THE EFFECT OF AGE ON THE CONCENTRATION OF POLY-CHLORINATED DIBENZO-P-DIOXINS, DIBENZOFURANS AND DIOXIN-LIKE POLYCHLORINATED BIPHENYLS IN BALTIC HERRING AND SPRAT

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SUMMARY

This paper reports on the concentrations of polychlorinated dibenzo-p-dioxins (CDDs), polychlorinated dibenzofurans (CDFs), and dioxin-like (planar) polychlorinated biphenyls (PPCBs) in 3-8 years old herring (*Clupea harengus membras* L.) and sprat (*Sprattus sprattus balticus* Schn.), caught in 2003. The concentrations are compared with those found in fish in 2002 and in the 1990s. The concentrations in fish of the same age do not differ noticeably between 2002 and 2003. The concentrations (pg/g lipid) of CDD and CDF in herring from 2002 and 2003 are lower than the concentrations in herring landed in the 1990s, partly because of a higher lipid content in the former. Consequently, the loads (pg/fish) are closer to one another in all sets. In contrast, the concentrations as well as the loads of the chlorobiphenyls 105, 118, and 156 are much higher in the 2003 herring than in the 1990s fish. In most cases, the concentrations in fish collected in a given year, increase with its age. This suggests that the age of the fish is more important than the year of capture for the concentrations of CDDs, CDFs, and PPCBs. The determination of long-term trends of persistent chemicals in Baltic fish requires monitoring of age-stratified samples, collected several years apart. The equation C or L = age/(a+b*age), where C and L are concentrations (pg/g lipid) or loads (pg/ fish) and 'a' and 'b' are constants, well describes the data.

KEYWORDS: Persistent organic pollutants, Baltic herring, Baltic sprat, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, dioxin-like polychlorinated biphenyls.

INTRODUCTION

The Baltic Sea is highly contaminated with polychlorinated dibenzo-p-dioxins (CDDs), polychlorinated dibenzofurans (CDFs) and dioxin-like (planar) polychlorinated biphenyls (PPCBs), see for example [1]. The concentrations of these compounds are most often measured [2-5] in the Baltic herring (*Clupea harengus membras* L.) and the Baltic sprat (*Sprattus sprattus balticus* Schn.) These fish are very good species for monitoring purposes, since they can be caught in all parts of the Baltic, their biology is fairly well-known [6], and they are of suitable size for pre-analytical sample treatment [3]. Baltic herring and sprat are also the most important fish species in the Baltic Sea, and are of considerable importance for the Estonian fish-processing industry. Consequently, the presence of toxicants in these fish is of concern from the point of view of human health [7].

A previous study [5] suggested that the age of the fish is a major factor affecting the levels, and, to some extent, also the congener profiles of CDDs and CDFs in herring and sprat. This paper reports the results of a similar study, performed a year later, and compares them with results, obtained by another laboratory, on herring collected in the 1990s [2].

MATERIALS AND METHODS

Samples

Baltic herring and Baltic sprat were obtained by Dr. Mart Simm from Estonian Marine Institute, University of Tartu, between May 2003 and June 2003 from industrial trawlers operating along the Estonian coast [3]. The fish were immediately frozen. Before analysis, their length, weight, gender, and the maturity of gonads were determined. Samples of muscle were submitted for chemical analysis.

Baltic herring (Table 1) were collected in the Central Baltic (sample R19), in the western Gulf of Finland (sample R20), and in the Gulf of Riga (samples R01, R03, R05 and R06). Baltic sprat (Table 2) were collected in the Gulf of Riga. In comparison with the samples collected in 2002 and in the 1990s, the codes of the former are as in the reference [5]. For the latter, the samples in Table 1 of reference [2] have the codes F2-F7, F815 and B815, and, those in Table 3 have the codes FS3, FS9, FL9, BS9, and BL9. The letters 'F' and 'B' refer to the Gulf of Finland and Gulf of Bothnia, respectively, the numbers 2-7 are ages of the fish; the number '815' indicates that the age ranged from 8 to 15 years. 'S3', 'S9', and 'L9' refer to small and large herring, caught in 1993 and in 1999, respectively.

Chemical analyses

The determination of dioxins and polychlorinated biphenyls was carried out by the National Research Centre for Environment and Health, Institute of Ecological Chemistry, 85764 Neuherberg, Germany, under quality control according to EN 17025 (accreditation license No. DAC-P-0141-01-00, valid until November 21, 2006).

Chemicals

All solvents were of trace analysis quality and purchased from LGC Promochem (Wesel, Germany), as were the silica, alumina and Florisil adsorbents. The C_{18}-modified silica (Isolute C18) was purchased from Separtis GmbH (Grenzach-Wyhlen, Germany). All ^{13}C-labeled standards were from Cambridge Isotope Laboratories (Andover, MA, USA) or Wellington Laboratories (Guelph, Ontario, Canada).

Sample processing

Measurement of lipids and CDDs, CDFs, and PPCBs

Each pool of the fish samples was freeze-dried and homogenized. Of each pool, 7.5 g were spiked with ^{13}C-labeled CDD and CDF standards and extracted by pressurized liquid extraction (ASE 200, Dionex GmbH, Idstein, Germany) with n-hexane/acetone 75/2. The lipid concentration in the extract was determined gravimetrically [8].

Cleanup

The fractions A and B from the alumina column (Fig. 1) were used for the measurement of PCBs. The solvents were changed to 0.2 ml acetonitrile. This acetonitrile solution was placed on a SPE cartridge filled with 1 g C_{18}-modified silica (conditioned with 6 ml acetonitrile) and rinsed firstly with two portions of 0.4 ml, and then with 3 ml of acetonitrile through the cartridge. All acetonitrile fractions were combined and reduced to 10 µl by a gentle stream of nitrogen.

Instrumentation

The measurements were performed with a high-resolution mass spectrometer (MS) Finnigan MAT 95S (Thermo Electron GmbH, Bremen, Germany) coupled to an Agilent GC 6890 (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separation was achieved by a splitless injection (cold injection system CIS4, Gerstel GmbH, Mülheim, Germany) of 1 µl on a Restek Rtx-2330 column (length 60 m, ID 0.25 mm, film thickness (ft) 0.1 µm, Restek GmbH, Sulzbach, Germany). The GC oven was programmed as follows: 90 °C initially, held for 1.5 min, increase at a rate of 30 °C/min to 200 °C, followed by an increase of 2 °C/min to 248 °C, then 20 °C/min to 260 °C, and a final hold at 260 °C for 15 min. The MS was operated in the SIM mode, at a resolution of 10000, and the two most intense ions of the molecular ion cluster were monitored for the unlabeled and labeled isomers.

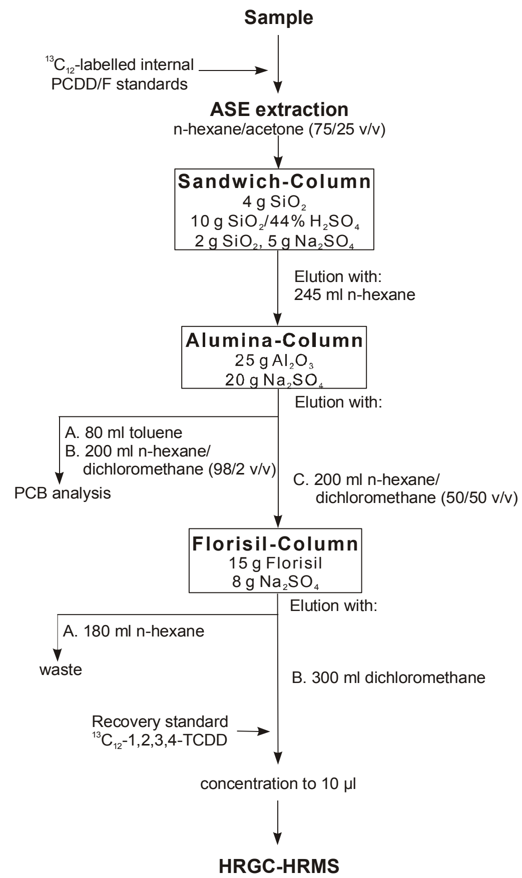

FIGURE 1 - The cleanup procedure.

The PCB measurements were performed with a high-resolution MS Finnigan MAT 95 (Thermo Electron GmbH, Bremen, Germany) coupled to an Agilent GC 5890 Series II (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separation was achieved by a splitless injection (cold injection system CIS3, Gerstel GmbH, Mülheim, Germany) of 1 µl on a J&W DB-XLB column (length 60 m, ID 0.25 mm, ft 0.25 µm, Agilent Technologies, Palo Alto, CA, USA). The GC-oven was programmed as follows: 90 °C initially, held for 1.5 min, increase at a rate of 15 °C/min to 200 °C, followed by an increase of 3 °C/min to 235 °C, held for 10 min, then 8 °C/min to 320 °C, and a final hold at 320 °C for 15 min. The MS was operated in the SIM mode at a resolution of 8000, and the two most intense ions of the molecular ion cluster were monitored for the unlabeled and labeled isomers.

Data evaluation

The relationship between the age (years) of the fish and the concentration (pg/g lipid) or load (pg/fish) of CDDs, CDFs, and PPCBs was evaluated by fitting the equation (A) to the data:

$$C \text{ or } L = \text{age}/(a + b*\text{age}) \qquad \text{(A)}$$

The constants 'a' and 'b' were determined by linear least squares regression of the transformed equation '1/y = a/age + b', where y = C or L. In a few cases, the fit was improved when some visually determined 'outliers' were not considered, or by a preliminary smoothing of the data with a quadratic polynomial.

The relationship between the age of the fish and their contaminant profiles (concentrations scaled to a sum of 100) was examined by Principal Component Analysis (PCA) of the samples as well as of 'theoretical' samples of different ages, predicted by the equation (A). The PCA was performed by PLS_Toolbox 2 [9], running in Matlab 5.0. Graphs were prepared in Excel (Microsoft). Labeler 2 [10] labeled the points in the graphs. Not detectable concentrations of CDDs, CDFs, and PPCBs were replaced by 0.5 * LOD values. The concentrations were then converted to profiles, centered (mean = 0), (std = 1), and subjected to the PCA. Individual CCDs and CDFs were identified by their 'shorthand' code (see Table 3). The IUPAC numbers were used for the chlorobiphenyls.

RESULTS AND DISCUSSION

Age and weight

Biological characteristics of the samples as well CDD, CDF and PPCB concentrations are in Tables 1 and 2. With the exception of three samples, the relationship (B)

$$\text{weight [g]} = 4.76*\text{age[years]} + 7.78 \qquad \text{(B)}$$

describes the increase of weight with age of the herring in all three data sets (this paper, [2], and [5], altogether 31 samples). The exceptions are the sample R01, in which the predicted median weight is 46 g, and the samples B815 and F815 [2] with reported median weights of 160 g and 150 g, respectively, as opposed to the median weight of 63 g, predicted by (B).

TABLE 1 - Biological characteristics and CDD, CDF, and PPCB concentrations (pg/g lipid) in herring.

Sample	R01	R03	R05	R06	R19	R20
Dry matter (%)	22.6	24.1	21.5	23.1	24.8	22.4
Lipids (%)	2.8	3.4	2.7	2.6	4.6	3.9
Number in pool	4	7	15	14	17	18
Length, cm	23.25	20.5	15.7	16	16.1	15.25
	(22.0-27.2)	(20.0-21.8)	(14.8-16.5)	(14.5-18.4)	(14.6-18.0)	(12.9-17.7)
Weight, g	92.95	62.7	23.7	24.8	24.2	20.85
	(78.8-160.7)	(57.1-70.8)	(20.4-28,7)	(21.2-40.1)	(16.0-39.8)	(12.7-30.4)
Age, years	8	8	3	3.5	5	4
	(8-9)	(5-10)	(3-6)	(3-5)	(2-6)	(2-8)
Gender M=1,F=2	2	1	2	1.5	1	2
Maturity	4	4	4	5	5	5
	(4-5)	(4-5)	(4-5)	(4-6)	(5-6)	(4-6)
2378D	5.7	6.8	6.1	7.6	4.5	4.9
12378D	19.8	27.6	9.4	9.4	9.1	23.2
123478D	3.3	4.4	3.6	1.4	2.3	3.2
123678D	10.7	12.9	6	7.2	7.9	12
123789D	0.88	0.71	0.44	0.83	0.4	0.77
1234678D	3.6	3.7	2.6	3.4	1.5	4
OCDD	4.6	3.7	4.1	3.5	7	7.3
2378F	86.9	101	88.2	85.9	62.3	71.4
12378F	24.5	32.2	15.5	17.2	13.1	18.6
23478F	119	144	95	89.3	74.3	128
123478F	5.3	10	4.2	4.1	3.3	5.7
123678F	7.9	11.8	4.4	5.8	4.3	9.3
123789F	1.1	0.55	0.96	0.98	0.68	0.51
234678F	8.9	10.6	3.4	5.3	5.2	10.2
1234678F	3.1	3.2	1.7	1.8	1.5	5.2
1234789F	1.1	0.99	0.65	0.15	0.71	0.09
OCDF	4	2.7	2.4	3.2	2	4.1
CDD/F TEQ	88.9	109.5	70.2	69.0	55.5	92.8
77	1488	2086	1400	1526	1383	1990
81	15.7	36.7	9	28.5	27.4	43.7
126	729	739	352	397	340	466
169	540	544	327	386	245	371
105	85843	70133	36487	39419	30018	44117
114	3790	3473	1488	1730	1312	2102
118	211288	197692	86206	97806	71540	108735
123	22878	20088	8360	9521	7298	10737
156	29905	25139	10519	12381	9254	15409
157	7119	5895	2533	2997	2226	3505
167	15256	13175	6062	6708	5272	7352
189	1938	1822	845	966	707	1227
PPCB TEQ	131.20	125.91	59.13	67.11	54.00	77.58
Total TEQ	220.11	235.40	129.38	136.12	109.53	170.41

TABLE 2 - Biological characteristics and CDD, CDF, and PPCB concentrations (pg/g lipid) in sprat.

Sample	L21	L22	L23	L24	L25
Dry matter (%)	27.3	27	29.9	29.3	28.4
Lipids (%)	9.7	9.8	11.2	10.4	9.4
Number in pool	28	21	36	33	36
Length, cm	12	12.6	11.4	11.9	11.7
	(10.8-12.9)	(11.4-13.7)	(11.0-12.7)	(11.2-12.7)	(11.2-12.6)
Weight, g	10.15	11.5	8.35	8.8	8.35
	(7.0-13.2)	(8.9-15.4)	(6.4-10.2)	(7.8-10.9)	(6.2-13.0)
Age, years	4.5	6	3	4	4
	(2-11)	(3-10)	(2-8)	(2-9)	(2-9)
Gender M=1,F=2	1	2	1	1	1
Maturity	4	4	4	4	4
	(3.5-4)	(4-4)	(4-4)	(4-4)	(4-4)
2378D	2.3	4.3	1.6	2.5	2.8
12378D	5.1	9.7	4.4	5.9	5
123478D	0.48	0.45	1.1	0.79	0.15
123678D	5.6	10.7	3.2	5.7	5.9
123789D	0.19	0.73	0.29	0.45	0.35
1234678D	1.1	1.3	2	1.1	1.8
OCDD	1.9	2.1	5	4.2	3.1
2378F	44.8	55.2	42	46.8	47.9
12378F	6.6	5.8	6	8.7	8.7
23478F	40.9	39.5	34.8	41.8	42.7
123478F	2.2	2.5	2	1.8	1.4
123678F	2.9	3.7	2.8	3.2	3.3
123789F	0.025	0.31	0.34	0.025	0.47
234678F	3.4	6	4	4.6	4.8
1234678F	6.3	12.2	2.8	3.1	2.8
1234789F	0.26	0.54	0.05	0.36	0.5
OCDF	0.84	1.6	2.2	0.96	1.4
CDD/F TEQ	31.67	37.29	27.12	33.17	33.56
77	1542	1608	1247	1412	1412
81	15.7	17.8	14.7	16.6	18.1
126	222	219	182	242	251
169	86.4	86.6	67	89.9	101
105	20107	19167	13237	18713	18504
114	939	871	652	859	867
118	50831	47161	34335	46341	47779
123	4694	4258	3266	4557	4542
156	6061	5975	4221	5673	5859
157	1448	1403	985	1413	1428
167	3128	3134	2397	3119	3235
189	497	476	352	445	490
PPCB TEQ	35.09	34.19	27.07	36.25	37.49
Total TEQ	66.75	71.48	54.19	69.42	71.06

The concentrations of CDDs, CDFs, and PPCBs in this and previous studies

The concentrations in pg/g lipid of all CDDs and CDFs in herring from the 1990s [2] are higher than those in the herring collected in 2002 [5] and this work (Table 3). A lower concentration of lipids in the herring analyzed by Kiviranta et al. [2] causes, at least in part, this difference. The difference is somewhat diminished by expressing the concentrations as 'loads' (pg/fish). Surprisingly, the concentrations of the chlorobiphenyls 105, 118, and 156 are much higher in the fish studied in this work, than in those reported in [2]. Unfortunately, a more detailed evaluation of complete chlorobiphenyl profiles cannot be carried out, because concentrations of nonplanar chlorobiphenyls are not available for herring in this work.

Effect of age on the concentration of CDDs, CDFs, and PPCBs

The CDD, CDF, and PPCB concentrations increased with the age of the fish in this as well as previous studies [2, 5]. Tables 4 and 5 present the constants 'a' and 'b' of the equation (A). This equation is formally the same as the Langmuir adsorption isotherm. It is worth-noting that, according to (A), the concentration of the contaminant will approach '1/b' in infinitely old fish when $b>0$. The initial rise in the concentration is proportional to '1/a'. When the constant 'b' is negative, the concentration increase with age shows no signs of slowing down. When 'b' is very small, the concentration increase approximates a straight line through the origin. The curve (A) has a discontinuity at $x = -a/b$, which may present fitting problems when $b<0$. The discontinuity may be avoided by removing 'outliers', or by smoothing the concentration vs age relationship by a quadratic polynomial.

TABLE 3 - A comparison of the median CDD, CDF, and PCB concentrations (pg/g lipid and pg/ fish) in herring, reported by Kiviranta et al. [2], Roots et al. [5], and in this work.

		Reference [2]	Reference [5]	This work	Reference [2]	Reference [5]	This work
	Lipids (%)	1.9	9.5	3.1			
	Length, cm	17.4	14.75	16.05			
	Weight, g	30	22.35	24.5			
	Age, years	5.5	3	4.5			
Code			pg/g lipid			pg/fish	
66d	2378D	15	1.65	5.9	8.55	3.50	4.48
76d	12378D	64	2.65	14.6	36.5	5.63	11.1
F6d	123478D	4.1	0.13	3.25	2.34	0.28	2.47
77d	123678D	66	2.3	9.3	37.6	4.88	7.06
7Ed	123789D	5.8	0.0975	0.74	3.31	0.21	0.56
F7d	1234678D	11	1.2	3.5	6.27	2.55	2.66
FFd	OCDD	34	5.6	4.35	19.4	11.89	3.30
66f	2378F	76	22.3	86.4	43.3	47.24	65.6
76f	12378F	52	2.45	17.9	29.6	5.20	13.6
E6f	23478F	390	22.3	107	222	47.24	81.3
F6f	123478F	20	0.835	4.75	11.4	1.77	3.61
77f	123678F	23	0.885	6.85	13.11	1.88	5.20
7Ef	123789F		0.0275	0.82	0	0.06	0.62
E7f	234678F	24	1.45	7.1	13.68	3.08	5.39
F7f	1234678F	11	1.20	2.45	6.27	2.55	1.86
FEf	1234789F		0.0725	0.68	0	0.15	0.52
FFf	OCDF	2.9	2.4	2.95	1.653	5.10	2.24
	CDD/F TEQ	271	18.1	79.6	155	38.5	60.4
	77	1200		1507	684		1145
	81			28.0			21.2
	126	1040		431.5	593		328
	169	480		378.5	274		287
	105	88		41768	50.16		31723
	114			1916			1455
	118	130		103271	74.1		78434
	123			10129			7693
	156	18		13895	10.26		10553
	157			3251			2469
	167			7030			5339
	189			1097			833
	PPCB TEQ	109		72.3	62.1		54.9
	Total TEQ	380		153	217		116

TABLE 4 - The effect of age on CDD , CDF, and PPCB concentrations (pg/g lipid) in herring , $C_{at\ age}$ = age/(a + b*age), where 'a' and 'b' are constants. Mean values are shown where trends were not apparent.

	Reference [2]		Reference [5]		This work	
	a	b	a	b	a	b
2378D	1.42E+00	-1.42E-01	1.35E+00	2.65E-01	5.93E+00	mean
12378D	4.79E-01	-5.70E-02	1.27E+00	-6.84E-02	2.73E-01	1.54E-02
123478D	1.11E+01	-1.34E+00	1.50E+01	-2.09E+00	7.96E-01	2.03E-01
123678D	4.05E-01	-4.71E-02	1.07E+00	1.78E-01	3.25E-01	4.31E-02
123789D	4.39E+00	-4.42E-01	2.23E+01	1.58E+00	2.34E+00	1.12E+00
1234678D	9.44E-01	-5.31E-03	1.28E+00	mean	1.47E-01	3.25E-01
OCDD	1.54E+01	mean	5.63E+00	mean	4.24E-02	2.07E-01
2378F	6.09E-02	9.22E-03	5.98E-02	2.26E-02	3.48E-03	1.16E-02
12378F	6.04E-01	-7.50E-02	1.33E+00	-7.12E-02	1.22E-01	2.74E-02
23478F	1.01E-01	-1.31E-02	1.32E-01	-1.24E-03	1.22E-02	7.04E-03
123478F	1.44E+00	-1.58E-01	3.41E+00	9.56E-02	4.10E-01	1.18E-01
123678F	1.52E+00	-1.97E-01	2.54E+00	1.87E-01	4.31E-01	6.38E-02
123789F			3.08E-02	mean	7.97E-01	mean
234678F	9.20E-01	-1.07E-01	1.07E+00	3.35E-01	6.94E-01	1.07E-02
1234678F	2.17E-01	1.34E-01	1.24E+00	mean	9.78E-01	2.25E-01
1234789F			9.00E-02	mean	2.81E+00	6.66E-01
OCDF	1.48E+00	mean	3.41E+00	mean	1.77E-01	3.10E-01
CDD/F TEQ	1.20E-01	-1.46E-02	1.52E-01	4.29E-03	1.66E-02	9.34E-03
77	5.24E-03	3.64E-04			4.34E-04	5.29E-04
81					1.72E-01	1.17E-02
126	1.75E-02	-1.56E-03			6.47E-03	7.73E-04
169	1.63E-02	-8.16E-04			4.60E-03	1.67E-03
105	2.65E-01	-2.67E-02			6.43E-05	8.31E-06
114					1.68E-03	1.38E-04
118	1.07E-01	-8.78E-03			2.91E-05	2.74E-06
123					3.21E-04	2.08E-05
156	2.10E+00	-2.57E-01			2.46E-04	1.63E-05
157					1.02E-03	7.23E-05
167					4.24E-04	3.71E-05
189					2.68E-03	3.29E-04
PPCB TEQ	1.71E-01	-1.56E-02			3.96E-02	4.42E-03
Total TEQ	7.11E-02	-8.06E-03			1.43E-02	3.33E-03

TABLE 5 - The effect of age on the concentrations (pg/g lipid) of CDDs, CDFs, and PPCBs in sprat, $C_{at\ age}$ = age/(a + b*age), where 'a' and 'b' are constants. Mean values are shown where trends were not apparent. For CDDs and CDFs, the constants were derived from the combined data from [5] and this work.

	Concentrations		Loads	
	a	b	a	b
2378D	2.82E+00	-1.50E-01	9.32E-01	2.57E-01
12378D	1.43E+00	-1.70E-01	5.13E-01	5.06E-02
123478D	5.89E+00	1.58E+00	5.82E-01	mean
123678D	1.15E+00	-8.41E-02	3.70E-01	9.47E-02
123789D	4.30E-01	mean	1.72E+01	-1.17E+00
1234678D	2.26E+00	mean	2.52E+00	mean
OCDD	4.26E+00	mean	8.35E+00	mean
2378F	1.37E-01	-7.12E-03	4.02E-02	1.42E-02
12378F	1.06E+00	-1.13E-01	3.51E-01	5.58E-02
23478F	1.85E-01	-1.58E-02	6.15E-02	1.21E-02
123478F	3.68E+00	-3.96E-01	1.13E+00	2.39E-01
123678F	2.09E+00	-1.74E-01	6.54E-01	1.64E-01
123789F	1.20E-01	mean	1.17E-01	mean
234678F	1.67E+00	-1.68E-01	5.58E-01	9.63E-02
1234678F	2.51E+00	-3.22E-01	4.03E-01	2.23E-01
1234789F	1.90E-01	mean	9.33E+00	4.55E+00
OCDF	2.47E+00	9.29E-02	1.84E+00	mean
CDD/F TEQ	2.14E-01	-1.64E-02	6.88E-02	1.66E-02
77	1.13E-03	4.22E-04	1.93E-03	2.77E-04
81	6.23E-02	4.55E-02	1.32E-01	3.26E-02
126	5.39E-03	3.22E-03	1.09E-02	2.14E-03
169	1.99E-02	6.96E-03	3.43E-02	4.15E-03
105	1.48E-04	2.08E-05	2.18E-04	7.65E-06
114	2.50E-03	6.02E-04	3.98E-03	3.23E-04
118	5.09E-05	1.01E-05	7.86E-05	4.83E-06
123	4.59E-04	1.27E-04	7.57E-04	6.95E-05
156	4.34E-04	7.70E-05	6.56E-04	3.54E-05
157	1.88E-03	3.06E-04	2.82E-03	1.27E-04
167	5.97E-04	1.91E-04	1.00E-03	1.14E-04
189	4.70E-03	1.10E-03	7.43E-03	5.85E-04
PPCB TEQ	4.67E-02	1.84E-02	8.31E-02	1.14E-02

TABLE 6 - The effect of age on the loads (pg/fish) of CDDs, CDFs, and PPCBs in herring , $C_{at\ age}$ = age/ (a + b*age), where 'a' and 'b' are constants. Mean values are shown where trends were not apparent.

	Reference [2]		Reference [5]		This work	
	a	b	a	b	a	b
2378D	1.42E+00	-1.42E-01	1.28E+00	-4.35E-02	9.09E-01	-2.53E-02
12378D	4.79E-01	-5.70E-02	9.42E-01	-1.35E-01	3.51E-01	-3.63E-02
123478D	1.11E+01	-1.34E+00	1.42E+01	-1.22E+00	2.87E+00	-2.08E-01
123678D	4.05E-01	-4.71E-02	8.37E-01	-3.07E-02	1.03E+00	-9.98E-02
123789D	4.39E+00	-4.42E-01	1.59E+01	-6.40E-01	1.17E+01	-8.36E-01
1234678D	9.44E-01	-5.31E-03	3.16E+00	mean	2.01E+00	-7.64E-02
OCDD	1.54E+01	mean	4.78E-02	1.08E-01	1.51E+00	-1.09E-01
2378F	6.09E-02	9.22E-03	6.51E-02	-6.46E-04	8.46E-02	-1.17E-03
12378F	6.04E-01	-7.50E-02	1.03E+00	-1.41E-01	4.15E-01	-3.19E-02
23478F	1.01E-01	-1.31E-02	1.09E-01	-1.46E-02	6.70E-02	-4.39E-03
123478F	1.44E+00	-1.58E-01	2.84E+00	-2.98E-01	1.56E+00	-1.16E-01
123678F	1.52E+00	-1.97E-01	1.95E+00	-1.04E-01	1.35E+00	-1.22E-01
123789F			1.66E+01	9.99E+00	6.07E+00	2.39E-02
234678F	9.20E-01	-1.07E-01	1.02E+00	-3.17E-03	1.74E+00	-1.93E-01
1234678F	2.17E-01	1.34E-01	2.87E+00	mean	3.58E+00	-3.06E-01
1234789F			1.88E-01	mean	9.58E+00	-7.54E-01
OCDF	1.48E+00	mean	8.36E+00	mean	2.20E+00	-1.25E-01
CDD/F TEQ	1.20E-01	-1.46E-02	1.28E-01	-1.49E-02	8.95E-02	-5.88E-03
77	5.24E-03	3.64E-04			4.21E-03	-2.77E-04
81					5.90E-01	-6.66E-02
126	1.75E-02	-1.56E-03			1.86E-02	-1.62E-03
169	6.06E-02	-6.97E-03			1.88E-02	-1.23E-03
105	2.65E-01	-2.67E-02			1.82E-04	-1.48E-05
114					4.38E-03	-3.87E-04
118	1.07E-01	-8.78E-03			7.66E-05	-6.50E-06
123					7.98E-04	-7.09E-05
156	2.10E+00	-2.57E-01			6.19E-04	-5.57E-05
157					2.57E-03	-2.29E-04
167					1.11E-03	-9.53E-05
189					7.60E-03	-6.40E-04
PPCB TEQ	1.71E-01	-1.56E-02			1.14E-01	-9.94E-03
Total TEQ	7.11E-02	-8.06E-03			5.08E-02	-3.96E-03

As can be seen from Tables 4 and 5, the values of the constants are quite close for almost all CDDs and CDFs in herring and sprat from this and the 2002 study [5]. This confirms the suggestion [5] that the age of the fish is more important than the year of collection in two successive years. On the other hand, the values of ‘a’ in herring from the 1990s [2] are generally higher, and values of b are negative, since the concentrations increase, without a tendency to level off, with age of the fish.

In contrast to herring, the concentrations of almost all CDDs and CDFs increase with the age of sprat (Table 5), without a tendency to level off. This may mean that sprat are more efficient than herring in the accumulation of these compounds, but less efficient than herring in the accumulation of PPCBs. The loads (pg/fish) of all the compounds in sprat show signs of ‘leveling off’ with increasing age (b>0, Table 5). This is generally not the case with herring (Table 6).

A few examples of the concentration vs age of herring relationships from this work and from [2] are in Fig 2, those of loads vs age in Fig. 3, and those of TEQ concentrations and loads in Fig. 4. As can be seen from Figs. 3 and 4, the loads have changed slightly from the 1990s to 2003. The concentration vs age of sprat relationships for combined 2002 [5] and 2003 data are shown in Fig. 5 There is a good agreement between the 2002 and 2003 data.

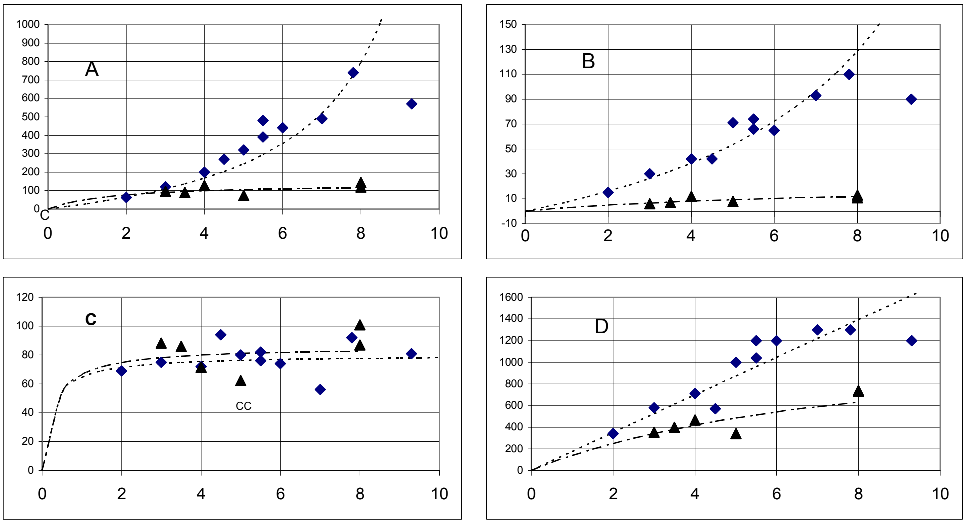

FIGURE 2 – Concentration vs. age of herring. A - 23478F, B - 123678D, C - 2378F, D - 126. Diamonds –data from [2], triangles – present work. Lines – fitted relationships C = age/(a+b*age).

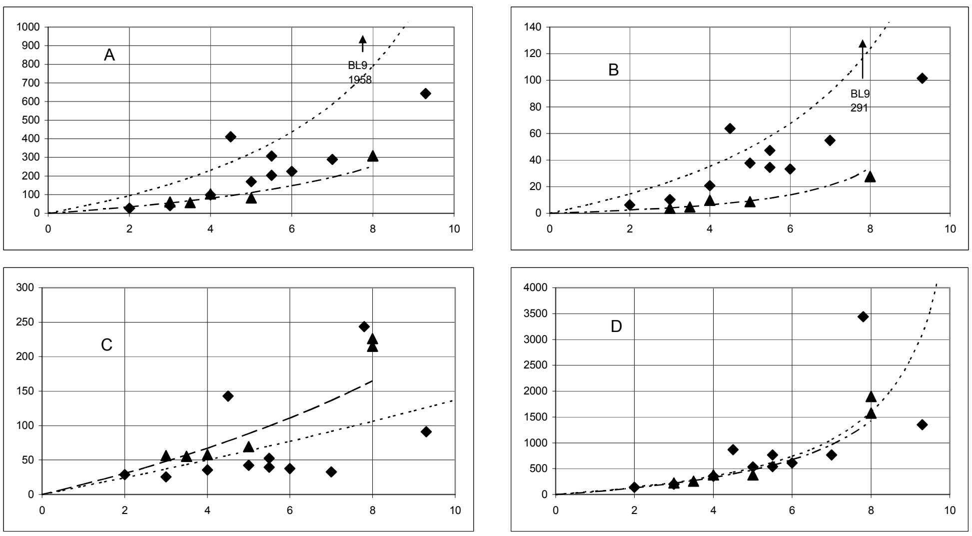

FIGURE 3 – Loads (pg/fish) vs. age of herring. A - 23478F, B - 123678D, C - 2378F, D - 126. Diamonds –data from [2], triangles – present work. Lines – fitted curves L = age/(a+b*age).

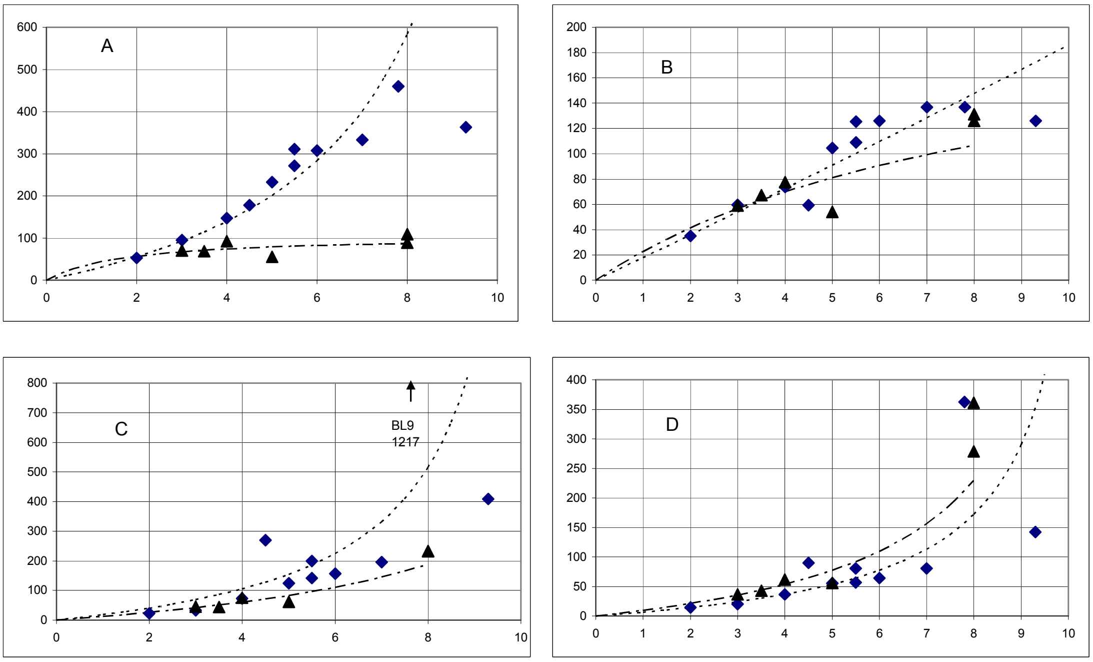

FIGURE 4 - Concentrations (pg/g lipid), A, B, and loads (pg/fish), C, D, of CDD/F TEQ (A, C) and PPCB TEQ (B, D) vs age of herring. Diamonds - data from [2], triangles - this work

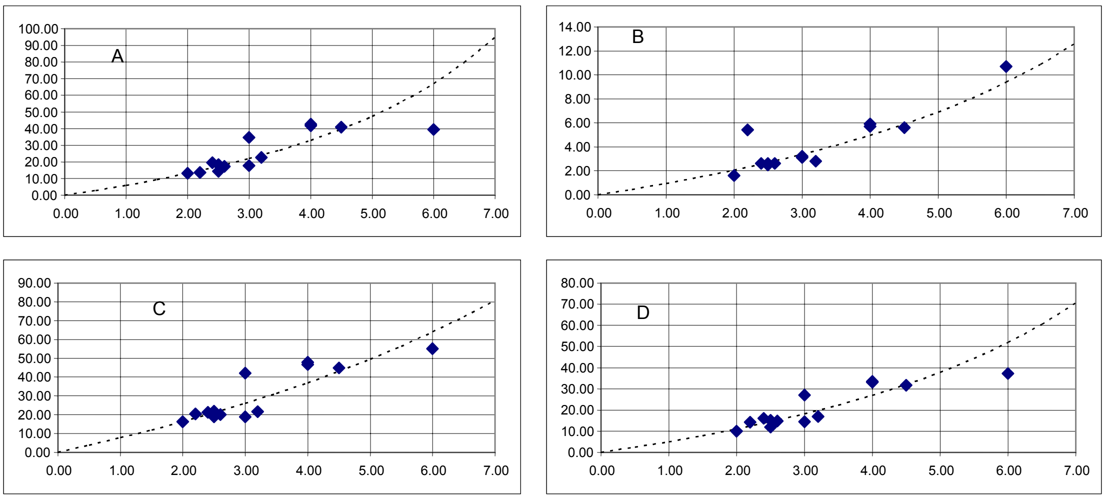

FIGURE 5 - Concentration (pg/g lipid) of CDDFs vs the age of sprat. Combined data from [4] and this work. A - 23478F, B - 123678F, C- 2378F, D- TEQ

The extrapolation from edible parts to the whole fish is justifiable for herring and sprat since both species have lipids quite evenly distributed throughout the body. The increase of the load with the age (and size) of the fish may also play a role in the food chain: a larger prey delivers a larger amount of the contaminant to the predator, than an equal weight of smaller prey.

Chemical structure of CDDs, CDFs, and PPCBs and the constants 'a' and 'b' (equation A)

2378-Tetrachloro- and 23478-pentachloro-dibenzofuran (66f and E6f) have the lowest values of the constants in all examined data sets (Table 7). Compounds exhibiting the highest values of the constants are less consistent. It would be interesting to examine additional data to see what patterns will emerge.

The constants 'a' and 'b' showed no discernible relation to the structure of the PPCBs in the set [2]. On the other hand, there is a linear log-log relationship between 'a' and 'b' for PPCBs in both, herring and sprat (Fig. 6). Three (81, 126, and 169) of the four di-ortho unsubstituted chlorobiphenyls have the highest 'a' and 'b' values in herring as well as in sprat. The fourth one (77) is preceded by the two mono-ortho unsubstituted heptachlorobiphenyls (157 and 159), and by the only mono-ortho unsubstituted pentachlorobiphenyl with a single chlorine in one of the benzene rings (114). More data have to be examined before making any conclusions.

TABLE 7 - Minimal and maximal values of the constants 'a' and 'b' (equation (A)) for CDDs and CDFs. R and L are herring (Table 1) and sprat (Table 2), respectively, other data are from references [2] and [5]. For CDD and CDF codes see Table 3.

References	66f		E6f		7Ed				FEf	
	a	b	a	b	a	b			a	b
	0.00348	0.0116	0.0122	0.0070	2.34	1.12			2.81	0.666
[5]	66f		E6f		7Ed		F6d			
	a	b	a	b	a	b	a	b		
	0.0598	0.0226	0.132	-.0012	22.3	1.58	15.0	-2.09		
[2]	66f		E6f		7Ed		F6d			
	a	b	a	b	a	b	a	b		
	0.061	0.00922	0.101	-.0131	4.39	-.442	11.1	-1.34		
L	66f		E6f				F6d		F6f	
	a	b	a	b			a	b	a	b
	0.137	-.00712	0.185	-.0158			5.89	1.58	3.68	-.396

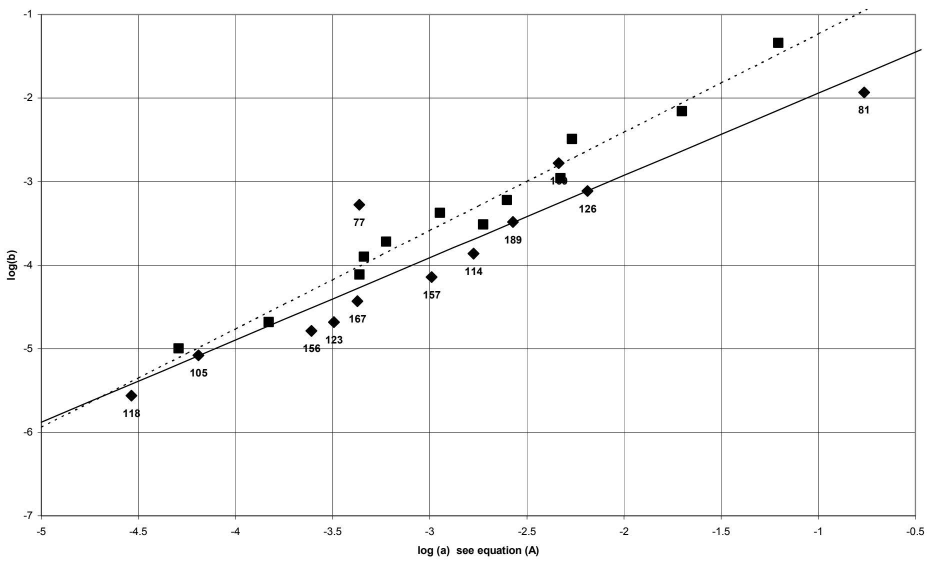

FIGURE 6 - The relationship of the constants 'a' and 'b' (equation (A)) of PPCBs in herring (diamonds and IUPAC codes) and sprat (Δ). The order of PPCBs is the same in both series.

Age and the profile of CDD, CDF and PPCB

Projections of the CDD and CDF (combined as CDDF) profiles of herring ([2] and present work) on the plane of the principal components 1 and 2 (pc-1 & pc-2), and of the profiles calculated from (A) and marked by age in years (numbers 1-10), are on the left-hand side of Fig. 7. It can be seen that the age is a major factor and accounts for 48% and 58% of the original variance. There is a good agreement between actual [2] and predicted profiles. The F7 profile is closer to that predicted for 6-years-old herring, and the FS9 and BS9 profiles are similar to that of 8-years-old herring. For the samples from this work, the profile of the 8-years-old R01 herring is more similar to that of younger fish, and the profile of R20 (age 4 years) to that of much older fish.

The right-hand side of Fig. 7 shows the effect of the original variables (individual CDDs and CDFs, for the codes see Table 3) on the principal components. Those with positive loadings in the pc-1 (ev-1>0) are more prevalent in the younger fish.

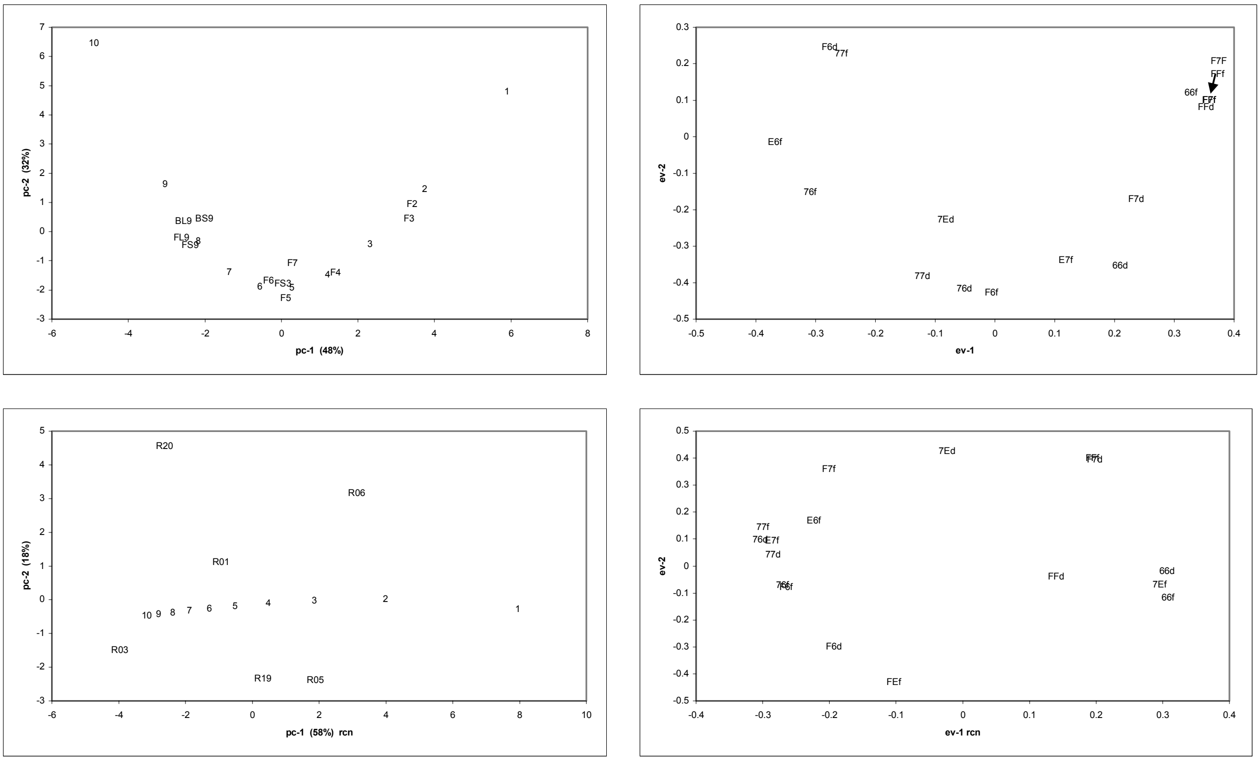

FIGURE 7 - Changes of CDDF concentration profiles with age of herring. On the left are projections on the plane of pc-1&pc-2, on the right are loadings of CDDFs on the principal components. In the upper and lower row are data from [2], and this work, respectively. For sample codes see Table 1 and 'Samples' in text, for CDDF codes see Table 3. Numbers alone mark profiles predicted from fitted curves.

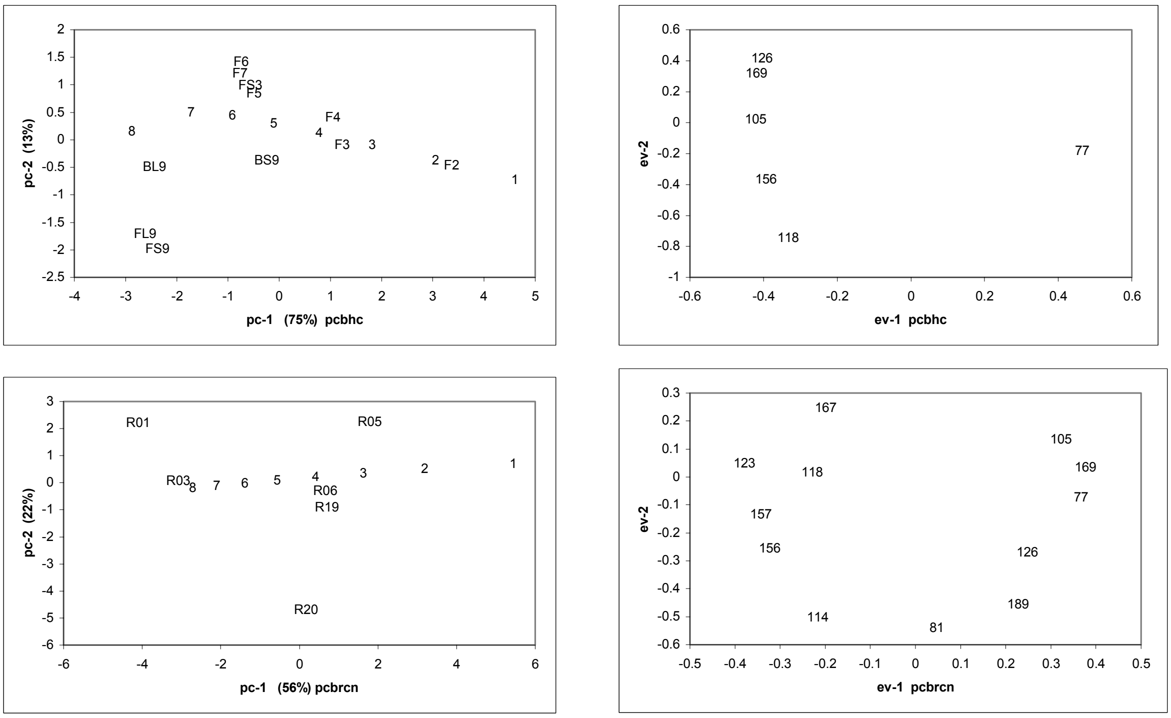

FIGURE 8 - Changes of PPCB profiles with the age of herring. Arrangements and sample codes are the same as in Fig. 7, and IUPAC codes are used for PPCBs.

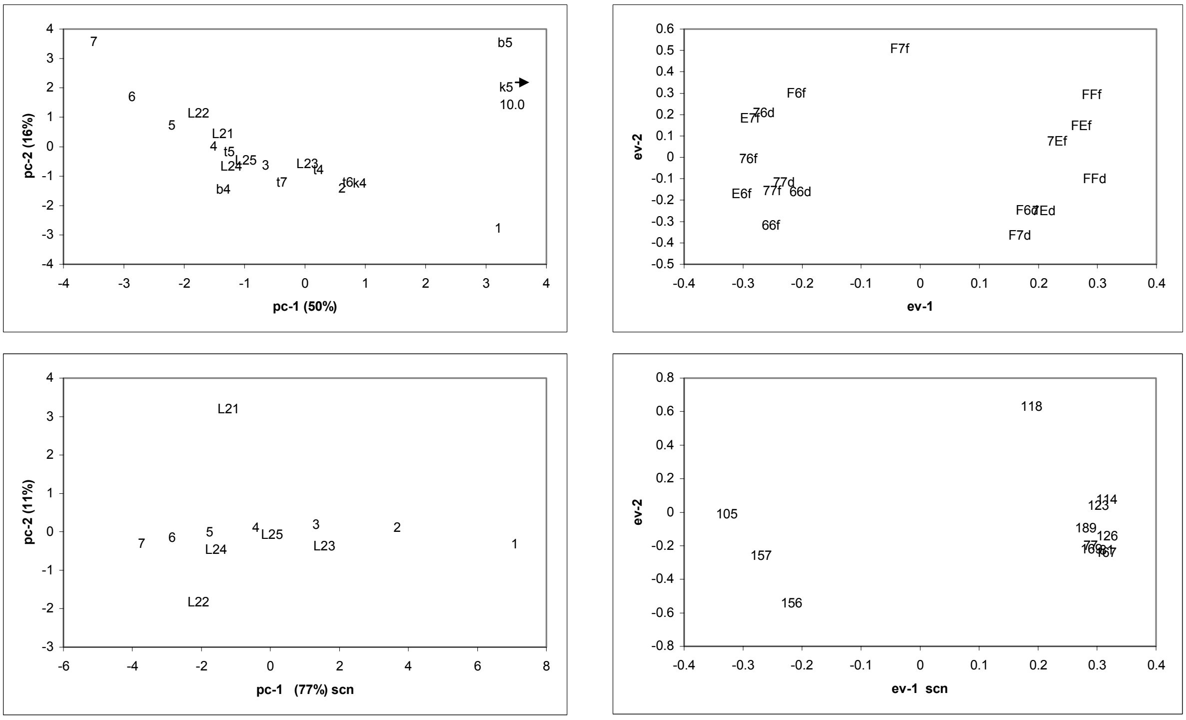

FIGURE 9 - Changes of combined (this work and [5]) profiles of CDDFs (upper plots) and profiles of PPCB (this work) with the age of sprat.

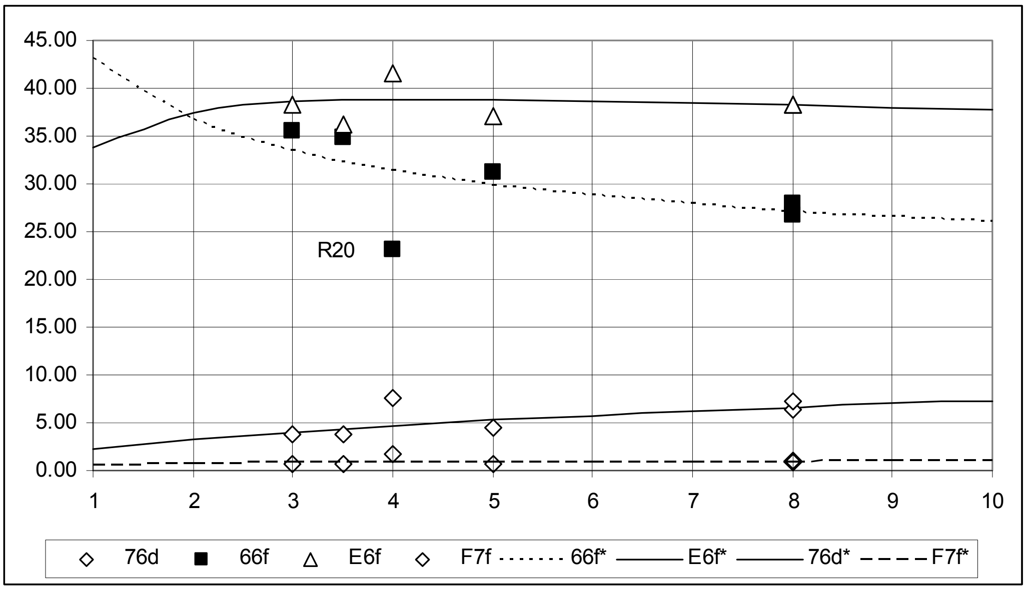

FIGURE 10 - Actual concentrations and that calculated (*) from (A) of 12378-pentachlorodibenzo-p-dioxin (76d), 2378-tetrachlorodibenzofuran (66f), 23478-pentachlorodibenzofuran (E6f), and 1234678-heptachlorodibenzofuran (F7f), plotted against the age of herring.

The changes of the PPCB profile with age of herring are shown in Fig. 8, and those of CDDFs and PPCBs of sprat in Fig. 9.

The deviations from the age trend (pc-2) are relatively minor, and illustrated in Fig. 10 for the major components of the herring profile from this work.

TABLE 8 - A summary of positive and negative effects of individual CDDFs and PPCBs on the principal component pc-1. For codes of CDDs &CDFs see Table 3, IUPAC number are used for PPCBs. Compounds with positive effect decrease, those with a negative effect increase with age in the profile (concentrations scaled to a sum of 100).

CDDF									
Positive effect on pc-1									
herring this work		7Ef				66f	66d		
herring [2]	FFd		FFf		F7f	66f			
herring [5]	FFd	7Ef		FEf	F7f			F7d	
sprat this work	FFd	7Ef	FFf	FEf					
Negative effect on pc-1									
herring this work			76d	E7f	77f	77d			
herring [2]	E6f	76f			77f		F6d		
herring [5]	E6f	76f	76d						
sprat this work	E6f	76f	76d	E7f					
PPCB									
Positive effect on pc-1									
herring this work	77	169	105						
herring [2]	77								
sprat this work	77	169		81	114	123	126	167	189
Negative effect on pc-1									
herring this work	156		157	123					
herring [2]	156	105			126	169			
sprat this work	156	105	157						

The effect of the original variables (CDDFs and PPCBs) on the principal components is shown in the ev-1 vs ev-2 plots in Figs. 7-9. Some patterns appear to be present (Table 8), and additional work in this direction is desirable.

CONCLUSION

The concentrations of CDDs and CDFs in the Baltic herring have not changed very much between the 1990s and 2003, and those in the Baltic sprat between 2002 and 2003. The concentrations of most of the PPCBs in herring, reported in this paper, are much higher than those found by Kiviranta et al. [2] in the 1990s. Unfortunately, that data on other chlorobiphenyls are not available. It should be a standard practice to report the concentrations of all the detectable chlorobiphenyls. An important factor for the concentration of CDDs, CDFs, and PPCBs is the age of the fish. Monitoring programs should be using fish samples of well-defined age. The equation (A), formally the same as the Langmuir adsorption isotherm, was very useful in the evaluation of the effect of age on the concentration of the contaminants. Its constants may provide some insights into the relationships between chemical structure and the accumulation of organochlorine compounds. Both equation (A) and the Principal Component Analysis are good tools for the evaluation of the data, and it would be desirable to use them also as a quality control before the data leave the laboratory. The analyses are expensive, but funds should be available for duplicate analyses of extracts as well as samples, to have an idea about the uncertainties in the data. Small numbers of samples in this work did not warrant an examination of regional differences in the concentration of CDDs, CDFs, and PPCBs in the fish.

ACKNOWLEDGEMENT

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