using chlorine gas was an important source of PCDD/Fs (Bignert et al. 2012).

By contrast, PCBs have been produced commercially since the 1920s by direct chlorination of biphenyls. PCBs were used for a range of applications e.g., inks, flame retardants and paints, but their primary use was in electronic appliances, heat transfer systems and hydraulic fluids (Baars et al. 2004). The use of PCBs in open systems was banned in many countries in the 1970s; however, they may still be in use in closed systems (Baars et al. 2004). Household and industrial waste disposal is considered to be the major source of PCBs, and hence DL-PCBs, to the environment (ATSDR 2000, in Baars et al. 2004). Therefore, PCDD/F and DL-PCB sources differ considerably.

High dioxin and DL-PCB levels within the Baltic Sea have caused concern for many years due to their impact on the environment and human health (HELCOM 2004). One major external source of dioxins to the Baltic Sea environment is from atmospheric emissions (Armitage et al. 2009; Wiberg et al. 2009), with combustion e.g., backyard burning, fossil fuel burning, and bio-fuel incineration, contributing to air emissions (Wiberg et al. 2009). Industrial emissions from e.g., the chemical and pulp/paper industry, have been major dioxin sources over the last decades (Wiberg et al. 2009). Long-range dispersal of dioxins is also a well-known transport mechanism (Tysklind et al. 1993; Kjeller et al. 1996; Lohmann & Jones 1998). As residence time of Baltic Sea water ranges from 25 – 35 years (Witt 2002), dioxin contaminants only move very slowly between Baltic basins (Armitage et al. 2009), and can therefore be retained for a long time within the Baltic Sea region, prolonging exposure time and bioaccumulation risk.

Environmental monitoring of different biota has been conducted by a number of EU countries, including Sweden, to follow temporal changes in dioxins (OSPAR 2007). Dioxin levels in Baltic herring, Clupea harengus, have been monitored at a number of sites within Sweden, and significant decreases have been observed at some sites (Bignert et al. 2011). However, despite continual decreases in dioxin air emissions (Quaß et al. 2004) brought about by numerous regulations and legislation, dioxin and DL-PCB concentrations in Baltic herring have been relatively stable since the mid to late 1990s, although significant decreases have also been observed at some sites (Bignert et al. 2012). It is unclear why dioxin and DL-PCB concentrations in Baltic herring are not following the observed decreases in air emissions.
Of further concern is that concentrations in Baltic herring occasionally exceed the limit set by the European Commission for human food, and feed for domestic animals used as human food (Wiberg et al. 2009) of 3.5 pg WHO\textsubscript{05}-TEQ/g w.w. (\(\sum\)PCDDs+PCDFs) or 6.5 pg WHO\textsubscript{05}-TEQ g/w.w. (\(\sum\)PCDDs+PCDFs+dl-PCBs; EC Regulation 1881/2006). Fish are one of the main sources of dioxins and DL-PCBs in humans. Elevated concentrations therefore constitute not only an environmental threat, but also a threat to the Baltic herring fishing industry, and potentially to human health. Furthermore, these high concentrations mean that the sale of herring from many Baltic Sea regions is restricted to domestic markets for both Sweden and Finland (Wiberg et al. 2009, EU Regulation 1259/2011). These restrictions on herring sales only apply to certain regions because distinct spatial variations in dioxin concentrations in Baltic herring have been observed (Karl & Ruoff 2007, Bignert et al. 2007, 2011). Concentrations from herring in the Bothnian Sea and Bothnian Bay are often elevated (Isosaari et al. 2006, Bignert et al. 2011). However, reasons for spatial differences in dioxin concentrations are not clear.

Baltic herring, the most commonly used indicator species for monitoring contaminants in biota within the Baltic Monitoring Programme (BMP) in the HELCOM convention area, is sampled by Finland, Estonia, Poland and Sweden (HELCOM 2004). Within the Swedish National Marine Monitoring Programme, herring have been sampled for more than 20 years at a number of sites. Baltic herring are a pelagic species belonging to the family Clupeidae, and are a sub-species of the larger Atlantic herring (\textit{C. harengus}). Young herring feed mainly on zooplankton, with the proportion of nektobenthos e.g., mysids, and fish in the diet increasing as herring size increases (Popiel 1951, in Parmanne et al. 2006; Casini et al. 2004). Diet can also vary depending on location, for example, herring from the Baltic Sea Proper feed mostly on zooplankton (Arrhenius & Hansson 1993), while zooplankton and mysids dominate herring diet from the Bothnian Sea and Bothnian Bay (Strandberg et al. 1998). Seasonal changes in diet have also been observed (Flinkman et al. 1992, Parmanne et al. 2006). Diet can be an important factor in dioxin and DL-PCB concentrations, due to the bioaccumulation and biomagnification of these chemicals through trophic levels, and because they do not rapidly degrade (HELCOM 2004, OPSAR 2007).

Sexual maturity of Baltic herring generally occurs between 2 – 4 years of age (Swedish Board of Fisheries 2010). In the Baltic Sea region, sexual maturity occurs at about 2 – 3 years of age, and on the Swedish west coast at 3 – 4 years of age (Parmanne 1999). Herring are the most dominant commercial fish species in the Baltic, and are important not only for human consumption but also as prey for several marine species. Predators include the Baltic grey seal (\textit{Halichoerus grypus}), cormorant (\textit{Phalacrocorax carbo sinensis}), cod (\textit{Gadus morhua}), salmon (\textit{Salmo salar}), trout (\textit{Salmo trutta}), pike (\textit{Esox lucius}), perch (\textit{Perca fluviatilis}) (Lundin 2011), the ringed seal (\textit{Phoca hispida}), and a number of other piscivores. Thus, bioaccumulation and biomagnification of dioxins and DL-PCBs is an ecotoxicological threat. Assessing temporal variation of dioxin and DL-PCB concentrations in Baltic herring is therefore essential because of their economic importance, their use for human consumption and their role as a keystone species in the Baltic ecosystem (Möllman et al. 2004).
Chapter 1. Long-term temporal trends in dioxins and dioxin-like PCBs in Baltic herring (Clupea harengus) within the Baltic Sea.
1.1 Introduction

High dioxins levels within the Baltic Sea have caused concern for many years due to their impact on the environment and human health (HELCOM 2004). Environmental monitoring of different biota has been conducted by a number of EU countries, including Sweden, to follow temporal changes in dioxins (OSPAR 2007). Dioxin levels in Baltic herring, *Clupea harengus*, have been monitored at a number of sites within Sweden, and significant decreases have been observed at some sites (Bignert et al. 2011). However, despite continual decreases in dioxin air emissions (Quaß et al. 2004) brought about by numerous regulations and legislation, a similar corresponding decrease in dioxin levels in herring has not been observed over the last 20 years (Bignert et al. 2011). As such, temporal trends of dioxins and DL-PCBs in Baltic herring were examined alongside a number of biological variables and stable isotope data, to see if any of these factors may explain why dioxin concentrations in herring have not been showing significant decreases in the last two decades.

Dioxin (PCDD/Fs) and dioxin-like PCBs (DL-PCB) levels in Baltic herring (*Clupea harengus*) have been relatively stable since the 1990s (Bignert et al. 2011), and in some areas, occasionally exceed the limit set by the EU for food and feed (Wiberg et al. 2009) of 4 pg WHO05-TEQ/g w.w. (∑PCDDs+PCDFs) or 8 pg WHO05-TEQ g/w.w. (∑PCDDs+PCDFs+DL-PCBs; EC Regulation 1881/2006). These high concentrations mean that the sale of herring is restricted to within domestic markets for both Sweden and Finland (Wiberg et al. 2009, EU Regulation 1259/2011). Assessing temporal variation of dioxin and DL-PCB concentrations in Baltic herring is therefore essential because of their economic importance, their use for human consumption, and their role as a keystone species in the Baltic ecosystem (Möllman et al. 2004).

Here, we examine long-term trends in dioxins and DL-PCBs in Baltic herring, and investigate whether the stability of dioxins observed in Baltic herring in the last 20 years can be attributed to a) fish bioenergetics e.g., growth (length, weight, age), lipid content etc., and/or b) shifts in herring diet.

1.2 Methods

1.2.1 Sampling Matrix and Sites

Baltic herring have been collected for more than 20 years at four sites along the Swedish coast – Harufjärden in the Bothnian Bay, Ängskärsklubb in the Bothnian Sea, Utlänge in the southern Baltic Proper, and Fladen in the Kattegatt, to within a circumference of 3 nautical miles around the central coordinates (Table 1.1, Figure 1.1) – for a long-term national temporal trend monitoring programme, financed by the Swedish Environmental Protection Agency. All sites used are reference sites, with no known local source points; however, several important paper/pulp mills are located along the coast outside Gävle, near to Ängskärsklubb. Near Harufjärden, there is considerable fresh water run-off from streams and rivers. All data presented here originate from samples collected in autumn (September – December). The number of herring sampled has varied over the years. In some years, 7-15 individuals were pooled at each site, while in other years 8-10 individual fish were analysed from each site. Geometric means have been calculated to give a single concentration for each congener in each year for each site.

Biological measurements were taken from all fish used for analysis – age (determined via scale reading), weight, total fish length, and reproductive phase. Fishing date was recorded each year,
and during dioxin analysis, lipid content was measured. To avoid between-year variance in dioxin concentrations due to gender and age, and as sexual maturation occurs anywhere between 2-4 years of age depending on site, female herring of 2-5 years old were selected for analysis as often as possible. At Ångskärsklubb, herring were sampled within this age range in only 12 of the 26 years sampled; mean herring age exceeded the 5 years in the other years. Poor age determination of herring from this site, in particular in earlier years of sampling, may be partly responsible for the overall higher herring age at this site. At both Harufjärden and Fladen, herring age was always within the 2-5 year range, while at Utlångan, herring age was within the 2-5 year range in 18 of 20 years sampled.

To minimize the between-year and spatial variation in concentrations of lipid soluble contaminants due to differences in the amount of subcutaneous fat, pure muscle tissue without subcutaneous fat was analysed. Dorso-lateral herring muscle tissue of approximately 10 g per specimen/pool was removed under strict laboratory protocols, and sent for analysis. Human consumers eat herring with the skin included, and may therefore be interested in the ratio of fat in the muscle to fat in the fillet (muscle and skin). Previous research has examined this issue, and more fat is contained in the fillet. A conversion factor of 1.64 was calculated (Bignert et al. 2005). Within the current research, temporal changes in this fat ratio have not been analysed.

<table>
<thead>
<tr>
<th>Site, Location</th>
<th>Season</th>
<th>Years sampled (Missing years)</th>
<th>Surface Salinity</th>
<th>Average Air Temperature</th>
</tr>
</thead>
</table>

### 1.2.2 Dioxin and DL-PCB Analytical Methods

The analyses of dioxins and DL-PCBs were carried out at the Department of Chemistry, Umeå University. The extraction method is described by Wiberg et al. (1998), the clean-up method by Danielsson et al. (2005), and the instrumental analysis (GC-HRMS) by Liljelind et al. (2003). The laboratory is accredited for dioxin analyses and participates in the annual FOOD intercalibration rounds, including laboratory reference material (salmon tissue) with each set of samples. Two dioxin analysis methods were used, one traditional for congener-specific determinations and one simplified for reduced costs and estimation of dioxin toxic equivalencies (TEQs). Both have been previously described (Haglund et al. 2007).

In the simplified method, only four marker congeners were analysed - 2,3,4,7,8-PeCDF and CBs 77, 126, and 157. The remaining sixteen PCDD/Fs, seven mono-ortho PCBs, and CB 169 were
estimated using their ratios to 2,3,4,7,8-PeCDF, CB157, and CB126, respectively, which was calculated using samples from 1995 only. The simplified method was used for herring samples from Harufjärden, Utlängan and Fladen, but not Ängskärsklubb, during 1996 - 2000. All other herring samples from these locations were analysed according to traditional analytical procedure. As 2,3,4,7,8-PeCDF was analysed using both methods, this congener was plotted and examined for the entire time series at these three sites. As no difference in this congener concentration or trend was seen whether these 5 years were included or not, and as the traditional and simplified methods differ somewhat, it was decided to only present results the traditional analysis.

1.2.3 Calculation of TEQs
Toxic equivalents, or TEQs, were calculated using the individual congener concentrations and the 2005 toxic equivalency factors, TEFs (WHO-TEFs) published by the World Health Organisation (Van den Berg et al. 2006). Unless otherwise stated, TEQ values referred to are the sum of the TEQ values for each year i.e., the TEQ values for each individual congener summed.

1.2.4 Stable Isotope Analysis (SIA), Ängskärsklubb
Muscle samples i.e., no skin or subcutaneous fat included, were taken from the same individuals/pooled sample herring examined each year for dioxins. Samples were analysed at the University of Jyväskylä, Finland, using a Carlo Erba Flash EA1112 elemental analyser connected to a mass spectrometer (CF-IRMS), via methods outlined in Kiljunen et al. (2006). All samples were freeze-dried to a constant weight and ground to fine powder before analysis. The international standards of Vienna Pee Dee belemnite (for carbon) and atmospheric N2 (for nitrogen) were used as reference materials, and dried pike muscle as an internal working standard. Results are expressed using the standard δ notation as parts per thousand (‰) difference from the international standards. Lipid normalisation was carried out for the δ\(^{13}\)C values using calculations presented in Kiljunen et al. (2006), as lipids are known to be \(^{13}\)C depleted relative to other major tissues (Bodin et al. 2007, Ehrich et al. 2010) i.e., fatty tissues can have lower δ\(^{13}\)C values than lean tissues (Enrich et al. 2010). No baseline data were available for comparison. Baseline data refers to stable isotope ratios for the basal resources within a food web e.g., planktonic or benthic primary consumers, which can vary over time (Solomon et al. 2008) and thus affect the stable isotope ratios of organisms feeding at higher trophic levels within the same food web.

1.2.5 Statistical treatment of the data
Data quality control was conducted for all sites. Any values below limit of quantification (LOQ) were replaced with LOQ divided by the square root of two. As herring from Ängskärsklubb were generally older than at the other three sites, data were age-adjusted to achieve comparable congener concentrations between sites and remove age as a confounding factor. Linear regression was carried out between the geometric mean congener concentration in each year and the median age of herring for each year to give the β value for adjusting data. The adjusted congener concentrations were calculated as follows:

\[
\text{Congener}_{\text{adjusted}} = \text{Congener}_{\text{observed}} + \beta * (\text{Age}_{\text{median}} - \text{Age}_{\text{observed}})
\]

where β is the beta value from the regression equation, and age\(_{\text{median}}\) was 5, based on median herring age from the other three sites. This calculation was also carried out using the arithmetic mean age of 3.3. However, age-adjusted log linear regression values decreased over time and showed a poorer relationship (age-adjusted log linear regression values decreased) compared to unadjusted log linear regression values for any of the examined data, and therefore was not used in the following analyses.
Congener patterns for each site are shown using stacked bar graphs for PCDDs, PCDFs, and DL-PCBs, as well as the TEQ values for each group. The relationship between biological variables and the summed TEQ values (l.w.) are presented. Log linear regression lines, equations, and \( r^2 \) values were added to scatterplots of each biological variable and \( \text{TEQ}_{\text{PCDD/F+DL-PCB}} \) values. Log linear regression was chosen rather than simple linear regression, as it assumes that a linear relationship exists between the independent variable and the logarithm of the dependent variable.

Correlation coefficients between \( \text{TEQ}_{\text{PCDD/F+DL-PCB}} \) values and biological variables were calculated and p values reported if <0.05. The range, arithmetic mean and standard deviation for each biological variable at each site are presented.

Trends in dominant congeners and TEQ values over the whole time period for each site are shown using scatterplots, with log linear regression indicating relationships between congener concentration and time. To assess whether these trends were statistically significant at \( p < 0.05 \), Mann Kendall trend tests were used (Statistica v10). This is the non-parametric alternative of the Pearson’s correlation coefficient. It is robust against outliers and does not rely on assumptions of the distributions of \( x \) and \( y \). Mann Kendall trend tests were conducted for dominant congeners and TEQ values at each site for the entire time series. The most recent 10 years of data were not examined separately for trends, because this would cause a decrease in statistical power, reducing reliability of results.

Stable isotope data from Ångskärsklubb spring- and autumn-caught herring are presented as scatterplots displaying \( \delta^{13}C \) and \( \delta^{15}N \) over time. Arithmetic mean ± standard deviation is presented for \( \delta^{13}C \) and \( \delta^{15}N \) for both spring- and autumn-caught herring.
Figure 1.1. Map of Scandinavia showing the Baltic Sea and surrounding countries. Red dots indicate the location of the four sites where sampling has occurred. From top of map – 1. Harufjärden (Bothnian Bay), 2. Ångskärsklubb (Bothnian Sea), 3. Utlångan (southern Baltic Proper), and 4. Fladen (Kattegatt).
1.3 Results

The temporal data series begin in different years for the different sites. At Ängskärsklubb, data collection began in 1979; at Fladen and Harufjärden, in 1990; and at Utlängan, data collection began in 1988 (Table 1.1). All temporal series for all sites are presented until 2009. Gaps are present between 1996 and 2000 for Fladen, Harufjärden and Utlängan because of the simplified methods used for dioxin analysis in these years (see 1.2.2). All results are presented on a lipid weight (l.w.) basis, unless otherwise stated.

1.3.1 Biological variables

Arithmetic mean age, weight, total length and lipid content (fat %) for each year were graphed with the summed TEQ\textsubscript{PCDD/F + DL-PCB} values (l.w.) over time for all sites (Figures 1.2-1.5, a – d). Herring age ranged from 2 - 3 years at Fladen, 3 - 4 years at Harufjärden, 3 - 7 years at Utlängan, and 3 – 9 years at Ängskärsklubb. Herring age showed a significant increase over time at Harufjärden (n=20, df=18, p<0.05) and significantly decreased over time at Fladen (n=20, df=18, p<0.05). Fish length across all four sites ranged from 14.2 – 22.9 cm and fish weight across all four sites ranged from 20.8 – 91.9 g (Table 1.2). Herring from Fladen were the largest, and herring from Harufjärden the smallest. Lipid content significantly decreased over time at both Harufjärden and Utlängan (n=20, df=18, p<0.05; n=21, df=19, p<0.05 respectively), whereas at Fladen a significant increase was seen over time (n=20, df=18, p<0.05). No other temporal trends were seen in the other biological parameters.

Table 1.2. Range, and arithmetic mean ± standard deviation of biological variables for herring sampled at each site (1 d.p.).

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Weight (g)</th>
<th>Total Length (cm)</th>
<th>Lipid content (fat %)</th>
<th>Reproductive Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harufjärden</td>
<td>3 – 4</td>
<td>21.6 – 32.0</td>
<td>14.2 – 16.9</td>
<td>1.9 – 3.9</td>
<td>1 – 4</td>
</tr>
<tr>
<td></td>
<td>3.6±0.5</td>
<td>25.2±2.4</td>
<td>16.0±0.6</td>
<td>2.6±0.5</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td>Ängskärsklubb</td>
<td>3 – 9</td>
<td>20.8 – 59.2</td>
<td>15.0 – 19.6</td>
<td>1.7 – 5.3</td>
<td>2 – 5</td>
</tr>
<tr>
<td></td>
<td>5.0±0.9</td>
<td>39.1±8.6</td>
<td>17.8±1.1</td>
<td>3.4±0.8</td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>Utlängan</td>
<td>3 – 7</td>
<td>30.2 – 67.1</td>
<td>17.1 – 21.8</td>
<td>1.6 – 9.3</td>
<td>2 – 5</td>
</tr>
<tr>
<td></td>
<td>4.1±0.6</td>
<td>37.8±7.7</td>
<td>18.2±1.0</td>
<td>2.9±1.6</td>
<td>3.3±0.4</td>
</tr>
<tr>
<td>Fladen</td>
<td>2 – 3</td>
<td>44.3 – 91.9</td>
<td>18.8 – 22.9</td>
<td>2.2 – 8.4</td>
<td>1 – 3</td>
</tr>
<tr>
<td></td>
<td>2.3±0.5</td>
<td>55.9±10.4</td>
<td>19.9±0.9</td>
<td>4.9±1.6</td>
<td>2.1±0.2</td>
</tr>
</tbody>
</table>

To explore the relationship between each biological variable and the TEQ\textsubscript{PCDD/F + DL-PCB} values at each site, log linear regression analyses and correlation coefficients were run (Table 1.3). For the whole time series, the strongest overall relationship was seen between lipid content and TEQ\textsubscript{PCDD/F + DL-PCB} value at Fladen. This was a significant negative relationship. At the other three sites, age showed the strongest relationship (all positive, although only significant at Harufjärden and Ängksarsklubb). Overall however, most of the relationships between any one biological variable and TEQ\textsubscript{PCDD/F + DL-PCB} value were non-significant.
Table 1.3. Regression (log linear) value ($r^2$, 2 decimal places) for each biological variable with the summed TEQ$_{PCDD/F+DL-PCB}$ concentration for each site. Significant correlations are indicated by a * (p<0.05).

<table>
<thead>
<tr>
<th>Site</th>
<th>n (years)</th>
<th>Fat %</th>
<th>Weight</th>
<th>Length</th>
<th>Fishing Date</th>
<th>Reproductive Phase$^1$</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harufjärden</td>
<td>18</td>
<td>0.05</td>
<td>0.09</td>
<td>0.36*</td>
<td>0.09</td>
<td>0.02</td>
<td>0.55*</td>
</tr>
<tr>
<td>Ångskärsklubb</td>
<td>26</td>
<td>0.01</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>0.10</td>
<td>0.20*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.53*2</td>
</tr>
<tr>
<td>Utlängan</td>
<td>18</td>
<td>0.25*</td>
<td>0.17*</td>
<td>0.27*</td>
<td>0.03</td>
<td>0.04</td>
<td>0.29</td>
</tr>
<tr>
<td>Fladen</td>
<td>18</td>
<td>0.47*</td>
<td>0.12</td>
<td>0.10</td>
<td>0.05</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$^1$Herring reproductive phase is equivalent to no detectable development (1), developing reproductive follicle (2), mature reproductive follicle (3), post-spawning reproductive follicle (4), and resorbing reproductive follicle (5) (Elston et al. 1997). Herring reproductive phase varied between sites.

$^2$Log linear regression for the first 10 years (1979 – 1988) of age data at Ängskärsklubb.

Figure 1.2. TEQ$_{PCDD/F+DL-PCB}$ (l.w.) and average herring age for each year for a) Harufjärden, b) Ångskärsklubb, c) Utlängan and d) Fladen.
Figure 1.3. TEQ$_{PCDD/F + DL-PCB}$ (l.w.) and average herring weight (g) for each year for a) Harufjärden, b) Ängskärsklubb, c) Utlängan and d) Fladen.

Herring weight (g) (Figure 1.3a-d) and length (cm) (Figure 1.4a-d) show little or no association with TEQ values over time at any of the sampled sites. However, correlation coefficient between TEQ$_{PCDD/F + DL-PCB}$ and biological variables at Harufjärden showed TEQ$_{PCDD/F + DL-PCB}$ significantly increased with increasing length ($n=18$, $df=16$, $p<0.05$). At Utlängan, TEQ$_{PCDD/F + DL-PCB}$ significantly increased as both weight and length increased ($n=18$, $df=16$, $p<0.05$).

Figure 1.4. TEQ$_{PCDD/F + DL-PCB}$ (l.w.) and average herring total length (cm) for each year for a) Harufjärden, b) Ängskärsklubb, c) Utlängan and d) Fladen.
At Fladen, lipid content shows some association with TEQ values in the last 6 – 7 years, with TEQ values decreasing as lipid content increases (Figure 1.5d). Correlation coefficient between TEQ$_{PCDD/F + DL-PCB}$ and biological variables at Fladen showed significantly decreased with increasing lipid content (n=19, df=17, p<0.05). Although no pattern is observed between TEQ values and lipid content at the other three sites (Figure 1.5a-c), correlation coefficient at Utlängan showed TEQ$_{PCDD/F + DL-PCB}$ significantly increased as lipid content increased (n=20, df=18; p<0.05).

Herring reproductive phase is equivalent to: 1 = no detectable development; 2 = developing reproductive follicle; 3 = mature reproductive follicle; 4 = post-spawning reproductive follicle; 5 = resorbing reproductive follicle (Elston et al. 1997). Average herring reproductive phase varied between sites over time. The lowest reproductive phase seen was 2, at Fladen, which corresponds to the young age of herring at this site. Reproductive phase 5 was only seen once, in 2003 at Ångskärsklubb. Herring sampled at both Harufjärden and Utlängan were generally between reproductive phase 2 and 3. At Fladen and Utlängan, reproductive phase was only measured since 2001 and 2002, respectively.

Fishing date has been relatively constant at all four sites. At Ångskärsklubb and Harufjärden, sampling has occurred in autumn. At Fladen and Utlängan, fishing also occurred in autumn with the exception of 2000 (Fladen), and 1999 and 2007 (Utlängan). However, both 1999 and 2000 fishing dates occurred during the use of simplified analysis methods.
1.3.2 Congener Patterns

Congener patterns were graphed for each site for PCDDs (Figure 1.6a – d), PCDFs (Figure 1.7a – d) and DL-PCBs (Figure 1.8a – d) on a lipid weight basis. The patterns look similar at each site. However, at Ångskärsklubb, the concentrations are higher compared to the other three sites.

The congeners 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD are the two dominant PCDD congeners for the majority of the time series, both on a TEQ and an absolute concentration basis (l.w.). At Fladen, OCDD is dominant early in the time series on an absolute concentration basis but not a TEQ basis. For both Fladen and Utlångan, various dioxin congeners were below LOQ in some years (Fladen: OCDD 1992, 1,2,3,4,6,7,8-HpCDD 2001, 1,2,3,4,7,8-HxCDD 2001; Utlångan: 1,2,3,7,8-PeCDD 1990, 1,2,3,4,7,8-HxCDD and 1,2,3,7,8,9-HxCDD 1992, 1,2,3,4,6,7,8-HpCDD 1992, 1995, 2001, OCDD 2001).

For the furans, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF are the dominant congeners for all sites on an absolute concentration (l.w.) basis, whereas on a TEQ basis, 2,3,4,7,8-PeCDF is dominant. Concentrations at Ångskärsklubb are again higher than those seen at the other three sites, possibly because of this site’s location near paper/pulp industrial sites near Gävle, and herring age. For both Fladen and Utlångan, various furan congeners were below LOQ in some years (Fladen: 1,2,3,7,8,9-HxCDF and 2,3,4,6,7,8-HcCDF 1992, 1,2,3,4,7,8-HxCDF 1994, OCDF 1995, 1,2,3,4,7,8,9-HpCDF 2002; Utlångan: 1,2,3,4,6,7,8-HpCDF 1992, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF 1992, 1,2,3,4,7,8,9-HpCDF 2002, 2003, 2005).

![Temporal congener patterns (absolute concentration l.w. basis) of polychlorinated dibenzo-p-dioxin (PCDD) for a) Harufjärden, b) Ångskärsklubb, c) Utlångan and d) Fladen. Some congeners at Fladen and Utlångan were below LOQ in some years.](image)
Figure 1.7. Temporal congener patterns (absolute concentration l.w. basis) of polychlorinated dibenzofurans (PCDFs) for a) Harufjärden, b) Ängskärsklubb, c) Utlängan and d) Fladen. Some congeners at Fladen and Utlängan were below LOQ in some years.

For the DL-PCBs, CB105 and CB118 are the dominant congeners at all sites on an absolute concentration (l.w.) basis. However, CB126 is the dominant congener on a TEQ basis. Again, concentrations at Ängskärsklubb are considerably higher than those seen at the other three sites.
Over time, a general decrease is seen for the TEQ2005 values for each of PCDD, PCDF and DL-PCBs (Figure 1.9a – d). This trend is seen most clearly at Ängskärsklubb (Figure 1.9b) most likely because this station has been monitored over a longer period (since the late 1970s).

In almost every year at every site, TEQPCDD contributed the least to the relative contribution of dioxins, in some years and sites, contributing less than 20% of the total dioxin toxicity. TEQPCDF make up the greatest relative contribution of dioxins at Ängskärsklubb since 1996; pre-1996, TEQDL-PCB dominated. At Harufjärden, TEQPCDF and TEQDL-PCB alternate in dominating contribution from 2001 onwards. TEQDL-PCB contributed the most in every year at Utlängan and Fladen, often between 40 - 60% of total toxicity. In 1990, no DL-PCBs were examined at Fladen, Harufjärden and Utlängan. Overall, PCDDs have begun to contribute less towards total toxicity at all sites.
1.3.3 TEQ and dominant congener concentrations

Below, graphs are presented on a lipid weight basis. However, for comparison, graphs for TEQ\textsubscript{PCDD/F} values are presented on a wet weight basis for each site. Log linear regression equations and regression value (r\textsuperscript{2}) is shown for each congener/TEQ value that is significant (Mann Kendall trend test) at each site. Where no significant change over time is observed, no regression line, equation or value is shown.

At Harufjärden, only 2,3,7,8-TCDD and TEQ\textsubscript{PCDD} showed a significant decreasing trend over time (l.w. basis) (Figure 1.10a). The other dominant congeners (absolute concentration, l.w.) and TEQ concentrations (l.w.) did not show any significant trends (Figure 1.10b-e). On a wet weight basis, TEQ\textsubscript{PCDD} was also the only TEQ value to show any significant trend. TEQ\textsubscript{PCDD/F} did not show any significant trend (Figure 1.10f). As outlined in section 1.3.1, herring age significantly increased and lipid content significantly decreased at this site over time. These are two biological parameters that would have a strong influence on dioxin concentration in herring, and probably contribute to masking dioxin concentrations seen in the environment.
Figure 1.10. TEQ concentrations (l.w.) for (a) PCDD, (b) PCDF, (c) DL-PCB, (d) PCDD/F, (e) PCDD/F + DL-PCB and (f) PCDD/F (w.w.) for the whole time series at Harufjärden. Log linear regression equation, $r^2$ value and $p$ values are shown only where there is a significant change over time.
Table 1.4. Mann-Kendall trend test results for Harufjärden, 1990 - 2009. Significant at p<0.05 (2 d.p.).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Valid N</th>
<th>Kendall Tau</th>
<th>Z</th>
<th>p-value</th>
</tr>
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<td>Year, 2378-TD</td>
<td>13</td>
<td>-0.54</td>
<td>-2.56</td>
<td>&lt;0.02</td>
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<td>-0.61</td>
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</tr>
<tr>
<td>Year, CB118</td>
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<td>-0.98</td>
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<td>TEQ PCDF</td>
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<td>-0.60</td>
<td>0.55</td>
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<tr>
<td>TEQ PCDD/F+DL-PCB</td>
<td>14</td>
<td>0.08</td>
<td>0.38</td>
<td>0.70</td>
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</table>

At Ångskärsklubb, all six dominant congeners (2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, CB105, CB118, absolute concentration, l.w.) showed significant decreases when the whole time series was examined. A decreasing concentration over the whole time series was also observed for the TEQ values (Figure 1.11a-d). All TEQ values on a wet weight basis also showed significant decreases over time. Here, only TEQ_{PCDD/F} is presented (Figure 1.11f). None of the biological parameters measured from herring at this site showed any change over time. Therefore, the higher herring age observed here is probably influencing the slightly elevated dioxin concentrations from this site, among other factors.
Figure 1.11. TEQ concentrations (l.w.) for (a) PCDD, (b) PCDF, (c) DL-PCB, (d) PCDD/F, (e) PCDD/F + DL-PCB and (f) PCDD/F (w.w.) for the whole time series at Ångskärsklubb. Log linear regression equation, r² value and p values are shown.
Table 1.5. Mann-Kendall trend test results for Ångskärsklubb, 1979 - 2009. Significant at p<0.05 (2 d.p.).

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</tr>
<tr>
<td>TEQ PCDD/F</td>
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<td>-0.61</td>
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<td>&lt;0.01</td>
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<tr>
<td>TEQ PCDD/F+DL-PCB</td>
<td>26</td>
<td>-0.62</td>
<td>-4.47</td>
<td>&lt;0.01</td>
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</table>

At Utlängan, the two dominant PCDD congeners showed significant decreases over time - 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD (l.w.). TEQ_{PCDD}, TEQ_{PCB} and TEQ_{PCDD/F+DL-PCB} showed significant decreases over time (1.12a, c, e) (l.w.). The significant decrease seen for TEQ_{DL-PCB} was largely caused by significant decreases in the non-dominant congeners. On a wet weight basis, all TEQ values showed significant decreases over time. Here, only TEQ_{PCDD/F} is presented (Figure 1.12f). Lipid content in herring at this site significantly decreases over time, which would indicate that concentrations of dioxins would show even stronger decreases if lipid content were to increase here.
Figure 1.12. TEQ concentrations (l.w.) for (a) PCDD, (b) PCDF, (c) DL-PCB, (d) PCDD/F, (e) PCDD/F + DL-PCB and (f) PCDD/F (w.w.) for the whole time series at Utlängan. Log linear regression equation and $r^2$ value are shown only where there is a significant change over time.

Table 1.6. Mann-Kendall trend test results for Utlängan, 1988 - 2009. Significant at p<0.05 (2 d.p.).

<table>
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<tr>
<td>Year, 23478-PF</td>
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<td>&gt;0.05</td>
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<td>0.07</td>
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<tr>
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<td>&lt;0.05</td>
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<tr>
<td>TEQ PCDF</td>
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<td>-1.14</td>
<td>0.26</td>
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<tr>
<td>TEQ CB</td>
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<td>-0.58</td>
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<td>&lt;0.01</td>
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<tr>
<td>TEQ PCDD/F</td>
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</tr>
<tr>
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<td>15</td>
<td>-0.47</td>
<td>-2.42</td>
<td>&lt;0.02</td>
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</tbody>
</table>

At Fladen, as at Ängskärsklubb, all dominant congeners showed significant decreases in concentration over the whole time series (l.w.). This was also the case for TEQ_{PCDD}, TEQ_{PCDF}, TEQ_{PCDD/F} and TEQ_{DL-PCB} (Figure 1.13a-d), whereas TEQ_{PCDD/F+DL-PCB} (Figure 1.13e) did not show a significant decreasing trend. This lack of significant trend is most likely because DL-PCBs were not analysed in 1990 at this site, and therefore, the summed TEQ value for this year is low. By contrast, no TEQ values showed any significant changes over time on a wet weight basis (TEQ_{PCDD/F}, Figure 1.13f), which may be explained by the changing lipid content in herring over time increasing variation in dioxin concentrations on a wet weight basis.
Figure 1.13. TEQ concentrations (I.w.) for (a) PCDD, (b) PCDF, (c) DL-PCB, (d) PCDD/F, (e) PCDD/F + DL-PCB and (f)PCDD/F (w.w.) for the whole time series at Fladen. Log linear regression equation and $r^2$ value are shown only where there is a significant change over time.
Table 1.7. Mann-Kendall trend test results for Fladen, 1990 - 2009. Significant at p<0.05 (2 d.p.).

<table>
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<th>Kendall Tau</th>
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<td>Year, 12378-PD</td>
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<td>-2.03</td>
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</tr>
<tr>
<td>Year, 23478-PF</td>
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<td>-0.58</td>
<td>-2.90</td>
<td>&lt;0.05</td>
</tr>
<tr>
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<td>-0.69</td>
<td>-3.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Year, CB118</td>
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<td>-0.62</td>
<td>-2.93</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TEQ PCDD</td>
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<td>-0.49</td>
<td>-2.46</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>TEQ CB</td>
<td>13</td>
<td>-0.49</td>
<td>-2.32</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>TEQ PCDF/F</td>
<td>14</td>
<td>-0.56</td>
<td>-2.79</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TEQ PCDD/F+DL-PCB</td>
<td>14</td>
<td>-0.34</td>
<td>-1.70</td>
<td>0.09</td>
</tr>
</tbody>
</table>

TEQ HALF-LIVES

Half-lives for TEQPCDD, TEQPCDF, TEQPCDD/F+DL-PCB were determined at the four sites (Figure 1.14-1.16), as depicted in each graph by e.g., Harufjärden, STEQPCDD t(1/2) = 20 yrs. TEQPCDD shows the shortest half-lives for all sites. TEQPCDF and TEQPCDD/F+DL-PCB will take the longest to decrease at Harufjärden (half-lives of 78 and 70 years respectively). Ångskärsklubb has the shortest half-lives of all four sites. This will be influenced by the higher concentrations seen in earlier sampling years.

![Graphs of TEQ half-lives for different sites](image-url)

Figure 1.14. TEQPCDD (l.w.) half-life for Harufjärden (Bothnian Bay), Ängskärsklubb (s.Bothnian Sea), Utlängan (s.Baltic Proper) and Fladen (Kattegatt).
1.3.4 Stable Isotope Analysis (SIA), Ångskärsklubb

Stable isotope data for both spring- and autumn-caught herring from Ångskärsklubb were examined. Stable isotope data from spring-caught herring were available from 1991 – 2004, while data from autumn-caught herring were available from 1979 – 2007. All $\delta^{13}$C values were lipid normalized, as fatty tissues can have lower $\delta^{13}$C values than lean tissues (Enrich et al. 2010), thus affecting
measurements and interpretation of δ^{13}C values (Bodin et al. 2007, Ehrich et al. 2010). Ångskärsklubb temporal data analysed for dioxins and DL-PCBs presented above are from autumn-caught herring.

Figure 1.17. Ångskärsklubb spring-caught herring stable isotope data over time for a) δ^{15}N and b) δ^{13}C (lipid normalized).

Figure 1.18. Ångskärsklubb autumn-caught herring stable isotope data over time for a) δ^{15}N and b) δ^{13}C (lipid normalized).

Figure 1.19. Ångskärsklubb a) spring-caught herring and b) autumn-caught herring stable isotope data.

δ^{15}N from spring-caught herring varied from 9.69 to 10.58 (mean 9.99 ± 0.27); lipid normalized δ^{13}C varied from -18.00 to -20.29 (mean -19.99 ± 0.75) (Figure 1.17a, b). In autumn-caught herring, δ^{15}N varied from 8.36 to 11.03 (mean 9.64 ± 0.57); δ^{13}C varied from -18.73 to -20.98 (mean -19.94 ± 0.51) (Figure 1.18a, b). Simple linear regression shows no significant relationship between δ^{13}C and δ^{15}N for the spring or autumn-caught herring (Figure 1.19a, b).
The δ13C values for both spring and autumn data indicate that all energy sources are Baltic i.e., neither fully marine nor fresh water. δ15N for spring-caught herring increased by approximately 1.5‰ over the examined period, while an increase of almost 3‰ was observed for the autumn-caught herring. Fractionation of 3.2‰ is generally accepted as a change in trophic feeding level (Michener & Schell 1994), therefore, autumn-caught herring appear to have shifted upward by close to one trophic level over time. However, no baseline data are available for comparison with this stable isotope data. Baseline data refers to stable isotope ratios for the basal resources within a food web e.g., planktonic or benthic primary consumers, which can vary over time (Solomon et al. 2008) and thus affect the stable isotope ratios of organisms feeding at higher trophic levels within the same food web. Therefore, these results must be interpreted with caution, and cannot be considered definitive until baseline data on the isotopic composition of zooplankton over time are obtained from ITM, Stockholm University.

1.4 Discussion

1.4.1 Temporal changes in dioxins

For the whole time series, PCDDs generally showed significant decreases at all sites (both l.w. and w.w., except Fladen, l.w. only). PCDFs and DL-PCBs showed some significant decreases at Ångskärsklubb, Utängan and Fladen (l.w. only). Consistency in decreases was generally lacking, with TEQPCDD decreasing at all sites, while the other TEQ values decreased at only some sites. However, some of these decreases were only seen on a lipid weight or wet weight basis, not both. Dioxin concentrations are not showing such large decreases in recent years as they were at the beginning of the monitoring period. Changes in herring biological parameters e.g., age, lipid content and slower growth rate, and ecological changes e.g., changes in diet, appear to be playing a role in the lack of decreases and where decreases have slowed in recent years, and will be discussed further below.

TEQ values (l.w. and w.w.) and absolute concentrations (l.w.) of PCDD/Fs and DL-PCBs at Ångskärsklubb were considerably higher than at the other three sites, especially in earlier years of monitoring. In more recent years e.g., 2005 and later, dioxin concentrations at Ångskärsklubb have become more in line with concentrations observed in herring at Harufjärden and Utängan, although continue to be higher than concentrations observed in herring from Fladen. As Ångskärsklubb is the longest time series here (30 years) and has very high concentrations at the start of the time series, it is not surprising to see significant decreases when the whole time series is examined.

TEQPCDD has contributed less and less to total toxicity at all sites, and is the least dominant group. Meanwhile, although generally decreasing, TEQPCDF and TEQDL-PCB have become more dominant in their contribution to total toxicity over time. This is reflected by the shorter half-life of TEQPCDD at all sites compared to TEQPCDF and TEQPCDDF+DLPCB. Contribution of DL-PCBs to the WHOsum-TEQ has been observed to be equal to or even greater than the contribution of PCDD/Fs (Isosaari et al. 2006), while DL-PCBs made up half or more of the total dioxin values in many of the herring sampled by Karl and Ruoff (2007), similar to what is seen here. Source strength may be a key factor in explaining these trends. As mentioned in the background to this report, sources of PCBs differ considerably compared to dioxins, and this may well be the major reason for the differences seen between dioxins and DL-PCBs here. PCBs were produced commercially for a number of years before being banned by many countries (Baars et al. 2004); however, some closed systems containing
PCBs still exist, and household and industrial waste continue to contribute to environmental levels of PCBs, and hence DL-PCBs (Baars et al. 2004). By contrast, dioxins are produced unintentionally. The major external source of dioxins to the Baltic Sea environment has been shown to be from atmospheric emissions (Armitage et al. 2009; Wiberg et al. 2010), with combustion e.g., backyard burning, fossil fuel burning, and bio-fuel incineration, still thought to be the primary producer of air emissions (Wiberg et al. 2010). Emissions from industrial sources e.g., municipal waste incineration, have decreased considerably over time (Quaß et al. 2004; Wiberg et al. 2010), although non-industrial sources are reported as having hardly decreased (Quaß et al. 2004). The difference in emission reduction from industrial verse non-industrial sources may be one reason why we see lowering PCDD emissions compared to PCDFs and DL-PCBs.

Congener patterns in herring did not differ much between sites, indicating similar sources of exposure over time. The dominant congeners here on an absolute concentration (l.w.) basis were 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, and CB105 and CB118. Parmanne et al. (2006) found 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF were the two dominant PCDD/F congeners in herring from the Bothnian Sea, with 2,3,4,7,8-PeCDF being the most problematic and abundant congener in herring due to it being only very slowly metabolised. Karl and Ruoff (2007) also found 2,3,4,7,8-PeCDF to be the dominant congener of all PCDD/Fs in herring, contributing approximately 50% of total toxicity. TEQ_{DL-PCB} was dominated by CB126. Karl & Ruoff (2007) found this congener contributed more than 60% of the WHO-TEQ. Certain congeners dominating over others can be due to a number of reasons, and may include source strength, differences in mobility and elimination rates for different congeners (Åberg et al. 2010; Muir & Yarechewski 1988), distance from source points (Parmanne et al. 2006), differences in exposure and/or bioaccumulation of different congeners, related to differences in herring diet between areas (Kiviranta et al. 2003).

Results here may also be related to accumulation efficiencies of DL-PCBs compared to PCDD/Fs. Accumulation efficiencies of DL-PCBs are significantly higher compared to PCDD/Fs in farmed salmon (Salmo salar) fed a controlled diet (Berntssen et al. 2005), and as both salmon and herring are fatty fish, accumulation efficiency may explain the high relative proportion of DL-PCBs to total toxicity in herring examined here. Results may also be explained by differing bioavailability of congeners being released into the environment from sediment reservoirs and resuspension (HELCOM 2004, Armitage et al. 2009) due to sedimentation rates and/or bioturbation, or other factors not examined here.

Residence time of Baltic Sea water ranges from 25 – 35 years (Witt 2002). Reduced water exchange will thus have some impact on dioxin concentrations at the three east coast sites (Harufjärden, Ängskärsklubb, Utlängan). By contrast, it is not surprising to see lower dioxin concentrations at Fladen on the west coast due to the mixing of coastal water with open ocean water, because export of dioxins to the open ocean is a major net loss mechanism for dioxins in coastal environments (Armitage et al. 2009; Wiberg et al. 2010). Higher salinity i.e., closer to open ocean salinity, will promote growth of a different zooplankton composition compared to within the Baltic Sea, and thus influence herring diet, growth and dioxin concentration.

1.4.2 Herring biological factors influence dioxin concentrations

Dioxin levels are known to be related to age, with older fish having higher concentrations compared to younger fish (Pandelova et al. 2008, Karl & Ruoff 2007, Isosaari et al. 2006). Herring age at Ängskärsklubb was higher compared to the other sites. Over time, herring age significantly
increased at Harufjärden, significantly decreased at Fladen, but did not show any change at either Ängskärsklubb or Utlängan. The overall higher age of herring from Ängskärsklubb would contribute to the observed higher dioxin levels. By contrast, the lower, and significantly decreasing age of herring from Fladen are likely to have contributed to the lower dioxin concentrations observed here.

Besides age, herring size (length/weight) did not change over time at any site. At Harufjärden, a lack of change in herring size coupled with the observed increase in herring age over time indicates a decrease in growth rate, which would counteract decreases in dioxins in the environment. One hypothesis to explain this could be that climate and ocean changes from greenhouse gas emissions are affecting marine fish body size – as ocean temperature increases, oxygen levels decrease. Oxygen is a key ingredient for growth, so with decreasing oxygen levels, larger fish size cannot be supported (Cheung et al. 2012). This theory, if correct, would explain the increased age but unchanged body size at Harufjärden, and would directly impact dioxin concentrations. Furthermore, slower growth rates in northern Baltic herring have been reported (Vuorinen et al. 2004, in Isosaari et al. 2006). This phenomenon has been attributed to lower salinity due to greater fresh water input in this area affecting e.g., zooplankton composition and abundance (Flinkman et al. 1998), and thus herring diet. A slower growth rate in the most northern site is supported by results here.

Lipid content is known to influence dioxin concentrations (HELCOM 2004, Berntssen et al. 2005, Bignert et al. 2011), with herring from the Baltic Proper and the Gulf of Bothnia having less fat compared to herring from the west coast (Ask & Westerberg 2009, in Persson 2010). This corresponds to findings here, with Fladen having the highest average lipid content and Harufjärden the lowest (4.9±1.6 and 2.6±0.5 respectively). At both Harufjärden and Utlängan, lipid content significantly decreased over time, while at Fladen it significantly increased. Higher dioxin concentrations (l.w.) are often seen in leaner fish (Bignert et al. 2011). The low and significantly decreasing lipid content observed in herring from Harufjärden and Utlängan will have influenced dioxin concentrations at those sites, and further contributed to masking decreases in dioxin emissions.

Reasons for changes in lipid content are varied, with spawning and diet being the most influential drivers of lipid changes. Lipid content of female Pacific herring (Clupea harengus pallasi) has been shown to decrease during spawning, along with a corresponding decrease in dioxins (Huynh et al. 2007). The majority of herring sampled here were female, and reproductive phase was investigated, although this parameter did not vary much at any site. Herring diet from the Baltic Sea is usually dominated by zooplankton and mysids, however, different dominant food items have been identified from Baltic herring diet during different seasons (Popiel 1951, Parmanne et al. 2004, both in Parmanne et al. 2006; Arrhenius & Hansson 1993, Möllman et al. 2004), and can also vary spatially (Parmanne et al. 2006; Arrhenius & Hansson 1993). Different food items e.g., zooplankton compared to mysids, contain different lipid content, and this is reflected in herring lipid content.

### 1.4.3 Ecology plays a role

Stable isotope analyses (SIA) conducted within this project indicated that changes in diet over time may have contributed to the observed temporal dioxin concentrations by counteracting emission reductions. SIA is used to examine diet - where the energy is sourced from, in this context, marine, brackish, freshwater, or terrestrial - and at what level in the trophic web an organism is feeding at. Stable isotope data was collected for herring from Ängskärsklubb for both spring and autumn-caught herring. However, no baseline data was available for comparison, and therefore results and subsequent discussion involving herring dietary changes cannot be treated as definitive.
Herring were feeding on Baltic (brackish water) food sources. Fractionation in δ^{15}N of approximately 3‰ was observed for autumn-caught herring and an increase of 1.5‰ was seen for spring-caught herring. Therefore, autumn-caught herring from Ångskärsklubb appear to have shifted up by one trophic feeding level. This potential upward shift in trophic level occurred over a number of years, but the biggest change occurred from the mid-1980s to the late 1990s. This timing coincides with the crash in cod stocks in the Baltic Sea (Flinkman et al. 1998, Möllman et al. 2004), and subsequent decreases in herring weight-at-age (WAA) (Cardinale & Arrhenius 2000, Persson 2010), which can influence dioxin concentrations. Reasons for the WAA decrease include the crash in cod stocks causing a decrease in predation pressure on herring (Flinkman et al. 1998, Cardinale & Arrhenius 2000, Möllman et al. 2004, Peltonen et al. 2007, Casini et al. 2010), and a sprat population explosion, leading to increased intra and inter specific competition (Flinkman et al. 1998), resulting in a change in herring diet.

Also coinciding with this timing was the introduction of the cladoceran Cercopagic pengoi to the Baltic Sea (Gorokhova et al. 2000). C. pengoi appears to have become a significant component of the diet of adult herring. It is also most abundant in August – September (Gorokhova et al. 2004), which coincides with the increase in δ^{15}N seen in autumn-caught herring but not spring-caught herring. Colonisation of the Baltic Sea by C. pengoi has in some areas (northern Baltic Proper) led to a shift in the feeding ecology of herring (Gorokhova et al. 2004). Furthermore, SIA carried out on C. pengoi and herring found that herring trophic position shifted before and after the invasion of this species, with higher ¹⁵N/¹⁴N ratios in year of the young herring to C. pengoi suggesting a linkage between the two species (Gorokhova et al. 2005). Introduction of this cladoceran could therefore offer an alternative explanation for changes in herring SIA seen here, by adding another level to the trophic web. At higher levels in the trophic web, dioxin concentrations become higher because dioxins bioaccumulate and biomagnify, and do not rapidly degrade (HELCOM 2004, OPSAR 2007). Therefore, an upward shift in trophic feeding level would result in stabilising, or even increasing, dioxin concentrations being observed in herring. However, as already noted, baseline SIA data is missing.

1.4.4 Summary

Overall, PCDDs showed the greatest number of significant decreases over time, while PCDFs and DL-PCBs showed fewer significant decreases. Decreases varied depending on whether results were presented on lipid or wet weight. This inconsistency was most apparent at Fladen, where almost all TEQ values decreased on a lipid weight basis, yet none decreased over time on a wet weight basis. Elimination rates were not examined here, but as dioxins are very stable chemicals (OSPAR 2007) with slow rates of degradation (HELCOM 2004), this is unlikely to be driving the decreases that are seen. Dioxin concentrations are not showing such large decreases in recent years as they were at the beginning of the monitoring period. This trend appears to be strongly related to herring biology, in particular a slower growth rate in the northern site, as shown by increased herring age but unchanged size (length/weight) at Harufjärden, and decreases in lipid content at both Harufjärden and Utlängan, masking any effect decreases in dioxin emissions could potentially have on concentrations in herring.

Dioxin concentrations observed at Ångskärsklubb are now more in line with concentrations observed in herring from Harufjärden and Utlängan, although Fladen on the west coast continues to have lower concentrations. A number of reasons, in particular the higher average herring age at Ångskärsklubb, as well as industrial usage of coastal areas, probably contributed to the high dioxin concentrations seen here in the early years of monitoring, while emission reductions undoubtedly
contribute to the decreases. Coupled to this is the apparent increase/addition of a trophic feeding level in herring at this site, which, along with the aforementioned factors, could explain the slower reductions in dioxin concentrations in herring from this site over time. Although SIA was only analysed from herring at one site, the ecological interactions that possibly contributed to this change in diet are more wide-spread than being restricted to just one Baltic basin. Diet changes due to changes in Baltic Sea ecology, e.g., the cod stock crash, or introduced species, probably affected other basins too.

Increasing lipid content in herring in the last 6 – 7 years, and decreasing herring age over time at Fladen, as well as overall efforts to reduce dioxin emissions, seem to be the strongest explanations for the observed concentrations here. However, decreases were seen from this site on a lipid weight basis only. Lipid weight results best reflect the abiotic environment e.g., effects of emission reductions, whereas wet weight results are dependent on lipid content of the fish that can confound environmental impacts.

A number of interacting chemical, biological and environmental factors are at play; however the contribution of each factor was not quantified here. It is apparent that herring biology and Baltic Sea ecological dynamics can and do play a part in observed temporal trends in dioxin concentrations in Baltic herring.
Chapter 2. Seasonal variation in dioxins and dioxin-like PCBs in Baltic herring *(Clupea harengus)* from the Bothnian Sea
2.1 Introduction

Previous research has shown evidence for seasonal variation in dioxin concentrations in Baltic herring (Bignert et al. 2009a, b) as well as other persistent organic pollutants (POPs) in fish species at other locations (e.g., Larsson et al. 1993, Stapleton et al. 2002). Seasonal variation in dioxin concentrations could be important in regards to the timing of herring sampling for monitoring of dioxins used for setting environmental target levels and safe food consumption levels. A number of biological and ecological parameters can influence dioxin concentration in herring e.g., lipid content of sampled individuals, diet/trophic feeding level, spawning, fishing date, age and habitat (Larsson et al. 1993), and thus should be considered when investigating dioxin concentrations in herring.

Here, we investigate whether annual variations in dioxin concentrations in Baltic herring are 1) due to seasonal changes in dioxin concentrations, and 2) if so, if they are related to biological parameters.

2.2 Methods

Herring was caught in most months between November 2005 to October 2006 at two sites, one in the northern and one in the southern Bothnian Sea (11 months sampled in southern Bothnian Sea; 9 months sampled in the northern Bothnian Sea) (Bignert et al. 2009c) (Figure 2.1). Herring size was representative of that usually taken for human consumption – approximately 20 cm length, equating to an age of between 5 – 10 years. A total of 15 individual herring were collected at each site on each sampling occasion. Biological measurements were taken, but age and sex were not determined. Muscle tissue from each individual fish was sampled by removing skin and subcutaneous fat and sampling the dorso-lateral muscle. Muscle was then pooled for each group of 15. Pooled muscle samples were sent to Umeå University Chemistry Department for analysis of PCDD/Fs, using traditional analytical techniques (see section 1.2.1, Dioxin Analytical Methods, for further details).

Results for seasonal data are presented in pg/g l.w., and, where specified, w.w. basis also. Toxic equivalency (TEQ) values were calculated using TEF2005 values, and congeners were summed for each site and month within the PCDD and PCDF group to give total TEQPCDD and TEQPCDF, and TEQPCDD/F values. Simple regression analysis and correlation coefficients were run on lipid content and TEQPCDD/F. Principal component analyses (PCA) were run to investigate patterns in PCDDs, PCDFs and PCDD/Fs at each site over the sampled time period using PIA Plot and Image Analysis (Bignert 2007).
Figure 2.1. Map of Scandinavia showing the Baltic Sea and surrounding countries. Red dots indicate the general location of the two sites where sampling occurred.
2.3 Results

There was little variation in fish size at either site. Total fish length i.e., length including the tail, ranged from 19.1 – 20.7 cm at the southern Bothnian Sea site, and from 18.7 – 20.9 cm at the northern site. Weight ranged from 42.5 – 51.3 g (southern site) and 40.6 – 52.3 g (northern site) equating to an age of between 5 - 10 years. Weight, total and body length i.e., length not including the tail, did not show any obvious pattern with TEQ_{PCDD/F} concentrations on either a l.w. (Figure 2.2, 2.3) or w.w. basis (only l.w. presented below). Simple linear regression on weight and TEQ_{PCDD/F} (w.w.) showed a reasonably strong positive relationship and a significant correlation at the northern site ($r^2 = 0.50$, $n = 9$, $df = 7$, $p<0.05$) but not at the southern site ($r^2 = 0.21$, $n = 11$, $df = 9$, $p>0.05$). Although simple linear regression for total length and TEQ_{PCDD/F} (w.w.) showed positive relationships, no significant correlations were seen ($r^2 = 0.28$, $n=11$, $df = 9$, $p>0.05$ southern site, $r^2 = 0.38$, $n = 9$, $df = 7$, $p>0.05$ northern site).

The highest TEQ_{PCDD/F} concentrations (l.w. basis) were observed in January (southern Bothnian Sea) and May (northern Bothnian Sea), and on a w.w. basis, January for both sites. An increase or decrease in lipid corresponds to a decrease or increase in TEQ_{PCDD/F} (l.w.) concentration (Figure 2.4, top). This trend is also seen on a w.w. basis, although not as pronounced (Figure 2.4, bottom). Lipid content showed a significant negative relationship and correlation with total TEQ_{PCDD/F} concentrations for both sites on a lipid weight basis ($n = 11$, $df = 9$, $p<0.01$, $r^2 = 0.79$ (southern); $n = 9$, $p>0.05$ northern site).
df = 7, p<0.01, $r^2 = 0.90$ (northern). On a wet weight basis, only the southern site showed a significant negative correlation between lipid content and TEQ_{PCDD} on a w.w. basis ($n = 11$, df = 9, p<0.05; $r^2 = 0.39$). The northern site showed a non-significant negative relationship ($r^2 = 0.39$).

The PCA examining TEQ_{PCDD} congener concentrations shows samples from the southern Bothnian Sea site have formed two groups – June to September and November to April (Figure 2.5). However the June to September group is near the centre of the PCA and therefore no one congener is exerting a particularly strong influence. The group formed by the November to April samples (no samples were taken in December) form a group close to OCDD. This indicates a change in dominant congeners between summer and winter/spring. October (10) and May (5) do not fit to either group. A similar pattern can be seen for the northern Bothnian Sea site, although the groups are not as tightly formed as for the other site, and November (11) sits with July, August, September. Again, both October (10) and May (5) do not fit to either group.

When examining TEQ_{PCDF} congener concentrations (l.w.) over the sampled months, no seasonal pattern is apparent for either site (Figure 2.6). While in some cases, the sampled months from both sites occur close together (e.g., January, February, September), there is no obvious pattern to their distribution, with no seasonal groups forming.
Figure 2.5. Principal component analysis (PCA) showing TEQ_{PCDD} congener loadings (l.w.) over the sampled months for both sites. S = southern Bothnian Sea, N = northern Bothnian Sea. Numbers indicate months e.g., 1 = January. Congener names are abbreviated. TD = 2,3,7,8-TCDD, PD = 1,2,3,7,8-PeCDD, HxD1 = 1,2,3,4,7,8-HxCDD1, HxD2 = 1,2,3,6,7,8-HxCDD2, HxD3 = 1,2,3,7,8,9-HxCDD3, HpD = 1,2,3,4,6,7,8-HpCDD, OD = OCDD.

Figure 2.6. Principal component analysis (PCA) showing TEQ_{PCDF} congener loadings (l.w.) over the sampled months for both sites. G = southern Bothnian Sea, H = northern Bothnian Sea. Numbers indicate months e.g., 1 = January. Congener names are abbreviated. TF = 2,3,7,8-TCDF, PF1 = 1,2,3,7,8-PeCDF1, PF2 = 2,3,4,7,8-PeCDF2, HxF1 = 1,2,3,4,7,8-HxCDF1, HxF2 = 1,2,3,6,7,8-HxCDF2, HxF3 = 1,2,3,7,8,9-HxCDF3, HxF4 = 2,3,4,6,7,8-HxCDF4, HpF1 = 1,2,3,4,6,7,8-HpCDF1.
When TEQ$_{PCDD/F}$ is examined (l.w.), again, the southern site appears to break into two groups (Figure 2.7), as seen for TEQ$_{PCDD}$. Samples from the southern Bothnian Sea form two groups again - June to September and November to April, corresponding to summer/autumn and winter/spring. For the northern Bothnian Sea site, a group consisting of July, Aug, Sept and November forms; however no corresponding winter/spring group can be seen for samples from this site. This indicates that the PCDD congeners have a stronger influence compared to the PCDF congeners.

2.4 Discussion

Changes in dioxin concentrations in Baltic herring were observed on a lipid weight basis. On a wet weight basis, seasonal changes in dioxins were not as apparent. There are a number of chemical, biological and ecological factors that could contribute to seasonal variation, but as lipid content was the biological parameter most strongly aligned with dioxin concentrations on both a lipid and wet weight basis, it seems likely that factors affecting lipid content e.g., spawning and seasonal changes in diet, are the drivers of the observed dioxin changes.

Lipid content (fat %) showed the strongest relationship to total TEQ$_{PCDD/F}$ congener concentration, with an increase in lipid content associated with a decrease in dioxin concentration, and vice versa. A strong negative correlation exists between organochlorine concentration expressed on a l.w. basis, and fat content in spring-caught herring (Bignert et al. 1993). A low fat content due to e.g., scarcity of food, can cause elevated concentrations of organochlorines on a l.w. basis, and vice versa (Bignert et al. 2011). A decrease in lipid content would appear as in increase in dioxin concentrations and vice versa, as observed here. In fact, rather than losing dioxins from the body, the redistribution of dioxins to other tissues in the body is the most likely explanation, as well as changes in the lipid normalised concentrations (l.w.). Different fish tissues can have quite different concentrations of PCDD/Fs (Wu et al. 2000, 2001). De Laender et al. (2010) modelled seasonal fluctuations in PCB concentration in three fish species, including herring, and found that lipid dynamics were the primary
driving force behind seasonal changes. This distinct pattern of increasing lipid content with decreasing dioxin concentrations was seen here at both sites throughout the sampled time period, although greater fluctuation in dioxin concentration from the southern site was observed.

Lipid content is closely related to spawning. Significant depletion of lipids due to spawning condition have been observed in Pacific herring (Clupea harengus pallasi) (Huynh et al. 2007). Changes seen in dioxin concentration here are possibly influenced by spawning activity and related lipid depletion. High concentrations of dioxins and corresponding lower lipid content in the first few months of the year for both sites indicate the sampled herring could be spring spawners. Observed changes in dioxin concentration may therefore be due to decreased lipid content resulting from spawning activity. Egg production has been reported as acting as a vector of depuration in female fish (Larsson et al. 1993, Miller 1993, Stapleton et al. 2002), which could also explain some of the observed dioxin fluctuations, although sex was not determined for these fish.

It seems very likely that diet plays a large role in influencing seasonal lipid content and therefore dioxin concentration. Seasonal fluctuations in prey source availability, shifts in dominant food source, inter and intra specific competition, and/or environmental conditions can all influence dioxin concentrations. Different dominant food items have been identified from Baltic herring diet during different seasons (Popiel 1951, Parmanne et al. 2004, both in Parmanne et al. 2006; Arrhenius & Hansson 1993, Möllman et al. 2004), leading to changes in bioaccumulation rate depending upon trophic level, dioxin concentration, and lipid content in the dominant prey source at the time.

In marine systems, diet is the dominant pathway of dioxin bioaccumulation in copepods and fish (Zhang et al. 2011). Herring diet from the Baltic Sea is usually dominated by zooplankton and mysids. Consumption of zooplankton varies with season and peaks between July and October (Arrhenius & Hansson 1993). The highest feeding activity for herring has been reported as occurring in spring and summer, which is the main reproductive period for calanoid copepods, one of the dominant zooplankton sources for herring (Möllman et al. 2004). Thus, large amounts of food from a low trophic level would be available at this time. This relates well to results reported here, with lower dioxin concentration and higher lipid content seen in this same period.

In late autumn and winter, herring food consumption decreases and a shift in diet to prey with higher energy values e.g., mysids, occurs (Arrhenius & Hansson 1993). As mysids are higher trophic level organisms than zooplankton, they bioaccumulate more dioxins (Strandberg et al. 1998) leading to greater dioxin bioaccumulation in herring as well. OCDD has been shown to be a dominant congener in mysids and zooplankton sampled within the Bothnian Bay, and here, appeared to have a stronger influence on herring during the winter months. A change in diet, along with the potentially slower metabolism/excretion of heavier PCDD/F congeners during winter when fat content in herring was lower, is one explanation for the observed grouping of herring in the PCA.

Therefore, seasonal dioxin changes are most likely due to the re-distribution of dioxins to other tissues when lipid content decreases, and a diluting of dioxin concentrations when lipid content increases and, on a lipid weight basis, lipid normalization of concentrations, rather than an actual loss of dioxins from the fish. The best way to determine this would be to conduct dioxin analyses on different parts of the fish body over season, as well as whole fish body dioxin analysis, to determine if dioxin concentrations vary by season between tissues.
Chapter 3. Spatial variation in dioxins and dioxin-like PCBs in Baltic herring (*Clupea harengus*) from the Bothnian Sea
3.1 Introduction

Distinct spatial variations in dioxin concentrations in Baltic herring have been observed (Karl & Ruoff 2007, Bignert et al. 2007, 2011). Concentrations from herring in the Bothnian Sea and Bothnian Bay are often elevated (Isosaari et al. 2006, Bignert et al. 2011). However, reasons for spatial differences in dioxin concentrations are not clear. Various hypotheses exist, such as differences in exposure to local sources, contaminated sediments acting as secondary dioxin sources, ecological and physical factors, or fish growth rate, metabolism or prey availability (Arrhenius & Hansson 1993, Peltonen et al. 2004). As herring are a keystone species within the Baltic ecosystem, and are popular for human consumption within the Baltic countries, improving knowledge about causes of spatial differences in dioxins is important from both an ecological and a human health perspective.

Here, we examine spatial variation of dioxin concentrations in herring, mysids, zooplankton, sediment and water data, as well as stable isotope analysis from herring, mysids and zooplankton, from four sites within a single basin, the Bothnian Sea, to investigate whether herring diet, biological variables or sediment/water concentrations can explain the observed spatial variations in dioxin concentrations in herring.

3.2 Methods

3.2.1 Sampling Sites and Matrix

Five sites were sampled along the coast of the Bothnian Sea in late summer (September) 2009 – Köpmanholmen, Lörudden, Hornslandet, Norrsundet and Skutskär (Figure 3.1). Based on previous observations (Bignert et al., 2007), Hornslandet can be used as a reference area, and concentrations of dioxins and DL-PCBS in herring from this site were expected to be more similar to offshore caught herring, while the other coastal sites are considered contaminated areas. From each site, Baltic herring (Clupea harengus), mysids and zooplankton were sampled. Herring (Clupea harengus) were collected by trawling and kept frozen (-20 °C) until further analysis. For comparison, herring were also sampled from four offshore sites by the Swedish Board of Fisheries (Figure 3.1). It must be noted that two of these sites, Utsjö 3 and 4, are located within the Baltic Proper, and it is known that dioxin levels in herring from this region differ from herring in the Bothnian Sea region. Therefore herring from these two sites are not directly comparable.

From each coastal site, 36 individual herring were sampled, except at Hornslandet where only 18 individuals were sampled, and Köpmanholmen where no herring was collected. From each offshore site, 24 individual herring were sampled. These were divided into three even pools for sampling (n = 12x3 at Skutskär, Norrsundet and Lörudden, n = 6x3 for Hornslandet, n = 8x3 for all offshore sites). Herring biological measurements were taken for each individual (sex, age, weight, body length, total length, liver weight, gonad weight, stomach weight). Scales were used for age determination.

Coastal mysids were collected using a 90 µm plankton net at depths generally >40 m. Two species (Mysis relicta and M. mixta) were identified and frozen separately. Coastal zooplankton were sampled using a 90 µm plankton net dragged behind a boat at a depth of 1 – 5 m, concentrated, and stored frozen in glass jars. A sub-sample of zooplankton from each site was fixed in 95% ethanol to determine the relative taxonomic composition. Mysids and zooplankton from offshore areas in the Bothnian Sea and Baltic Proper (fig. 1) were collected by the Finnish marine research vessel “Aranda” during August 16-26, 2010 by dragging a plankton net (mesh size 200 mm) from the bottom to the
surface. These offshore sampled areas differed to the offshore sites as they were collected within another project, but were still within the Bothnian Sea.

For dioxin analysis, three pooled samples of 30 g w.w. herring fillet (muscle with subcutaneous fat and skin) were sampled for each site. Muscle only (skin and subcutaneous fat removed) were simultaneously sampled from the same individuals from two coastal (Lörudden, Skutskär) and two offshore (2, 4) sites. There was only enough biomass of *M. relicta* and *M. mixta* for dioxin analysis from Lörudden and Skutskär.

For stable isotope analysis, dorso-lateral herring muscle tissue of approximately 0.5 g w.w. from each individual was sampled following strict laboratory protocol. Five individual *M. relicta* and 3 *M. mixta* from each site were analysed for stable isotopes. Bulk zooplankton samples from all coastal (excluding Köpmanholmen for dioxin analysis) and offshore sites were analysed for dioxins and stable isotopes

3.2.2 Dioxin Analytical Methods

The analyses of dioxins and dioxin-like PCBs from herring fillet, muscle, mysids and zooplankton were carried out at Eurofins Ökometri laboratory in Bayreuth, using dioxin analysis standard method EPA 1613 B High Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS). Fat extraction was done via column extraction using hexane:dichloromethane 1:1 as per the German extraction method ASU L00.00-12; ASU L00.00-38.

3.2.3 Stomach Content Analysis

Herring stomachs from each coastal site were removed, and preserved in glass jars with 95% ethanol. Following laboratory protocols, each stomach was cut open, contents removed and examined under a binocular microscope. Contents were divided into groups e.g., zooplankton, mysids, fish, other, and the number in each group was recorded.

3.2.4 Stable Isotope Analysis (SIA), Ångskärsklubb

Muscle samples were analysed at the University of Jyväskylä, Finland, using a Carlo Erba Flash EA1112 elemental analyser connected to a mass spectrometer (CF-IRMS), via methods outlined in Kiljunen et al. (2006). All samples were freeze-dried to a constant weight and ground to fine powder before analysis. The international standards of Vienna Pee Dee belemnite (for carbon) and atmospheric N2 (for nitrogen) were used as reference materials, and dried pike muscle as an internal working standard. Results are expressed using the standard δ notation as parts per thousand (‰) difference from the international standards.

Lipid normalisation was carried out for the δ¹³C values for herring, mysids and zooplankton, using calculations presented in Kiljunen et al. (2006), as lipids are known to be ¹³C depleted relative to other major tissues (Bodin et al. 2007, Ehrich et al. 2010) i.e., fatty tissues can have lower δ¹³C values than lean tissues (Enrich et al. 2010).

3.2.5 Statistical treatment of the data

Data quality control was conducted for all sites. Any values below limit of quantification (LOQ) were replaced with the LOQ divided by the square root of two. Herring biological variables were compared between coastal and offshore sites using student’s t-tests (Excel 2010); differences between coastal sites, and between offshore sites, were examined using one-way analysis of variance (ANOVA) and Tukey’s post-hoc tests (Statistica v10). As there were significant differences (p<0.05) seen in herring biological variables between coastal and offshore sites, with coastal herring being
older, data were age adjusted. Age was chosen as both length and weight are closely related to age, and because dioxin levels are known to be related to age, with older fish have higher concentrations compared to younger fish (Pandelova et al. 2008, Karl & Ruoff 2007, Isosaari et al. 2006). As there was some spread in the age data e.g., a 10 year old herring from Skutskär, and 2 year old herring from Norrsundet and Hornslandet, the median age of the offshore fish was chosen as the age to correct coastal fish age to. Data from coastal-caught herring were age adjusted using the following equation:

\[
\text{Congener}_{\text{adjusted}} = \text{Congener}_{\text{observed}} + \beta \times (\text{Age}_{\text{median}} - \text{Age}_{\text{observed}})
\]

where \(\beta\) is the beta value from the regression equations (herring age vs. the absolute concentration (l.w.) for the dominant congeners for PCDDs, PCDFs and DL-PCBs), and \(\text{age}_{\text{median}}\) was 5, based on herring age from the offshore sites. At four of the eight sites, both fillet and muscle samples were taken, resulting in half as many muscle samples compared to fillet. This led to a very poor fit when age and TEQ values were regressed together, and therefore age adjustment of herring muscle data was poor. Data adjustment for herring fillet was better. As such, it is indicated throughout the results section where adjusted and/or unadjusted data have been used, as appropriate.

Student’s t-tests were used to examine differences in absolute concentration of the dominant congeners between coastal and offshore herring, and one-way ANOVAs with Tukey’s post hoc tests (Statistica v10) were used to examine differences in absolute concentration of the dominant congeners between coastal sites, and between offshore sites, using age adjusted fillet data. Pearson’s correlation coefficients were used to attempt to establish a link between dioxin concentrations in sediment and herring fillet. A conversion factor between congener concentration in herring fillet and muscle was calculated. Principal component analysis (PCA) with Hotellings tests were used to examine any patterns in congener groups between coastal and offshore herring, and between coastal sites, using normalised data (each congener divided by the sum of all congeners for each of PCD, PCDF and DL-PCBs). Any concentrations below LOQ were removed (PIA, Plot and Image Analysis, Bignert 2010). Graphs of zooplankton composition from each coastal site, and herring stomach content were compiled. Stable isotope data were used to determine dietary proportion of herring at the four coastal sites where herring was sampled. Bi-plots and correlation coefficients were calculated and graphed using SIAR (2011), an R statistical package developed specifically for stable isotope data analysis.
Figure 3.1. Map of Scandinavia showing the Baltic Sea and surrounding countries. Coastal sites within the Bothnian Sea are indicated by red dots with site name alongside. Offshore sites are indicated by green dots with site name alongside.
3.3 Results

As only zooplankton was caught at Köpmanholmen, this site was removed from all but the zooplankton graphs in the following analyses. Results are presented in pg/g l.w. unless otherwise specified. Skutskär, Lörudden, Utsjö 2 and Utsjö 4 had both fillet (muscle, skin and subcutaneous fat) and muscle (skin and subcutaneous fat removed) sampled from herring. At all other sites, only fillet samples were taken from herring. Mysids were only caught from Skutskär and Lörudden.

3.3.1 Biological variables of herring at coastal and offshore sites

Age, weight, total length, lipid content (fat %), as well as liver, gonad and stomach weight were measured in all herring sampled. Here we focus on age, weight, total length and lipid content (fillet only, as muscle samples were only taken at half of the sites). The oldest herring were caught from Skutskär, and the youngest from Utsjö 1. Largest herring (weight and length) were from Lörudden, and smallest herring were from Utsjö 1 (Table 3.1).

| Table 3.1. Range, and arithmetic mean ± standard deviation (1 decimal place) for herring biological variables from all sites, and total number of individuals from each site (n) |
|---|---|---|---|
| Age (years) | Weight (g) | Total Length (cm) | Lipid Content (fillet only) (fat % w.w.) |
| Skutskär | 6 – 10 | 39.1 – 53 | 17 – 20.9 | 3.3 – 4.0 |
| n=36 | 7.7±1.0 | 43.7±3.2 | 18.7±0.6 | 3.6±0.5 |
| Norrsundet | 2 – 9 | 23.1 – 38.1 | 15.5 – 17.7 | 8.5 – 10 |
| n=36 | 4.8±1.9 | 31.4±8.8 | 16.6±1.4 | 9.4±0.8 |
| Hornslandet | 2 – 8 | 22.2 – 28.7 | 14.6 – 16.6 | 3.8 – 5.1 |
| n=18 | 5±1.6 | 29.6±7.2 | 16.5±1.3 | 4.5±0.7 |
| Lörudden | 5 – 9 | 46.3 – 51.2 | 19.2 – 19.9 | 8.1 – 11.1 |
| n=36 | 6.9±1.0 | 45.5±4.5 | 19.3±0.6 | 9.3±1.6 |
| Utsjö 1 | 3 – 7 | 15.4 – 23.3 | 12.7 – 15.2 | 4.2 – 6.3 |
| n=24 | 4±1 | 16.3±1.5 | 13.5±0.6 | 5.2±1.1 |
| Utsjö 2 | 4 – 9 | 26.6 – 45.2 | 16.2 – 20.3 | 5.8 – 6.9 |
| n=25 | 6.4±1.5 | 33.0±5.3 | 17.7±1.0 | 6.2±0.6 |
| Utsjö 3 | 4 – 7 | 22.3 – 36.6 | 16.2 – 18.5 | 4.8 – 7.1 |
| n=24 | 5.6±1.0 | 28.3±3.1 | 17.2±0.6 | 5.7±1.2 |
| Utsjö 4 | 3 – 8 | 28.1 – 37.4 | 16.4 – 17.9 | 4.0 – 10.3 |
| n=24 | 5.1±1.1 | 31.2±2.8 | 16.9±0.4 | 8.0±3.5 |

Significant differences in age, weight and length (p<0.01) were seen between coastal and offshore caught herring with coastal herring being older and larger (Table 3.2). One-way ANOVAs with Tukey’s HSD post-hoc tests showed significant differences in herring weight (f = 52.6, df_between = 3, df_within = 123, p<0.01), length (f = 63.2, df_between = 3, df_within = 121, p<0.01) and age (f = 32.4, df_between = 3, df_within = 123, p<0.01) between the four coastal sites. In all cases, herring from Skutskär and Lörudden were similar, as were herring from Norrsundet and Hornslandet; herring from Skutskär and Lörudden differed significantly (were larger/older) than herring from Norrsundet and Hornslandet. For the offshore sites, significant differences were seen for weight (f = 116.4, df_between = 3, df_within = 96, p<0.01), length (f = 192.2, df_between = 3, df_within = 95, p<0.01) and age (f = 18.9, df_between = 3, df_within = 95, p<0.01). Tukey’s HSD post hoc tests revealed that for weight, herring from Utsjö 2 and 4 did not differ significantly but all other combinations of sites did, while for length and age, herring from Utsjö 3 and 4 did not differ significantly, but all other combinations of sites did. As Utsjö 3 and 4 are located within the Baltic Proper this result is expected.
Table 3.2. Student’s t-test results for age, weight, total length and lipid content for coastal versus offshore herring. Arithmetic means, degrees of freedom (df) and p values are shown.

<table>
<thead>
<tr>
<th></th>
<th>Coastal Sites</th>
<th>Offshore Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6.2</td>
<td>5.3</td>
</tr>
<tr>
<td>df = 127</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>38.6</td>
<td>27.2</td>
</tr>
<tr>
<td>df = 225</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Total Length (cm)</td>
<td>18.0</td>
<td>16.3</td>
</tr>
<tr>
<td>df = 197</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Lipid Content (fat % w.w.)</td>
<td>7.0</td>
<td>5.5</td>
</tr>
<tr>
<td>df = 16</td>
<td></td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

3.3.2 Congener Patterns and Concentrations

HERRING

Congener pattern did not vary much between coastal and offshore caught herring on either a l.w. or w.w. basis (only l.w. presented below). Abbreviations used in the below figures are as follows: SK = Skutskär, NS = Norrsundet, HL = Hornslandet, LU = Lörudden, Ut 1 = Utsjö 1, Ut 2 = Utsjö 2, Ut 3 = Utsjö 3, Ut 4 = Utsjö 4, f = fillet, m = muscle, 1, 2 or 3 refers to which pooled sample is represented, e.g., Ut f1.1 refers to herring fillet sampled at Utsjö 1, pooled group 1. Unadjusted data is used. PCDDs, PCDFs and DL-PCBs (l.w.) show very similar congener patterns and concentrations between coastal and offshore sites (Figure 3.2a, b; fig 3.3a, b; Figure 3.4a, b). The dominant PCDF and DL-PCB congeners are the same as that seen in the temporal dioxin data; however dominant PCDD congeners seen here differ to that in the temporal data (2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, chapter 1, 1.3.3).

Figure 3.2. PCDD (l.w.) congener pattern for a) coastal and b) offshore herring.
Figure 3.3. PCDF (l.w.) congener pattern for a) coastal and b) offshore herring.

Figure 3.4. DL-PCB (l.w.) congener pattern for a) coastal and b) offshore herring.

Figure 3.5. TEQ values for PCDD, PCDF and DL-PCBs (l.w.) for a) coastal and b) offshore herring.
In both coastal and offshore herring, TEQ\textsubscript{PCDD} contributed the least to total toxicity on both a l.w. and w.w. basis (Figure 3.5, 3.6a, b, only l.w. presented). This result is in agreement with results seen from the temporal data (chapter 1), where TEQ\textsubscript{PCDD} contributed the least, and alternatively TEQ\textsubscript{PCDF} or TEQ\textsubscript{DL-PCB} contributed the most, depending on site (see 1.3.3).

a)  

Figure 3.6. Relative proportion to total toxicity contributed by TEQ\textsubscript{PCDD}, TEQ\textsubscript{PCDF} and TEQ\textsubscript{DL-PCB} (l.w.) for a) coastal and b) offshore herring. Unadjusted data.

When the TEQ\textsubscript{PCDD}, TEQ\textsubscript{PCDF}, TEQ\textsubscript{PCDD/F}, TEQ\textsubscript{DL-PCB} and TEQ\textsubscript{PCDD/F+DL-PCB} (l.w.) values were compared between coastal and offshore herring using student’s t-tests, no significant differences were found when unadjusted fillet or muscle data was used (fillet and muscle examined separately). When age adjusted data was used, TEQ\textsubscript{PCDD} and TEQ\textsubscript{PCDF} concentrations showed significant differences for muscle samples only (df = 4, p<0.01, arithmetic mean 19.0, 6.6, coastal and offshore; df = 4, p<0.02, arithmetic mean 32.6, 13.0 coastal and offshore respectively). Fillet samples continued to show no significant differences in concentrations between coastal and offshore herring. It must be kept in mind that muscle samples had a very low number of observations (n = 6) and therefore confidence in muscle t-test results is poor.

Student’s t-tests were repeated using both unadjusted and age-adjusted fillet data on a wet weight basis, but not muscle data, because of the low number of muscle observations. No significant differences were seen between any TEQ values from herring sampled coastally or offshore for the unadjusted data. However, when the age-adjusted data was examined, a significant difference between coastal and offshore sites was seen, but only for TEQ\textsubscript{PCDD/F} values (df = 14, p<0.04, arithmetic mean 2.7, 1.7, coastal and offshore respectively).

When the four coastal sites were compared for differences in TEQ\textsubscript{PCDD}, TEQ\textsubscript{PCDF}, and TEQ\textsubscript{DL-PCB} values (l.w.), and dominant congeners (l.w.) using one-way ANOVAs (age-adjusted fillet data), a significant difference was only seen for TEQ\textsubscript{DL-PCB} (f = 9.05, df = 3, p<0.01). Skutskär and Lörudden had significantly higher values compared to Hornslandet and Norrsundet. However, as Hornslandet is a reference site, it is expected that herring from this site would have lower concentrations than herring from the other three sites. When the offshore sites were examined in the same way, no significant differences in dominant congener concentrations or TEQ values were seen.

By contrast, when one-way ANOVAs were carried out using age-adjusted wet weight data, significant differences were seen between all sites for TEQ\textsubscript{PCDD}, TEQ\textsubscript{PCDF}, TEQ\textsubscript{PCDD/F} and TEQ\textsubscript{DLPCB} (f = 7.24, df = 3, p<0.02; f = 4.45, df = 3, p<0.05; f = 16.99, df = 3, p<0.01; f = 4.41, df = 3, p<0.05 respectively). Tukey’s post hoc tests showed that Hornslandet had significantly lower values.
compared to Lörudden for all TEQ values. Norrsundet did not differ to any of the other three sites, except in the case of TEQ_{PCDD}, when Norrsundet had a significantly lower value compared to Lörudden. Skutskär had significantly lower TEQ_{PCDD} value compared to Lörudden, and significantly higher TEQ_{PCDD} value compared to Hornslandet. However, for the offshore sites, no significant differences were seen.

MYSIDS AND ZOOPLANKTON

OCDD was highly dominant in both mysids and zooplankton from all sites (Figure 3.7a). 2,3,7,8-TCDF was dominant in mysids from Skutskär, while OCDF dominated in zooplankton from Skutskär, and mysids and zooplankton from the other sites (Figure 3.7b). For PCDFs and DL-PCBs, mysids show higher concentrations than zooplankton. This is not the case in PCDDs.

The relative proportion that TEQ_{PCDD}, TEQ_{PCDF} and TEQ_{DL-PCB} contributed to total toxicity in mysids (Figure 3.8a) and zooplankton (Figure 3.8b) is similar to that seen in herring. The main difference is that TEQ_{DL-PCB} contributes far more to total toxicity in zooplankton than it contributes in herring (coastal or offshore) and mysids.
SEDIMENT AND WATER

Sediment data are from Task 1D of this project (please refer to BalticPOPs final report 2012), and the chosen sites were in close proximity to where coastal herring were sampled (Fig. 3.9).

Pearson’s correlation coefficients were used to try to establish a statistical link between dioxin concentrations in sediment and herring. The sum TEQ_{PCDD} values and 1,2,3,7,8-PeCDD concentrations for the closest sediment sites to the coastal sampling sites were examined; however no significant correlations were found, probably due to the low number of sites (n=4) (Figure 3.10).
Figure 3.9. The average sum PCDD/F (avgsPCDD/F) and average sum DL-PCB (avgsDLPCB) (pg/g l.w.) concentration from herring fillet for each coastal site, and for the closest sediment sample to each site, the sumPCDD/F and sum DL-PCB (pg/g d.w.) concentration. Red dots indicate approximate location of coastal sites.

<table>
<thead>
<tr>
<th>Coastal Site</th>
<th>avgsPCDD/F fillet (pg/g l.w.)</th>
<th>avgsPCDD/F sediment (pg/g l.w.)</th>
<th>avgsDLPCB fillet (pg/g l.w.)</th>
<th>avgsDLPCB sediment (pg/g d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skrugår</td>
<td>180</td>
<td>470</td>
<td>1752</td>
<td>690</td>
</tr>
<tr>
<td>Hornsundet</td>
<td>210</td>
<td>507</td>
<td>883</td>
<td>225</td>
</tr>
<tr>
<td>Mörrum</td>
<td>104</td>
<td>1919</td>
<td>5620</td>
<td>5666</td>
</tr>
<tr>
<td>Lörudden</td>
<td>94</td>
<td>351</td>
<td>351</td>
<td>225</td>
</tr>
<tr>
<td>Norrsundet</td>
<td>92</td>
<td>1919</td>
<td>5620</td>
<td>5666</td>
</tr>
</tbody>
</table>
Congener pattern for sediment is presented on a dry weight (d.w.) basis, while water is presented on a m³ basis. Congener concentrations at Norrsundet were considerably higher than at all other sites (in extreme cases up to 80x higher for a single congener (e.g., OCDD)), thus graphs are presented on a percentage basis so congener patterns can be seen at all sites.
Sediment congener patterns were dominated by OCDD (Figure 3.11a), and OCDF (Figure 3.11b). CB118 and CB105 dominate the DL-PCBs for sediment on an absolute concentration basis (d.w.). In sediment, CB167 (+128) appears to be at least as dominant as CB105; however this could be an artefact of coeluted congeners raising concentrations (Figure 3.11c). Sediment from Lörudden is marked as a chemical district; however there does not appear to be any obvious differences in congener patterns between this site and the three with current usage of pulp/paper mills. For PCDFs, 2,3,7,8-TCDF and 1,2,3,4,6,7,8-HpCDF are more dominant, while OCDF contributes much less to total PCDF toxicity.
The relative proportion of the summed TEQ values from each group for sediment shows that DL-PCBs contribute the least, and PCDDs contribute the most to total toxicity (Figure 3.12a – d). Although DL-PCBs are shown as contributing 0% at Norrsundet (Figure 3.12b), in fact, this is 0.5%. Of the four sites, PCDDs contribute the least towards total toxicity at Lörudden (Sundsvall District, 50%).

Overall, congeners in herring did not differ much in concentration or pattern between coastal or offshore sites. Mysids and zooplankton had similar a pattern to each other. Sediment patterns were more similar to zooplankton, while water was different again, in line with previous investigations (Wiberg et al. Report 5912). Contributions to total toxicity of the three groups of dioxins varied – in all biota, PCDDs contributed the least, and PCDF/DL-PCBs contributed the most, while in sediment PCDDs contributed the most and DL-PCBs contributed the least to total toxicity.

CONVERSION BETWEEN FILLET AND MUSCLE CONCENTRATIONS

As fillet (skin and subcutaneous fat) and muscle (skin and subcutaneous fat removed) contain different amounts of fat, an attempt at calculating a conversion factor between muscle and fillet was made using wet weight concentrations, following a procedure similar to Bignert et al. (2005). A ratio between the average fillet:muscle fat content was calculated. Here, that ratio was 7.6:4.8 fillet:muscle. Average fillet fat was divided by average muscle fat content, giving a factor of 1.6. Thus, the summed concentrations e.g., summed PCDD, for fillet was divided by the conversion factor of 1.6 to give an estimation of muscle concentration, as shown by the following example:

\[ C_m = \frac{C_f}{(Av_{fl}/Av_{ml})} \]

where \( C_m \) is the concentration in muscle, \( C_f \) is the concentration in fillet, \( Av_{fl} \) is the average fillet lipid content, and \( Av_{ml} \) is the average muscle lipid content.

e.g., \( C_m = \frac{\text{sumPCDD Skutskär}}{1.6} \)

\[ = \frac{2.88}{1.6} \]

\[ = 1.80 \]

The actual summed PCDD concentration for muscle in this case was 1.73. For summed PCDD concentrations, this equation was relatively accurate, giving estimated muscle concentrations very close to actual concentrations. For both PCDF and DL-PCBs, the estimated values were slightly higher each time (Table 3.3). However, a slight over estimation of concentration leaves a safety margin.
Converting from muscle concentration to fillet concentration can be done by using this equation in reverse i.e., multiplying the muscle concentration by the conversion factor.

Table 3.3. An example of estimated muscle concentrations calculated using the above equation. These results are for Skutskär pooled group 1, 2, and 3. Concentrations are in pg/g w.w. (2 s.d.).

<table>
<thead>
<tr>
<th>Fillet Concentrations</th>
<th>Actual Muscle Concentrations</th>
<th>Estimated Muscle Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>sPCDD 3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>sPCDF 16</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>sDL-PCB 8500</td>
<td>3600</td>
<td>3100</td>
</tr>
</tbody>
</table>

3.3.3 Congener Influence on Offshore and Coastal Herring

Principal component analysis (PCA) was carried out to investigate what, if any, congener exerts the greatest influence on herring contamination load between coastal and offshore sites. This was done using normalised data, with values that were below LOQ removed. As muscle samples were taken from only half of the sites, muscle was removed from the analyses, making a total of three pooled herring samples at each site. As no significant differences were found in TEQ concentrations between coastal and offshore herring for fillet samples for either adjusted or unadjusted data, it was decided to present the PCAs using unadjusted data.

In the following figures, numbers are equivalent to sites, where 1,2,3 = Sk (Skutskär), 4,5,6 = NO (Norrsundet), 7,8,9 = HO (Hornslandet), 10,11,12 = LO (Lörudden), 13,14,15 = Ut1 (Utsjö 1), 16,17,18 = Ut2 (Utsjö 2), 19,20,21 = Ut3 (Utsjö 3), 22,23,24 = Ut4 (Utsjö 4).

For PCDDs and PCDFs, there is no pattern discernible in the data (Figure 3.13a, b). Points are scattered over the whole graph, with no one congener showing a strong influence in PCDDs (fig 3.13a). No particular pattern is seen for PCDFs, although coastal herring appear to group more closely towards 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF (Figure 3.13b). The DL-PCBs show coastal and offshore herring forming two groups, which are significantly different (Hotellings t test, f = 5.8, df = 12, p<0.02) (Figure 3.13c), however there is still some scatter and overlap between the two groups. Offshore and coastal herring largely group together on opposite sides of the PCA; however, no one congener appears to be exerting a dominating affect. Overall, there does not seem to be much difference in congeners influencing concentrations in herring caught from coastal and offshore sites.
3.3.4 Zooplankton Composition

Zooplankton samples were taken at each site. At Skutskär and Norrsundet, copepods were the dominant zooplankton, comprising 67 and 50% of total zooplankton composition respectively (Figure 3.14a, b). At Hornslandet, Lörudden and Köpmanhölmen, Bosmina species were the dominant zooplankton, comprising 63, 50 and 60% of total zooplankton respectively, with copepods being the secondary dominant group (Figure 3.14 c, d, e). Skutskär and Norrsundet occur in the southern Bothnian Sea, while Köpmanhölmen, Lörudden and Hornslandet are located from the central to northern Bothnian Sea (see Figure 3.1). Zooplankton community structure is largely regulated by the environment e.g., hydrography and stratification within the Baltic Sea (Flinkman et al. 1998 and references therein). Therefore, distances and different environmental conditions between sites could explain the observed differences in zooplankton composition.
3.3.5 Herring Stomach Content

When the stomach contents of herring from Skutskär were examined, 25% were empty. Between 40 – 60% of herring stomachs contained stones. Where only stones were present i.e., no other food was found, these herring were added to those with empty stomachs, increasing the number of empty stomachs from 25% to 36%. Less than 20% of stomachs contained mysids (both *M. relicta* and *M. mixta*). The only other food found was fish scales. However no fish were found, and other decomposed contents were unidentifiable. No zooplankton were found in these stomachs, and one herring stomach contained grass/algae. Stomach content did not differ with herring size.

At Norrsundet, 5% of herring stomachs were empty (2 of 36). No mysids were found, and the majority of the rest of the stomach contained unidentifiable zooplankton, and also Bosmina, Copepoda, Podon and Cercopagis species. Composition of stomach contents did not change with herring size.

At Hornslandet, herring stomach content was primarily comprised of Gammarus species, a type of amphipod; chironomid pupae, a life history stage of nematoceran flies; and fish of indeterminate species. Chironomid pupae were present in fish of all lengths, while Gammarus were only present in the two largest groups (14 – 15 cm, n = 9) and at very low numbers, comprising <5% of total stomach contents. Fish were present across the whole range of sizes. Empty stomachs accounted for 10% (2 of 19) of herring. No zooplankton was found in herring stomachs from Hornslandet.

At Lörudden, 8% (3 of 36) of herring stomachs were empty. Bosmina species dominated herring in the size range 15 – 16 cm length, while copepods and then Bosmina species were dominant.
in the largest size (17 cm length). Mysids (both *M. relicta* and *M. mixta*) were present in 60% of herring stomachs. Usually only 1 – 3 mysids were present per stomach, but in one case, 10 mysids were recovered from a single stomach.

Overall, the stomach contents of the four coastal sites varied from each other somewhat, with zooplankton (various species) dominating at Norrsundet, both zooplankton and mysids occurring in most stomachs from Lörudden, while mysids and other food items e.g., chironomid pupae, fish, Gammarus etc., dominated at Hornslandet and Skutskär. Empty stomachs are not included here; however Skutskär had the highest percentage of empty stomachs (25%).

### 3.3.6 Stable Isotope Analysis (SIA)

![Stable isotope bi-plots of coastal herring and their prey items (zooplankton and mysids) in a) Skutskär, b) Norrsundet, c) Hornslandet, d) Lörudden.](image)

From the SIA conducted, it was apparent that herring diet from all sites is dominated by mysids (coastal and/or offshore), with little input from zooplankton. In particular, coastal zooplankton is not dominant in herring diet at any site (Figure 3.15). Herring from Skutskär and Lörudden have a higher proportion of coastal mysids in their diet compared to offshore mysids and zooplankton. The diet of herring from Norrsundet is dominated by offshore mysids, with coastal mysids and zooplankton making up a smaller proportion of the diet. Herring diet at Hornslandet is dominated...
firstly by offshore mysids, and then by coastal mysids and zooplankton (Figure 3.16). While zooplankton plays a small role in the diet of herring at any site, offshore zooplankton features more prominently than coastal zooplankton in herring diet from Norrsundet and Hornslandet. In stomach content analyses (section 3.3.5), mysids were only found from herring at Skutskär and Lörudden. However, stomach content analysis only gives a snapshot overview of diet, which is why SIA is important.

Figure 3.16. Proportion of coastal and offshore prey items in coastal herring diet estimated with the SIAR model for a) Skutskär, b) Norrsundet, c) Hornslandet, d) Lörudden.
The proportion of herring diet that is comprised of coastal sourced food resources is greater at Skutskär and Lörudden compared to herring from Norrsundet and Hornslandet (Figure 3.17). When herring biological parameters were measured and compared between these four sites (section 3.3.1), herring from Norrsundet and Hornslandet were similar to each other, as were herring from Skutskär and Lörudden, while Norrsundet and Hornslandet herring differed significantly in weight, length and age to herring from Skutskär and Lörudden. These biological differences may be a reflection of herring diet.

### 3.4 Discussion

#### 3.4.1 Biological Variables, Congener Pattern and Concentrations

Investigation of herring from coastal and offshore sites from the Bothnian Sea revealed little variation in congener patterns or spatial difference in absolute congener concentrations and TEQ values (l.w. basis). Principal component analyses (PCAs) showed both coastal and offshore herring to form one group for PCDD/Fs (l.w.). Two groups were formed for DL-PCBs; however, there was a large amount of scatter with considerable overlap. Offshore and coastal herring largely grouped together on opposite sides of the PCA; however, no one congener appeared to be exerting a dominant affect. Overall, there did not appear to be much difference in congeners influencing concentrations in herring caught from coastal and offshore sites.

However, on a wet weight basis and using age adjusted data, a difference between coastal and offshore herring was seen, but only for TEQ_{PCDD/F}, with this value being higher in coastal compared to offshore herring. This difference was not seen when unadjusted data was used. The relative proportion to total toxicity contributed by TEQ_{PCDD}, TEQ_{PCDF} and TEQ_{DLPCB} also did not differ between coastal
and offshore caught herring. TEQ$_{PCDD}$ contributed the least to total toxicity, the same as was seen in the temporal data (chapter 1). Some populations of herring are known to feed offshore, and migrate to coastal areas for spawning (Lundin 2011). This phenomenon may have influenced results seen here. However, this does not account for the difference seen in TEQ$_{PCDD}$ on a wet weight basis. The overall lack of differences may also be because a limited geographical range was examined here.

By contrast, significant differences in TEQ$_{DL-PCB}$ (l.w.) were observed between coastal sites. TEQ$_{DL-PCB}$ values in herring from Skutskär and Lörudden were significantly higher than in herring from Hornslandet and Norrsundet. The pattern of these differences, with two sites grouping together, is the same as seen for the biological differences. On a wet weight basis, and using age adjusted data, all TEQ values differed significantly between coastal sites. The sites that differed varied depending on which TEQ value was being examined. In general, however, Hornslandet had lower TEQ values compared to the other sites, while Lörudden had higher values. Age adjusting the data removes age, and consequently size, as potential confounding factors. Therefore, there appears to be perceptible differences between herring sampled at different sites within the one basin on a wet weight basis. Depending on how far these fish travel/migrate, variable local sources of dioxin, or variation in herring diet and consequently lipid content, may be responsible for the observed differences.

SIA results support the observed differences in coastal sites on a wet weight basis. Herring caught from Skutskär and Lörudden had a higher proportion of coastal mysids present in their diet, whereas Hornslandet and Norrsundet had a greater proportion of offshore mysids in their diet. Hornslandet had lower TEQ values compared to Lörudden in all cases, and a lower TEQ$_{PCDD}$ value compared to Skutskär. Norrsundet had a lower TEQ$_{PCDD}$ value compared to Lörudden. It appears that differences in diet (predominantly coastal versus offshore) explain much of the variation seen between the four sites.

Congener patterns and congener absolute concentrations (l.w. basis) in mysids and zooplankton differed somewhat from herring. Different dominant congeners for both PCDDs and PCDFs but not DL-PCBs, as well as lower concentrations, were observed. Reasons for different dominant congeners between herring and mysids/zooplankton may be related to differences in biotransformation ability, differential accumulation efficiencies and different diet between species (HELCOM 2004, Armitage et al. 2009). As dioxins bioaccumulate through trophic levels, congener concentrations should be lower in zooplankton and mysids compared to herring (HELCOM 2004, OSPAR 2007), exactly what was seen here.

Sediment and water patterns were, as expected, quite different to both coastal and offshore herring, but showed similarities with mysids and in particular with zooplankton patterns, with OCDD being the overall dominant congener on an absolute concentration (l.w.) basis for PCDD/Fs. CB118 was the dominant DL-PCB congener, the same as for all biota. No significant correlation could be established between PCDD/F and DL-PCB concentrations in sediment and herring, indicating a lack of local environmental contamination in these herring; however a low number of samples could also be influencing this lack of significant result; therefore, a link between local sources from sediment to herring contaminant loads cannot be ruled out. Bignert et al. (2007) were also unable to establish a link between PCDD/F patterns in sediment and herring from the southern Bothnian Sea. It is unsurprising that sediment patterns differ to herring, as dioxins have low water solubility and therefore settle in the sediments close to the source point, receiving the full spectrum of locally produced pollutants, whereas herring only receive what they accumulate through diet and/or diffusion across the gills (Wiberg et al. 2009). Only one data set for water was available, and as it did not include DL-
PCBs, it is difficult to make any inference about similarities or otherwise with the biota or sediment patterns. For this reason, water was not included on any PCA.

Contribution to total toxicity of TEQ\textsubscript{PCDD}, TEQ\textsubscript{PCDF} and TEQ\textsubscript{DL-PCB} differed considerably between sediment and biota. In herring, mysids, and zooplankton, TEQ\textsubscript{PCDF} and TEQ\textsubscript{DL-PCB} contributed the most, and TEQ\textsubscript{PCDD} the least, with this trend even more pronounced in zooplankton. However, in sediment, TEQ\textsubscript{DL-PCB} contributed negligible amounts, while TEQ\textsubscript{PCDD} contributed the most to total toxicity, the complete opposite to biota. Results from biota are similar to results from Isosaari et al. (2006), where the contribution of DL-PCBs to the WHO\textsubscript{sum}-TEQ was equal to or greater than the contribution of PCDD/Fs. As mentioned above, dioxins have low water solubility and sediments receive the full spectrum of locally produced pollutants. Over time, small amounts of dioxins are released from sediment and settling particulate matter (SPM) to become biologically available in the food web. Biota are then exposed to these small amounts, but at the same time, biological organisms are often able to biotransform the accumulated pollutants, creating species specific congener patterns. Thus the differing water solubility of the congeners and biotransformation explains the difference in contribution to total TEQ seen between biota and sediment.

Overall, coastal and offshore herring do not differ from each other in congener pattern or absolute concentrations (l.w.), while only TEQ\textsubscript{PCDD/F} differed between coastal and offshore herring on a wet weight basis and using age adjusted data, indicating the sampled herring may not be strictly coastal/offshore populations. Coastally caught herring differed between sites for all TEQ values on a wet weight basis, but only in TEQ\textsubscript{DL-PCB} value on a lipid weight basis. Congener patterns of mysids and zooplankton show a slight shift away from the herring pattern, while sediment is even further removed, although showing similarities to mysids and zooplankton. SIA results indicate that herring diet from Hornslandet and Norrsundet includes a greater proportion of offshore food resources while coastal dietary resources predominate in herring from Skutskär and Lörudden. These differences likely explain the differences observed in TEQ values between sites on a wet weight basis. Coastal caught herring appear little influenced by dioxins in coastal sediments, as seen from the lack of significant correlation between sediment and herring dioxin concentrations, although the low number of sites included here may have influenced the outcome. However, zooplankton and mysids were coastal, and thus reflected the local environment to a greater degree.

3.4.2 Zooplankton Composition and Herring Stomach Analyses

In the marine environment, diet is the primary route of dioxin exposure in fish (Zhang et al. 2011). Zooplankton samples from each coastal site were examined. At Skutskär and Norrsundet, copepods and then \textit{Bosmina} species dominated the zooplankton. At Hornslandet, Lörudden and Köpmanholmen, the opposite was seen, with \textit{Bosmina} species followed by copepods dominating the zooplankton.

Herring stomach contents varied – herring from Skutskär and Hornslandet contained no zooplankton, while those from Norrsundet and Lörudden did contain zooplankton. A variety of mysids, unidentified fish, and chironomid pupae were found in stomachs between sites, as well as some herring stomachs being empty.

In coastal feeding herring, mysids are more common, while in offshore feeding herring, zooplankton is more common in the diet (Arrhenius & Hansson 1993). As zooplankton dominated herring stomachs from Lörudden and Norrsundet, it may indicate that these fish were feeding offshore.
before they were sampled, but this idea is only supported by SIA results for Norrsundet (see 3.4.3, below). In the Bothnian Sea, herring larvae dominate the diet in autumn/summer (Parmanne et al. 2006). Unidentified fish were found in the stomachs of herring from Hornslandet, and sampling was conducted in late summer, so these unidentified fish may in fact be herring larvae. Numerous chironomid pupae were also found in herring sampled at Hornslandet. Some chironomid species are marine e.g., *Telmatogon japonicus*, which has been found near Kalmar in the Baltic Sea (Brodin & Andersson 2008), although chironomids are not usually reported as herring prey. However, this gives no indication of whether herring sampled from Hornslandet were predominantly coastal or offshore. The majority of herring from Skutskär appeared to be hungry at the time of sampling, and many contained stones. Stones in fish stomachs can be used for grinding or crushing shells of prey; however, herring diet does not generally contain prey items requiring such processing. Such snap-shot sampling can only indicate what these fish ate directly prior to sampling, and no definitive statement can be made about how diet may have influenced dioxin concentrations based on stomach analyses presented here.

### 3.4.3 Stable Isotope Analyses

SIA was conducted on herring from coastal sites. Contrary to the stomach analysis, which only gives a snapshot of herring diet, SIA from fish muscle can retrospectively examine what the fish has been feeding on for approximately a month before sampling (Kiljunen, *pers. comm.*). SIA showed that zooplankton featured very little in herring diet, with mysids being the dominant food source for all four sites. Herring diet from Skutskär and Lörudden included more coastal mysids, while herring from Hornslandet and Norrsundet had a greater proportion of offshore mysids present in the diet. The differences seen in TEQ values (w.w.) could well be explained by differences in diet observed here.

### 3.4.4 Summary

Earlier studies have shown spatial variation in dioxin concentration of herring in the Baltic Sea (Bignert et al. 2007, 2011). However, that spatial variation could not be linked to variation in sediment (Bignert et al. 2007), and neither could a link be established here between sediment and herring dioxin concentrations. It should be noted that sediment levels at coastal and offshore sites are decreasing (please refer to BalticPOPs final report 2012). The importance of contaminated sediments as a local source for fish is therefore decreasing over time.

Here, spatial differences between coastal sites sampled within the Bothnian Sea were seen, but only on a wet weight basis. A single difference was seen between coastal and offshore sites also on a wet weight basis (TEQ$_{PCDD/F}$), but no differences were seen on a lipid weight basis. As mentioned in the methods (3.2.1), two of the offshore sites were sampled within the Baltic Proper and were therefore not directly comparable to the coastal sites sampled, and may thus explain the difference that was seen. Herring diet may offer some explanation for differences seen in dioxin concentrations between coastal herring, as shown by SIA. These results supported the differences seen on a wet weight basis between the four coastal sites, indicating that variations in diet were overall a large contributor to dioxin levels in herring in this basin. However, the overall lack of differences between coastal and offshore herring can probably be attributed to the migratory nature of herring populations within the Bothnian Sea, feeding mainly offshore and migrating to coastal areas for spawning for only a few weeks in the spring/summer (Lundin, 2011).
General Discussion

Processes involved in dioxin concentrations observed in herring are complex and difficult to disentangle. Chemical, biological and environmental factors all play a role, yet it is difficult to quantify the contribution of each, especially as each set of factors varies spatially and temporally, and are inter-related. From data examined here, Baltic Sea ecological processes and physical environmental conditions appear to have affected herring biology, which in turn played a role in the observed slowing of temporal decreases, seasonal observations of dioxin concentrations, and the spatial differences seen between coastal sites in the Bothnian Sea on a wet weight basis.

Temporal data presented here came from four sites – three sites throughout the Baltic Sea region, and one from the Swedish west coast. Because of the marked differences in environmental conditions, dioxin concentrations were not directly comparable between sites. However, the differing environmental conditions are likely directly contributing to differences in herring biology and bioenergetics, and therefore indirectly to dioxin concentrations. Such processes would also contribute to explaining inter-basin dioxin differences, and possibly, intra-basin variations seen here.

The Bothnian Bay has lower salinity and average air temperatures compared to the Bothnian Sea, the Baltic Proper and the Swedish west coast (Table 1.1). Since the late 1980s, oceanographic conditions linked to atmospheric forcing have caused increasing average water temperature and consequently decreasing salinity in the central Baltic Sea (Möllman et al. 2005). As salinity decreases, the abundance of small plankton species decreases (Rönkkönen et al. 2004), meaning fewer/poorer food resources are available for herring. Decreasing salinity and increasing temperature also decreases oxygen levels, another limiting factor for herring growth (Rönkönnen et al. 2004, Cheung et al. 2012), and presumably zooplankton. Thus, under different salinity regimes, different zooplankton species dominate (Rönkkönen et al. 2004, Möllman et al. 2005), meaning zooplankton community structure varies spatially (between basins) but also, according to Möllman et al. (2005) temporally. The differing availability of zooplankton species would influence herring diet – depending on the abundance and energy content of the zooplankton, energy available for growth in herring would also vary – thus herring growth, and in turn, dioxin concentrations.

Various hypotheses exist to explain decreased herring growth, from the cod stock crash working as a top-down process (Flinkman et al. 1998, Möllman et al. 2004); increasing temperature and decreasing salinity causing changes in zooplankton community structure, a bottom-up process (Rönkkönen et al. 2004, Möllman et al. 2005); to climate changes causing increased temperature, which in turn lowers oxygen levels and makes oxygen become a limiting factor earlier in pelagic fish growth (Cheung et al. 2012). Any, or all, of these processes could drive a decrease in herring growth. Each of these processes will have stronger or weaker impacts within each basin because of the original environmental conditions in the basins, thus contributing to not only temporal but also spatial changes in dioxin concentrations.

Although differences in dioxin concentrations between basins was not a focus of this work, there are some factors highlighted within the current project that stand out as potentially having an influence on this issue. Different basins in the Baltic are subject to differences in salinity and temperature. Variations of both of these abiotic parameters may increase energetic costs in herring, thereby reducing energy that can be spent on growth. In turn, this would contribute to lower growth rates, and a lack of decreases in dioxins, as already discussed. Growth rate modeling carried out within this project (BalticPOPs) showed that a slower growth rate in the north of the Baltic Sea was an important factor impacting dioxin concentrations in herring. Herring diet may impact dioxin levels.
also, due to e.g., salinity affecting zooplankton composition and/or differences in trophic level between basins. As has been observed here, lipid content in herring varies between basins, with lower lipid content seen in herring from further north. As already discussed, lipid content strongly influences dioxin concentrations, and is linked to herring diet. Therefore, differences in physical conditions between basins, coupled with herring biology, can probably explain much of the spatial variation observed between basins in the Baltic Sea.

Within this report, we observed that herring biology appeared to be very important in observed dioxin concentrations, but their precise contribution was not quantified, and such quantification may never be possible because of the complex ways these factors interact with environmental conditions. However, chemical factors such as dioxin emissions, source strength, distance from source etc., which are also known to be important in dioxin concentrations (Wiberg et al. 2009) were not investigated here, and are certainly not ruled out. Chemical factors will also play a major role in dioxin concentrations; however, here we investigated, and were able to see, that herring biology is also important in observed dioxin concentrations.
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