PCDD, PCDF, PCB and thiamine in Baltic herring (*Clupea harengus* L.) and sprat [*Sprattus sprattus* (L.)] as a background to the M74 syndrome of Baltic salmon (*Salmo salar* L.)

Pekka J. Vuorinen, Raimo Parmanne, Terttu Vartiainen, Marja Keinänen, Hannu Kiviranta, Olli Kotovuori, and Folke Halling

Baltic herring and sprat were sampled between October 1994 and January 1995 off the southern coast of Finland and around the Åland Islands. From this material fish of 135–159 mm in length, a size class that was assumed to be preferred as prey by Baltic salmon, were selected and grouped according to species, sex and age. Herring were 1–3 years and sprat 3–13 years old. In addition, one group of smaller, two-year-old, female sprat was selected. Thus twenty groups were formed and prepared as whole-fish homogenates. Thiamine was quantified from all homogenates, while female and male sprat and female herring were analysed for polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs). There were no significant differences between sexes within each species in the total thiamine concentrations, but the mean total thiamine concentration in 1 to 3-year-old herring (8.6 nmol g⁻¹) was higher than that in 2 to 13-year-old sprat (6.7 nmol g⁻¹). The mean-fresh-weight concentrations of total PCBs, coplanar PCBs, PCDDs and PCDFs were on average 2–3 times higher in 2 to 10-year-old sprat than in 1 to 3-year-old herring of a similar size, although the difference in PCDD concentrations was not significant. In sprat females there were no significant correlations between age or fat content and the fresh-weight concentrations of coplanar PCBs or PCDFs. However, the higher OC concentrations in sprat could be explained both by their higher fat content, with the younger age groups being the most fatty, and by their age, which reflects their slower growth rate. The fat-weight-based total PCB, PCDD, PCDF and coplanar PCB concentrations increased with age in sprat and, after controlling for age, there was a significant positive relationship between the fat content and the fresh-weight-based concentrations of OCs, apart from total PCBs. On a fat-weight basis the concentration of coplanar PCBs was also higher in sprat than in herring. It is concluded that the Baltic salmon is provided with an adequate supply of thiamine, at least for growth, from its two main prey species, Baltic herring and sprat. Furthermore, sprat might have been the principal source of organochlorines, particularly coplanar PCBs and PCDFs, for salmon. The concentrations of these compounds were earlier found to have increased in salmon coincidently with the outbreak of the M74 syndrome.

Keywords: herring, sprat, M74, Baltic salmon, thiamine, PCDD, PCDF, PCB, organochlorine.

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Introduction

Herring (Clupea harengus L.) and sprat [Sprattus sprattus (L.)] together formed approximately 90% of the prey of Baltic salmon (Salmo salar L.) feeding in the eastern and southern parts of the Baltic proper in samples collected from 1995–1997, although there were spatial and seasonal differences (Karlsson et al., 1999a; Karlsson et al., 1999b; Hansson et al., 2001). This area is the main feeding ground for salmon that originate from the stocks of rivers around the Gulf of Bothnia and that ascend these rivers to spawn (Karlsson et al., 1996). At the beginning of the 1990s these stocks experienced a dramatic increase in yolk-sac fry mortalities due to the M74 syndrome, a reproductive disturbance of Baltic salmon (Norrgren et al., 1993; Vuorinen et al., 1997). The causes of the syndrome are unknown but it is commonly believed to be linked to the food of salmon (Bengtsson et al., 1999), i.e. herring or sprat, or both.

The cod (Gadus morhua L.) stock of the Baltic Sea has decreased since the early 1980s (ICES, 2001) due to unsuccessful reproduction caused by a decrease in salinity and oxygen content in the spawning areas (Wieland et al., 1994) and extensive fishing pressure (Jonzén et al., 2001), and the stock is currently at a very low level (Vallin et al., 1999; ICES, 2001). The cod is the main predator of herring and sprat (Sparholt, 1994), and reduced cod predation allowed the sprat to increase in abundance and biomass from the late 1980s towards the beginning of the 1990s (Figure 1) when the M74 mortalities became prevalent (Bengtsson et al., 1999). Sprat abundance levelled off in the mid-1990s but in 2000 it was estimated to be approximately three times as high as on average during the 1980s, while the biomass has doubled (ICES, 2001). The biomass of herring in the southern and central Baltic Sea has declined from the early 1980s but abundance has decreased only slightly. At the same time the mean weight of herring in all year classes has decreased, even to less than half of the previous values (Figure 1) with this decrease being greater in the northern than in the southwestern part of the Baltic proper (Cardinale and Arrhenius, 2000). The mean weight-at-age of sprat also decreased during the 1990s (ICES, 2001).

In the period 1995–1997 herring and sprat dominated in the stomach contents of salmon, both in terms of weight and number of prey items (Karlsson et al., 1999a; Karlsson et al., 1999b). Karlsson et al. (1999a) did not record any sprat in the stomachs of salmon sampled in the Gulf of Bothnia. However, the salmon diet has changed in that it now feeds on smaller and relatively older herring and sprat than in the 1980s. Toxicant contents in herring increase with age (Perttilä et al., 1982; de Wit et al., 1994; Vartiainen et al., 1995b), but corresponding information from sprat is lacking thus far. Salmon have probably increased their feeding on sprat as their relative abundance has increased (ICES, 2001) and Hansson et al. (2001) observed that in the period 1995–1997 salmon consumed a greater proportion of younger sprat than in the 1960s.

It has been proposed that the rising frequency of the M74 syndrome is associated with the increase in abundance of sprat (ICES, 1994). Sprat have been supposed to have a high thiaminase activity (Soivio and Hartikainen, 1999) that would result in an inadequate supply of thiamine for salmon. Thiamine deficiency has been connected with the M74 syndrome: eggs that developed into yolk-sac fry suffering from M74 symptoms had a low thiamine content (Amcoff et al., 1998b; Vuorinen and Keinänen, 1999), and thiamine treatment of the M74 fry ameliorated the symptoms and decreased mortality (Bylund and Lerche, 1995; Amcoff et al., 1998a). Thiamine deficiency in adult specimens of several fish species results in anorexia, poor growth, depigmentation and the loss of equilibrium, reviewed by Millikin (1982), and related symptoms are found in yolk-sac fry with the M74 syndrome (ICES, 1994). However, during the 1990s the growth rate of adult salmon on their feeding migration was high and their mean weight increased (Karlsson et al., 1999a).

In River Simojoki salmon the 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic equivalent (TEQ) concentrations of certain dioxin-like organochlorines, i.e. coplanar PCBs and polychlorinated dibenzofuran (PCDF) congeners, increased coincidentally with an outbreak of M74, and certain congeners were significantly associated with the increase in yolk-sac fry mortality due to the M74 syndrome (Paasivirta et al., 1995; Vuorinen et al., 1997). The increase in these compounds in salmon remains unexplained. However, it has been reported that the total concentrations of PCBs and DDT in Baltic fish and the concentrations of polychlorinated dibenzo-p-dioxin (PCDD) and PCDF congeners and coplanar PCBs in guillemot (Uria aalge) eggs have decreased from the peak values in the 1970s (Haahiti and Perttilä, 1988; Kannan et al., 1992; de Wit et al., 1994; Vuorinen et al., 1998; Olsson et al., 2000). On the other hand, Falandysz et al. (1994) found no temporal decrease in the concentrations of coplanar PCBs in liver oils from Baltic cod during the period 1971–1989.

There could be differences in thiamine content between herring and sprat (Soivio and Hartikainen, 1999) and currently salmon might obtain more toxicants by eating slower-growing, and thus relatively older, prey specimens than before (Vuorinen et al., 1997). The aims of the present study were to determine the PCB, PCDD and PCDF congener and thiamine concentrations in sprat and herring sampled from 1994–1995 when the M74 mortality of salmon yolk-sac fry was high. Whole-fish homogenates were used, as the focus was on examining the intake of these substances by salmon as a background to investigating the M74
syndrome. Organochlorine (OC) concentrations in sprat of different age groups and sexes were determined and compared with OCs in herring of a similar size. The selected length class was that which was assumed to be preferred by salmon as prey (Andersson, 1980). To our knowledge there is little published data on OC contents in sprat. Roots and Aps (1993) analysed fillets of sprat sampled in 1986 and 1991, and Vartiainen and Hallikainen (1995) reported total PCB and PCDD/F concentrations in three pooled, sprat-fillet samples.
Materials and methods

Fish samples and preparation of homogenates

Between October 1994 and January 1995 420 herring and 420 sprat were sampled from the catches of professional fishermen. The fish were caught off Hanko headland in the western Gulf of Finland and around the Åland Islands (Figure 2). Only fish with a total length of 12–16 cm were taken. All the fish were measured – total weight and length to the nearest 0.1 g and 1 mm respectively – and otoliths were removed for age determination. The fish were individually sealed in mini-grip polyethylene bags and stored at −20°C for subsequent analysis. The otoliths were illuminated from above against a dark background and “age” was determined as the number of hyaline rings, the individual being moved to the next age group on 1 January. The condition factor (CF) of each fish was calculated from the equation:

\[
\text{CF} = \frac{w \times l^3}{80} \times 100, \quad \text{where} \quad w = \text{total weight (g)} \quad \text{and} \quad l = \text{total length (cm)}. 
\]

The fish were divided into 5-mm length classes ranging from 135 to 159 mm, with 19 classes in total. In addition, a sample of smaller (120–129 mm total length), two-year-old female sprat was collected. Twenty groups of fish were then formed: three groups of female and three of male herring, six of male sprat and eight of three-year-old female sprat. A sample of smaller (120–129 mm total length), with 19 classes in total. In addition, a sample of smaller (120–129 mm total length), two-year-old female sprat was collected. Twenty groups of fish were then formed: three groups of female and three of male herring, six of male sprat and eight of two-year-old female sprat. A sample of smaller (120–129 mm total length), with 19 classes in total. In addition, a sample of smaller (120–129 mm total length), two-year-old female sprat was collected. Twenty groups of fish were then formed: three groups of female and three of male herring, six of male sprat and eight of two-year-old female sprat.

Thiamine analysis

Thiamine contents were determined by HPLC according to Brown et al. (1998), with some modifications, in the laboratory of the Finnish Game and Fisheries Research Institute in Helsinki. Approximately 500 mg of homogenate was weighed into a chilled potter tube. The sample was homogenized (IKA Euro-ST P DV) with 2.0 ml of 2% TCA and the tube was incubated at 100°C for 10 min. The sample was briefly mixed with a vortex-mixer and cooled on ice. Homogenization was repeated with 1.5 ml of 10% TCA. The sample was centrifuged (Beckman Avanti J-30I) at +4°C, 14 000 × g for 15 min. The supernatant was washed four times with an equal volume of ethyl acetate and hexane (3:2). In order to convert thiamines into their corresponding thiochromes an aliquot of 425 µl of supernatant was taken and 75 µl of 0.1% potassium hexacyanoferrate in 1.2 M sodium hydroxide was added. The sample was filtrated (Spartan 13/0.45 RC, S&S) prior to HPLC. Standards were subjected to the same procedure as the tissue samples. The injection volume was 20 µl and the analysis time was 27 min using a linear gradient flow of eluents: eluent A was 0.5% acetonitrile and 99.5% 25 mM phosphate buffer, pH 8.4, and eluent B 25% dimethylformamide and 75% 25 mM phosphate buffer, pH 8.4. All reagents were of analytical grade or better. The HPLC apparatus consisted of two Waters 510 pumps, a Waters 717 Plus Autosampler equipped with a cooling unit (sample tray at +4°C), a thermostated (+35°C) column oven, a Waters 474 fluorescence detector and Millennium^2 Chromatography Manager.

Analysis of organochlorines

Determination of polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans in homogenates, marked with an asterisk in Table 1, was performed in the laboratory of the National Public Health Institute in Kuopio as described in Vartiainen et al. (1995a). About 10 g of freeze-dried fish sample was soxhlet-extracted for 24 h with toluene. The fat content was determined gravimetrically and the raw extract was purified over a silica gel column, fractionated using activated carbon column containing Celite and further cleaned with an activated alumina column. The analyses were performed with a fused silica capillary column (DB-DIOXIN) and a VG 70 SE mass spectrometer (resolution 10 000). The separated PCB fraction was further purified with another activated carbon column (without Celite) and coplanar PCBs were also analysed with a high-resolution mass spectrometer equipped with the fused silica capillary column. Nine 13C-labelled PCB congeners (100 pg/sample of coplanar PCBs and 900 pg/sample for the others, Cambridge Isotope Laboratories) were used as internal PCB-standards as well as a total of 16 13C-PCDD/Fs congeners (100 pg/sample, Cambridge Isotope Laboratories), added to the samples before silica gel column extraction. To test the recoveries, 13C-1,2,3,4-TCDD and 13C-1,2,3,7,8,9-HxCDD or PCB159 were added to the final concentrate before GC-MS analysis; the recoveries for all 13C-labelled PCDD/F and PCB congeners were within the range 60–110%. The toxic equivalent concentrations (TEQs) for PCDDs and PCDFs were calculated (with human/mammal TEFs) according to Van den Berg et al. (1998).

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Figure 2. The sampling areas of herring and sprat from October 1994–January 1995 and the ICES subdivision numbers.
Organochlorines and thiamine in prey of M74 salmon

Table 1. Whole fish homogenates of Baltic herring and sprat analysed for organochlorines (marked with an asterisk*) and thiamine (all). A dissimilar letter as a superscript indicates significant difference (p<0.05) between age groups (i.e. homogenates) in mean (± s.e.) total weight, total length or condition factor (CF) within the species and sex.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Weight (g)</th>
<th>No. fish</th>
<th>Fat %</th>
<th>Weight (g)</th>
<th>Length (mm)</th>
<th>CF</th>
</tr>
</thead>
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<td>F</td>
<td>1</td>
<td>848</td>
<td>52</td>
<td>9.1</td>
<td>16.3±0.3*</td>
<td>140±0.4*</td>
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</tr>
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<td>186</td>
<td>11</td>
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<td>16.9±0.5*</td>
<td>142±0.8*</td>
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<td>27</td>
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<td>15.8±0.2*</td>
<td>138±0.4*</td>
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<td>26</td>
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<td>139±0.6*</td>
<td>0.59±0.01*</td>
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<td>Herring</td>
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<td>190</td>
<td>12</td>
<td>5.8</td>
<td>15.8±0.3*</td>
<td>140±0.6*</td>
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<td>7</td>
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<td>124±0.7*</td>
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<td>17</td>
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<td>15.5±0.4b</td>
<td>137±0.4b</td>
<td>0.61±0.01*</td>
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<td>140</td>
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<td>14.6±0.5a</td>
<td>142±1.7a</td>
<td>0.51±0.02a</td>
</tr>
</tbody>
</table>

thiamine. The analysis results were not corrected for these recoveries.

Statistical analysis

The mean weight, length and condition factor of homogenized fish were compared within each species and sex using one-way analysis of variance (ANOVA) coupled with the post-hoc Tukey’s test (p<0.05 for statistical significance). ANOVA was also used to test the differences between the fat content of homogenates within a species and sex. The relationships between total PCB, coplanar PCB, PCDD and PCDF concentration and fish age or fat content were examined by Pearson’s correlation analysis. Differences between sprat and herring in the OC concentrations calculated on a fresh- and fat-weight basis respectively were tested by ANOVA. The dependence of OC concentrations based on fat-weight and fresh-weight on age or fat content respectively was tested by regression analysis. Differences in the total PCB, coplanar PCB, PCDD and PCDF concentrations between herring and sprat females were also tested by analysis of covariance (ANCOVA), the fat content alone or together with the age of the homogenate being the covariates (Hebert and Keenleyside, 1995). Sprat aged 13 years were excluded from Pearson’s correlation and regression analysis, ANOVA and ANCOVA because the toxicant results for this group clearly deviated from those of other groups. All statistical analysis was performed by using the Statistical Analysis System, version 8 (SAS Institute Inc., 1988).

Results

The mean length and weight of individual male and female herring and male sprat in the homogenates did not vary significantly according to age (p>0.05, ANOVA (Table 1). However, the mean length and weight of two-year-old female sprat, which were out of the range of selected size classes, were significantly lower than older sprat females in other homogenates. There was no significant difference in mean weight between female sprat from the 3–13 years age groups (p>0.05), but the mean length of those aged 8–13 years was significantly greater than that of 3 to 6-year-olds (p<0.05, Table 1). The condition factors (CF) of female sprat from the 3–13 years age groups (p<0.01), although all the fish were within a narrow length class. In herring, however, there were no marked differences in CF between the age groups of 1 to 3 years (Table 1). The fat content of sprat females and males also decreased as age increased (r²=0.922, p<0.0001, r²=0.892, p<0.003, respectively); in herring the differences were not significant (Table 1). The mean fat content of herring females and males (8.4±0.4% and
6.9 ± 0.5%, respectively) were lower than in sprat (11.7 ± 1.3% and 9.7 ± 0.8%, respectively), the difference between males of the two species being significant (p<0.05, ANOVA).

The mean total thiamine concentration in 1 to 3-year-old female and male Baltic herring was 7.3 and 9.9 nmol g⁻¹, respectively (Figure 3). In female sprat aged 2–13 years the mean total thiamine was 6.9 nmol g⁻¹ while in 4 to 13-year-old males it was 6.4 nmol g⁻¹ (Figure 3). Thus, the overall mean thiamine concentration of herring was significantly higher than that of sprat (8.6 vs. 6.7 nmol g⁻¹, p<0.05, ANOVA). The mean proportion of the free thiamine was also significantly higher in herring than in sprat (72.4 ± 3.6% vs. 60.3 ± 1.7%, p<0.01, ANOVA; Figure 4). In herring there were no significant differences in the total thiamine concentration between the sexes or between the three-year-old and one to two-year-old fish of either sex (Figure 3). In female sprat there was no clear relationship between age and total thiamine concentration, although it was lowest in the two-year-olds and somewhat higher in six-year-olds than in other age groups: in male sprat the total thiamine concentration also peaked in the 6 to 8-year-olds (Figure 3).

The mean total PCB, coplanar PCB and PCDF concentrations on a fresh-weight basis were significantly higher in 2 to 10-year-old female sprat than in 1 to 3-year-old female herring (p<0.05, ANOVA): on a fat-weight basis only the concentration of coplanar PCBs was significantly higher (Table 2). When tested by ANCOVA with fat content and age as covariates, the concentration of coplanar PCBs was also significantly higher.
higher in sprat than in herring and the fat content had no significant effect but “age” significantly affected the total PCB and PCDD concentrations (Table 3). The fresh-weight-based total PCB and PCDD concentrations in female sprat increased with age up to the ten-year-olds but then declined in the 13-year-old fish. However, the coplanar PCB and PCDF concentrations were not associated with the age of the fish (Table 3, Figure 5). In 1 to 3-year-old female herring no significant age-related increases were recorded in the total PCB and PCDD concentrations due to the presence of only three age groups. There were no significant correlations between the fresh-weight-based concentrations of coplanar PCBs and PCDFs and the age or fat content of female sprat, which is consistent with the results of ANCOVA (Tables 3 and 4). However, the fat-weight-based concentrations of all four OC groups significantly increased with age (Figure 6). Because fresh-weight-based concentrations had either a negative relationship or none with the fat content (data not shown), apparently due to the masking effect of age (Figure 5), age-related correction

<table>
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<tr>
<th>Source of variation</th>
<th>PCB</th>
<th>CoPCB</th>
<th>PCDD</th>
<th>PCDF</th>
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<td>Age</td>
<td>0.0244</td>
<td>0.6903</td>
<td>0.0025</td>
<td>0.0570</td>
</tr>
<tr>
<td>Fat</td>
<td>0.5989</td>
<td>0.4229</td>
<td>0.0675</td>
<td>0.1787</td>
</tr>
</tbody>
</table>

Figure 5. The concentrations of total polychlorinated biphenyls (PCB), coplanar PCBs, dibenzo-p-dioxins (PCDD), and dibenzofurans (PCDF) on a fresh-weight basis in homogenates prepared from whole female Baltic herring and female and male sprat of different age groups (see Table 1).

Table 3. Significance of differences in organochlorine concentrations between female sprat (age 2–10 years) and herring (1–3 years); results of ANCOVA with the fat content (upper part) or age and fat content (lower part) as a covariate for the polychlorinated biphenyls (PCB), coplanar biphenyls (CoPCB), dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF).

Table 4. The Pearson coefficients of correlation (and their significances) between the fat content, age and organochlorine concentrations of female sprat (2–10 years, N=7) homogenates (Table 1).
factors were calculated. From the linear regressions of fat-weight-based OC concentration in relation to age (Figure 6), concentrations at each age were calculated and divided by the concentration in one-year-old fish, and the fresh-weight-based OC concentrations were then divided by these age correction factors. Regression analysis then revealed a significant linear relationship between the age-independent, fresh-weight-based
and the salmon data were obtained from 14 females from River Simojoki sampled at the ‘‘stripping of eggs’’ in October 1993.

<table>
<thead>
<tr>
<th>Toxicant</th>
<th>Salmon/herring</th>
<th>Salmon/sprat</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB</td>
<td>9.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Coplanar PCB</td>
<td>9.5</td>
<td>3.2</td>
</tr>
<tr>
<td>PCDD</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>PCDF</td>
<td>3.9</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Concentrations of coplanar PCBs, PCDF and PCDD and the fat content of female sprat (Figure 6).

The PCB congener patterns were similar (p>0.05, paired t-test) in herring and sprat when the percentage proportions of congeners were calculated from the means of the results of all the homogenates. They were also similar (p>0.05, paired t-test) to the congener pattern in the muscle of River Simojoki salmon (mean of 14 female fish) that were sampled in 1993 during the spawning period, when the fat content of muscle had decreased due to fasting during exogenous vitellogenesis (Figure 7). The PCDD and PCDF congener patterns were also similar (p>0.05, paired t-test) in herring, sprat and salmon (Figure 8). The most common PCDDs were 2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and OCDD, while for PCDFs they were 2,3,7,8-TeCDF, 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF. However, the concentrations of 2,3,7,8-TeCDF and 2,3,4,7,8-PeCDF were many times higher than those of all other detected PCDD/F congeners (Figure 8).

The total PCB and coplanar PCB concentrations, whether calculated on a fat or fresh weight basis, were approximately 2–3 times higher in sprat than in herring, and those of PCDDs and PCDFs were 1.5–2 times higher (Table 2). In salmon the mean concentrations of the OCs were 2–9 times higher than in herring and 2–3 times higher than in sprat, i.e. all the analysed OCs were highly bioaccumulated in salmon (Table 5, Figure 9).

Discussion

Thiamine

The hepatic thiamine content in Baltic salmon was reported to be higher in males than in females (Amcoff et al., 1999). However, this study found no significant differences in total thiamine concentrations between male and female sprat or herring, or any large difference in abundance between sexes that would be of importance for the supply of thiamine to salmon. Overall, the total thiamine concentration was somewhat higher in herring than in sprat, which contradicts the study of Soivio and Hartikainen (1999) in which total thiamine in sprat was nearly three times higher than in herring. However, these authors found that thiamine degraded faster in sprat homogenate than in herring homogenate when incubated at 6°C (Soivio and Hartikainen, 1999). Furthermore, their initial thiamine concentrations were considerably lower than in the present study (0.34 and 0.88 µg g⁻¹ in herring and sprat, respectively, compared to 3.1 and 2.6 µg g⁻¹ in this study). The differences between the present study and that of Soivio and Hartikainen (1999) might be explained by the analytical methods and the sampling season. Soivio and Hartikainen (1999) collected their samples in late spring when the body stores of herring and sprat might be at their lowest levels after the winter, and in the present study the samples were collected in late autumn to early winter after the feeding period of Baltic herring (Arrhenius and Hansson, 1993). Moreover, the total thiamine method used by Soivio and Hartikainen (1999) has been shown to give up to 40–60% lower results in muscle samples than the method used in the present study (Löflund et al., 1999). Presumably there are differences in thiamine concentrations between seasons in both species that should be investigated more thoroughly.

The thiamine requirements for maximal weight gain of fish have been presented or reviewed by Halver (1989), Morito et al. (1986) and Cowey and Cho (1993). In reviewing these recommendations, Woodward (1994) considered the latest as the most reliable, i.e. 1 mg kg⁻¹ in dry feed, which is approximately 0.3 µg g⁻¹ (0.9 nmol g⁻¹) in fresh feed. The thiamine contents in herring and sprat in the present study were several times higher than the recommended values. Therefore, salmon apparently obtain adequate amounts of dietary thiamine, at least until midwinter, as they also feed to a considerable extent during winter (Karlsson et al., 1999b).

Salmonids in the Great Lakes of North America that have been suffering from Early Mortality Syndrome (EMS), an M74-like syndrome (Bengtsson et al., 1999; Fitzsimons et al., 1999), were also considered to have obtained adequate amounts of thiamine from their prey species (Fitzsimons et al., 1998), which contained similar or lower concentrations of thiamine than Baltic herring and sprat. According to Karlsson et al. (1999b), salmon may even increase their food intake at the beginning of their spawning migration in March, when the thiamine content of herring and sprat is expected to be at its lowest. Soivio and Hartikainen (1999) reported thiamine levels in late spring to be 0.34 and 0.88 µg g⁻¹ in herring and sprat, respectively, which were at or a little above the recommended level even without taking the analytical method into consideration.

If thiaminase in ingested herring and sprat degrades thiamine to a considerable extent, as has been suggested but not shown to be the case in Lake Ontario Atlantic...
Figure 7. The PCB congener pattern as percentage proportions of congener concentrations in Baltic herring and sprat and female salmon. The proportions were calculated from the grand mean of the congener concentrations in all three herring homogenates and those of nine spart homogenates of different age groups (see Table 1), and for salmon from 14 females from River Simojoki sampled (epaxial muscle) at the stripping of eggs in October 1993.
Figure 8. The congener pattern of polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) as percentage proportions of congener concentrations in Baltic herring and sprat and female salmon. The proportions were calculated from the grand mean of the congener concentrations in all three herring homogenates and those of nine sprat homogenates of different age groups (see Table 1), and for salmon from 14 females from River Simojoki sampled (epaxial muscle) at the stripping of eggs in October 1993.
salmon feeding on alewife (Ketola et al., 2000), salmon may not be able to replenish their thiamine stores adequately before the spawning run. However, thiaminase is probably of little significance in relation to the low thiamine contents in Baltic salmon tissues, because salmon have always fed on sprat and herring (Karlsson et al., 1999a), i.e. even before the appearance of the M74 syndrome. On the other hand, dioxin-like toxicants and PCBs might reduce the thiamine content of salmon tissues, as they have previously been observed to do in the rat (Yagi et al., 1979; Pélissier et al., 1992).

Organochlorine levels

Comparison of the organochlorine data with published figures is difficult because others have analysed concentrations in fillets rather than whole-fish homogenates. In four salmonid species from the Great Lakes the total PCB concentrations in muscle were less than 50% of those in whole fish (Niimi and Oliver, 1989), and in another study on coho salmon (Oncorhynchus kisutch) and rainbow trout (O. mykiss) from Lake Michigan, respective PCB concentrations were, on average, 1.70 and 1.47 times higher in whole fish than in fillets (Amrhein et al., 1999). The fat content of whole-fish homogenate is obviously higher than that of fillets as the former includes intraperitoneal fat, for example. The fat contents of herring and sprat homogenates ranged from 5.8–9.1% and 5.4–16.2%, respectively, depending on the age of the fish, while the fat content of fillets of two-year-old Baltic herring sampled in autumn has been reported as 1.5–6.3% (Perttilä et al., 1982; Vuorinen et al., 1998) and the values obtained by Roots and Aps (1993) varied from 1.5 up to a maximum value of 19.6% for herring-muscle samples collected in spring. In the present study the fat contents in herring were lower than in sprat, as was also the case in the autumn samples of Roots and Aps (1993). The fat content of sprat and herring decreased in older fish. However, with herring this may have partly resulted from the sampling of fish with a defined length: faster growing and perhaps more fatty fish may not have been selected. The lower accumulation of coplanar PCBs and PCDD/Fs (on a fresh-weight basis) in 13-year-old sprat is explained by their lower fat content. In these fish the fat-weight-based concentration of toxicants also conformed with the linear fit on age, as did the age-corrected fresh-weight-based concentrations on the fat content.

Although the total PCB concentration in Baltic herring decreased from approximately 4 μg g⁻¹ to ca. 1 μg g⁻¹ (in fat) from 1969 to 1986 (Olsson and Reutergårdh, 1986), the decrease has been negligible since 1986 (Haathi and Perttilä, 1988; Vuorinen et al., 1998). On a fresh-weight basis the total PCB concentration in fillets of 1 to 3-year-old herring in 1981 was 16–34 ng g⁻¹ (Perttilä et al., 1982), i.e. slightly lower than in the present study. However, in addition to the sample type, the analytical method of Perttilä et al. (1982) also differed from the present study. The PCDD/F concentrations as TEQs in fat of two-year-old Baltic herring from 1988–1993 (de Wit et al., 1994) were at a similar level as in the present study. On a fresh-weight basis the TEQs in fillets of herring caught in 1994 in the same areas as in the present study were somewhat lower (Vartiainen et al., 1995b). Thus, assuming that the whole-herring toxicant concentrations also are higher in whole-fish homogenates than in fillets, the concentrations of PCBs and TEQs in herring of the present study should be at about the same level or only slightly lower than those detected by earlier authors. The concentrations of coplanar PCBs in herring females in the present study did not increase with age in age groups 1–3 years and the increase in PCDF concentrations was not as evident as that of total PCBs and PCDDs.

The fresh-weight concentrations of total PCBs and PCDD/Fs in the female sprat fillets (two samples) reported by Vartiainen and Hallikainen (1995) were about one third and one fifth of the respective concentrations in female sprat of the present study. This could be explained by differences in sample type (fillet vs. whole fish), fat content and sampling time, since the samples in the above study had a lower fat content. However, Vartiainen and Hallikainen (1995) recorded a total PCB concentration in 7 to 8-year-old sprat that was twice as high as in 2 to 4-year-olds, similarly to the present study, but the difference in the concentration of PCDD/Fs was smaller. In sprat females (up to ten years

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**Figure 9.** The mean concentrations of total PCBs, coplanar PCBs and polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) and toxic equivalent concentrations (TEQ) of PCDDs and PCDFs on a fresh weight basis in Baltic herring, sprat and salmon. For herring and sprat (whole body homogenates) the means were calculated from the results for different age groups (Table 1 and Figure 5) and for salmon from the epaxial muscle of 14 females (total body length 74–93 cm) from the River Simojoki sampled at the “stripping of eggs” in October 1993.
Organochlorines and thiamine in prey of M74 salmon

Organochlorine bioaccumulation

The PCB congener patterns in herring, sprat and salmon were quite similar. The dominant congener, PCB153, has been reported to be the most common congener in fish samples (Niimi and Oliver, 1989; Roots and Aps, 1993; Atuma et al., 1996). The second “most frequent” congener was PCB138 and the third PCB101 followed, in turn, by PCB110, PCB118 and PCB180 which were also among the most common in four salmonids from the Great Lakes (Niimi and Oliver, 1989). The proportion of the PCB congener 101 seemed to be higher in salmon than in its prey species. Of the PCDD/F congeners, the proportion of 2,3,7,8-TCDD was very low in both herring and sprat and, consequently, also in salmon. On the other hand, the two dibenzofuran congeners, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF, together comprised over 70% of all the PCDD/Fs. However, in herring the proportions of these two congeners were approximately equal but in sprat the relative proportion of the tetrachloro congener was much lower than its pentachloro counterpart. In salmon the proportions of these congeners were close to each other but the proportion of the 2,3,4,7,8-PeCDF was higher, suggesting that sprat may have been more important than herring as prey for salmon. This is very likely to have reflected the increase in abundance of sprat during the 1990s (ICES, 2001).

Concentrations of PCDFs and coplanar PCBs in salmon were earlier shown to have increased coincidentally with the increase in salmon yolk-sac fry mortality, i.e. the outbreak of the M74 syndrome at the beginning of 1990s (Paasivirta et al., 1995; Vuorinen et al., 1997). As a result of the reduced growth rate of sprat and herring, salmon were probably feeding on relatively older specimens in the 1990s than the 1980s and the older fish contained more toxicants (Perttilä et al., 1982; de Wit et al., 1994; Vartiainen et al., 1995b). However, as the mean concentrations of the coplanar PCBs and PCDFs in particular were clearly higher in sprat than in herring, and similarly high in all age groups of sprat, it is possible that salmon have accumulated these compounds largely from sprat. This is supported by the fact that the abundance and biomass of sprat were higher than those of herring during the 1990s (see Figure 1), with all the age groups of sprat appropriate as prey for salmon whilst only 1–3-year-old herring – 60% of the herring biomass in the Baltic proper (ICES, 2001) – could be taken. Moreover, salmon preyed on younger sprat in 1994–1997 than in the early 1960s (Hansson et al., 2001), indicating that the number of young sprat, which in the present study were shown to be more fatty than older ones, was high in the mid-1990s. The growth rate of salmon increased and was higher during the 1990s than the 1980s (Karlsson et al., 1999a).

Conclusions

Thiamine concentrations in both Baltic herring and sprat in autumn–winter samples from the years of high M74 incidence in Baltic salmon have been at a level that would provide an adequate supply for salmon growth. The total PCB concentration increased with age in sprat as has been previously detected in herring, and the present study revealed the same pattern for coplanar PCBs, PCDDs and PCDFs. Therefore, the accumulation of organochlorines in salmon might have been increased by their feeding on relatively older specimens of herring and, more especially, on the whole age-range
of the more slowly growing sprat. Moreover, the concentrations of all measured organochlorines, most clearly those of coplanar PCBs and PCDFs, were related to the fat content of sprat and were therefore also high in young specimens. It is concluded that sprat might have been the principal source of organochlorines, particularly coplanar PCBs and PCDFs, for Baltic salmon since the beginning of the 1990s, when there was an alarming rise in the incidence of the M74 syndrome at the same time as the abundance of sprat in the Baltic Sea increased. The results of this study therefore offer an explanation for the earlier observations of Paasivirta et al. (1995) and Vuorinen et al. (1997) of increased coplanar PCB and PCDF concentrations in River Simojoki salmon coinciding with an outbreak of M74 mortality in their offspring at the beginning of the 1990s.

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