POPs, PFAS and metals in ringed seals *(Pusa hispida botnica)* from the Baltic 1978-2015

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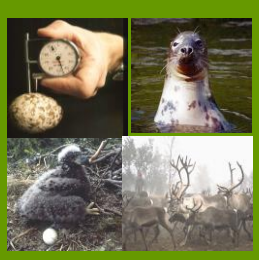

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1974–2015

Sammanfattning
Twenty two pooled samples from 69 juvenile ringed seals collected between 1974 and 2015 were analyzed for PCB (22 congeners), pesticides, PFAS, metals and selenium. Most seals were bycaught in fishing gear.

All CB congeners were above the detection limits. CB-138 and -153 accounted for ca 50% of ∑PCB. CB-180 and -101 stood for ca 10 % each. ∑PCB decreased over the study period with approximately -4.6% annually and since 2000 the mean concentrations (*i.e.* in pooled samples) were below 9 mg/kg in these juvenile seals, which is the lowest suggested threshold for onset of physiological effects in experimental marine mammal studies.

sDDT decreased with a rate of ca -8.9% yearly. The proportion of p,p'DDE increased over time as p,p'DDT decreased. Chlordanes and nonachlor also decreased over time (-4.6%). Trans-nonachlor was the most predominating compound. Finally, mirex decreased over time at a rate of approximately -2.3% annually.

Nine PBDE congeners were analyzed in blubber. The predominating BDE was BDE-47 (ca 70%) followed by BDE-99 and BDE-100. Most BDE congeners peaked during the 1990s.

Eleven perfluorinated substances were analysed. PFHxA and PFHpA were below detection limits in all but three samples not included in the statistical analyses. All but FOSA showed increasing trends over the study period, with increasing rates of +5-9% annually. However, if only looking at the last 15 years the concentrations appear stable (*i.e.* not increasing or decreasing). FOSA showed decreasing rates over the full period as well as the last 15 years. PFOS was observed in the highest concentrations (range 9.4-400 ng/g ww) and was the predominant PFAS. PFNA was the predominant PFCA followed by PFDA and PFUnDA.

Arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), (Cr), mercury (Hg), manganese (Mn), nickel (Ni), selenium (Se) and zinc (Zn) were analysed in seal liver. Cr was below detection limit in all samples but one, which was at detection limit. Ni was below detection limit in all samples. Only Co showed slight decreasing concentrations, the concentrations of the other metals were stable over time. However, concentrations of Pb were very low, and after 2000 below detection limit (<0.03 mg/kg ww), indicating decreasing concentrations. The relationship Hg vs Se on a molar basis showed no trend over time. Generally, the ratio was slightly below 1, but in five samples it did exceeded 1 (mean ratio was 0.9).

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1. Aim

Little is known of contaminant concentrations in Baltic ringed seals. The population is slowly recovering but not as fast as would be expected. Data on PCB and other contaminants in ringed seals is needed so the environmental status of ringed seal within HELCOM can be evaluated. The aim of the study was therefore to analyze time trends of a number of contaminants in juvenile ringed seals.

2. Introduction

The ringed seal is a circumpolar species, found in Arctic waters. It was landlocked in the Baltic after the last ice age, in Lake Saima in Finland and Lake Ladoga in Russia, resulting in several subspecies (*Pusa hispida botnica, P.h. saimensis and P.h. ladogensis*). In the Baltic, ringed seals (*P. h. botnica)* are mostly found in the northern parts (Bottenhavet, Bottenviken, Norra Kvarken), but also in the Bay of Finland and the Bay of Riga.

The population of ringed seals in the Baltic was estimated to be around 190 000-220 000 individuals in 1900 and decreased to approximately a few thousand in the late 1970s [1]. This decrease was more dramatic than what was seen for the grey seal (*Halichoerus grypus*) [1]. Both species decreased in numbers initially due hunting but after the 1970s due to problems with reproduction. A disease complex among ringed and grey seals was identified, including sterility among females and elevated concentrations of organochlorines were believed to be the cause for this sterility [2]. Now the population of ringed seals is recovering. Surveys on ringed seals starting in 1988 indicate a +4.5% yearly increase. This increasing rate is only approximately half of what could be expected from a healthy population and what has been seen for grey seal. Although not common, occluded uteri are still found among ringed seals, but not in grey seals [3]. The ringed seal populations in The Bay of Finland and in the Bay of Riga are decreasing of unknown reasons.

As an aquatic top predator seals are subjected to elevated concentrations of contaminants from their diet. Bans of PCBs and DDT have led to improved reproductive success for several apex species in Sweden, resulting in increases in population sizes [4]. However, little is known about contaminant burden in Baltic ringed seals. Previously concentrations of polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) and dioxin like CBs (dl-PCB) in juvenile ringed seals from the Baltic over a time limit of over three decades have been reported [5].

Although PCB has been banned for half a century and has decreased in concentrations in biota it is still of concern for marine mammal health [6, 7]. Recently it has been suggested to use PCB as a health indicator for marine mammals within OSPARCOM and HELCOM.

3. Material and Methods

3.1 Ringed seals

The aim of this study was to study general temporal trends of different contaminants in ringed seals and therefore we aimed to base the study on pooled samples of five individuals in each pool instead of individual samples. Due to few ringed seals in the Environmental Specimen Bank, this was not

always possible. In five cases individual seals were analyzed, and otherwise 2-5 seals were included in each sample (Table 1). Altogether 69 seals were included in this study, pooled into 22 samples, collected between 1974 and 2015. The seals were necropsied at the Swedish Museum of Natural History and samples from them were stored in the Environmental Specimen Bank (ESB) at the museum, and subsampled from there for this study. The majority of the seals drowned in fishing gear (54 individuals) but in recent years some were also shot during a domestic hunt (8 individuals 2007-2015). Seven individuals were found dead with no known cause of death (in 1974-1978). Both sexes were included (43 females and 26 males). Twenty-seven seals were collected from Bay of Bothnia, 33 from Bothian Bay and nine from Baltic Proper. The animals were all juvenile seals (0-3 years old), 20-49 kg (1 unknown), and 84.5-119 cm long (11 unknown).

Table 1. Biological information of the seals included in this study. Twenty two samples were analyzed. A yearling is <1 year old.

Sample number	Year	Month	Cause of death	Agegroup	Sex	Weight (kg)	Total length (cm)	Area
1	1974	6	Bycaught	Yearling	F	23		Bothnian Bay
	1974	6			M	28		Bothnian Sea
2	1975	8			F	26		Bothnian Bay
3	1977	7			M	20		Bothnian Bay
	1977	10			M	26		Bothnian Bay
	1977	5			M	45		Bothnian Sea
4	1978	8			F	48	119	Bothnian Bay
	1978	11			F	25		Baltic Proper
5	1980	6	Bycaught		F	35	106	Bothnian Bay
6	1982	9	Bycaught	Yearling	F	20	85	Bothnian Bay
7	1985	9	Bycaught	Yearling	F	36	93	Bothnian Sea
	1985	10	Bycaught	Yearling	F	45	108	Bothnian Sea
	1985	10	Bycaught	Yearling	M	38	95	Bothnian Sea
	1985	12	Bycaught	Yearling	F	43	104	Bothnian Bay
8	1986	8	Bycaught	Yearling	M	34	96	Baltic Proper
	1986	8	Bycaught	Yearling	M	30	95	Baltic Proper
	1986	9	Bycaught	Yearling	F	31	94	Bothnian Bay
	1986	10	Bycaught	Yearling	F	46	101	Bothnian Sea
	1986	10	Bycaught	Yearling	F	38	96	Bothnian Sea
9	1987	8	Bycaught	Yearling	F	32		Bothnian Bay
	1987	9	Bycaught	Yearling	M	43		Bothnian Sea
	1987	9	Bycaught	Yearling	F	48		Baltic Proper
	1987	11	Bycaught	Yearling	M	48		Bothnian Sea
10	1988	5	Bycaught	Yearling	M	39	111	Baltic Proper
	1988	6	Bycaught	Yearling	F	34	108	Bothnian Sea
	1988	9	Bycaught	Yearling	F	38	99	Baltic Proper
	1988	10	Bycaught		F	46	112	Baltic Proper
11	1991	9	Bycaught		M	41	108	Baltic Proper
12	1995	9	Bycaught		F	34	100	Baltic Proper
	1995	10	Bycaught		M	29	92	Bothnian Bay
13	1996	6	Bycaught		F	31	99	Bothnian Bay
14	2001	5	Bycaught		F	35	110	Bothnian Bay
	2001	5	Bycaught		F	33	109	Bothnian Bay
	2001	5	Bycaught		M	28	103	Bothnian Bay
	2001	5	Bycaught		F	33	119	Bothnian Bay
	2001	7	Bycaught	Yearling	M	26	95	Bothnian Sea
15	2004	7	Bycaught		F		96	Bothnian Bay
	2004	6	Bycaught		F	22	93	Bothnian Sea
16	2007	11	Shot	Yearling	F	34	95	Bothnian Bay
	2007	8	Bycaught	Yearling	M	35	101	Bothnian Bay
	2007	7	Bycaught		F	31	109	Bothnian Sea
	2007	10	Bycaught	1 year	M	40	106	Bothnian Sea
	2007	10	Bycaught		F	28	113	Bothnian Sea
17	2008	6	Shot	1 year	M	23	109	Bothnian Bay
	2008	10	Shot	Yearling	F	29	92	Bothnian Bay
	2008	6	Shot	1 year	F	31	102	Bothnian Bay
	2008	5	Shot	1 year	M	36	112	Bothnian Bay
	2008	6	Bycaught	Yearling	M	20	91	Bothnian Sea
18	2010	10	Bycaught	Yearling	F	39	103	Bothnian Sea
	2010	10	Bycaught	Yearling	F	34	98	Bothnian Bay
19	2012	5	Bycaught	1 year	F	30	105	Bothnian Bay
	2012	9	Bycaught	Yearling	F	23	92	Bothnian Bay
	2012	7	Bycaught	Yearling	F	26	99	Bothnian Sea
	2012	11	Shot	1 year	M	46	117	Bothnian Bay
	2012	9	Bycaught	Yearling	M	31	102	Bothnian Bay
20	2012	8	Bycaught	Yearling	F	25	96	Bothnian Bay
	2012	8	Bycaught	Yearling	F	26	88	Bothnian Bay
	2012	8	Bycaught	Yearling	F	20	91	Bothnian Sea
	2012	8	Bycaught	Yearling	M	24	92	Bothnian Sea
	2012	9	Bycaught	Yearling	M	25	94	Bothnian Sea
21	2014	10	Bycaught	Yearling	M	28	95	Bothnian Sea
	2014	8	Bycaught	Yearling	F	24	91	Bothnian Sea
	2014	9	Bycaught	Yearling	F	25	96	Bothnian Sea
	2014	11	Bycaught	Yearling	F	44	108	Bothnian Sea
22	2015	4	Shot	3 years	F	48	105	Bothnian Sea
	2015	9	Bycaught	Yearling	M	42	107	Bothnian Sea
	2015	10	Bycaught	2 years	F	49	116	Bothnian Bay
	2015	10	Bycaught	1 year	M	43	111	Bothnian Bay
	2015	6	Shot	2 years	F	37	115	Bothnian Bay

3.2 Chemical analyses

3.2.1. PCBs, pesticides and PBDE

The analyses of PCBs, pesticides and PBDEs were carried out at the Department of Chemistry, Örebro University. All samples were weighed and freeze-dried for at least 36 hours to remove excessive water from storage. This was done to prevent clean-up column to clog during extraction and clean-up. The samples were then homogenized with dry (baked in oven at 300˚ for 24 hours) sodium sulphate (Sigma-Aldrich (Steinheim, Germany). A lipid extraction, representing 0.2-0.5 g lipids for each sample, was carried out by using 1:1 hexane-dichloromethane. Isotopically labelled standards were added prior to this step to monitor the recovery of compounds of interest for the entire sample preparation procedure. Initial lipid removal was performed by adding the eluted lipids with a small volume of n-hexane to the flask with 20% H_2SO_4 acidic silica. Thorough shaking was performed and additional small amounts of acidic silica was added until the recently added silica did not show any discoloring from the lipids in the sample. This was followed by sample clean-up using multilayer silica columns composed of (bottom to top) KOH silica, neutral activated silica, 40% H_2SO_4 silica gel, 20% H_2SO_4 silica gel, neutral activated silica gel and Na_2SO_4 as described previously (https://doi.org/10.1016/j.talanta.2016.10.060). Columns were rinsed with 50 ml of n-hexane prior to elution of compounds of interest using 100 ml n-hexane. Extracts were evaporated to 25 µL in tetradecane including ^{13}C-labeled recovery standards. Instrumental analysis was performed using gas chromatography coupled to mass spectrometry. PCBs and OCPs were analysed using GC electron ionization (EI) low resolution single quadrupole mass spectrometry. Due to extremely high concentrations of mainly p,p-DDE and p,p-DDT samples were re-injected using GC high resolution mass spectrometry with a 200:1 split injection. PBDEs were analysed using GC atmospheric pressure ionization coupled to tandem mass spectrometry. Isotope dilution was used for quantification. Blank and reference samples were analysed concomitantly with each batch of samples extracted. Method detection limits were calculated from co-extracted blank samples using a signal-to-noise ratio of 3.

3.2.2 PFAS

The analyses of PFAS were carried out at ACES, Stockholm University. Approximately 1 gram of liver was used.

Samples were extracted and analyzed using a previously reported method [8]. The liver sample was fortified with a mixture of isotopically labelled internal standards and acetonitrile before homogenizing with an Ultra-Turrax hand blender and then sonicated for 15 min. The supernatant was removed and the extraction procedure was repeated once, after which all extracts were combined, reduced in volume, and then cleaned-up using dispersive carbon. After spiking with recovery standard, a portion of the extract was subjected to instrumental analysis using a Waters ultra-performance liquid chromatograph coupled to a Waters triple quadrupole mass spectrometer (UPLC-MS/MS) operated under negative ionization, multiple reaction monitoring mode. Quantification was carried out by isotope dilution using a 5-point calibration curve. In each batch of samples, blanks and replicate control fish tissue were analyzed to assess lab contamination and ongoing accuracy and precision of the method. Method detection limits were calculated based on the concentration producing a signal-to-noise ratio of 3.

3.2.3 Metals and Selenium

The analyses of metals and selenium were carried out at ALS Analytica using ICP-SFMS. Approximately five gram liver was used for the analyses.

3.3 Statistical analyses

Concentrations of contaminants were logged before statistical analysis, in order to approach normal distribution. Then simple regression analysis was performed on each compound.

Values below the MDL (non-detects) were replaced by MDL/√2 prior to statistical treatment in less than 10% of the samples [9].

The temporal trend of contaminants was initially scrutinized. For PCBs, OC compounds and metals, the trend appeared to follow well by an exponential decrease and were analyzed by log-linear regression. As both individual and pooled samples were included, the samples were weighted by the number of individuals in the regression analyses. For BDEs and PFASs (except PFOA), the trend obviously did not follow an exponential decrease. A locally weighted scatterplot smoothing (loess) was applied to describe the temporal trends. The loess smoothing was similarly weighted by the number of individuals.

In this study, $p<0.05$ was used as a limit for statistical significance. In the diagrams, statistically significant regressions are shown as a red regression lines. Also, it is indicated how many individuals is included in each analysis.

5. Results

The results from this study must be treated with caution since the sample size is small. However, it seems like the trends approximately follow many other trends seen in biota. The data are presented in wet weight (ww). The blubber had a lipid content of 88-100%, mean 94%.

5.1 PCBs

Twenty one PCB congeners were analyzed: CB-28, -52, -74, -66/70, -101, -110, -118, -105, -153, -138, -167, -156, -157, -187, -180, -170, -189, -194, -206 and -209.

All congeners were above the detective limits. The predominant congeners were CB-138 and -153 which accounted for ca 50% of sPCB. CB-180 and -101 stood for ca 10 % each (Figure 1).

sPCB decreased over the study period with approximately -4.6% annually ($p<0.001$, Figure 1) and since the millennium the mean concentrations (*i.e.* in pooled samples) were found below 9 mg/g ww, which is the lowest suggested threshold for onset of physiological effects in experimental marine mammal studies (for a summary see Jepsen et al [7]). The concentrations in adult ringed seals from the Baltic has not been studied.

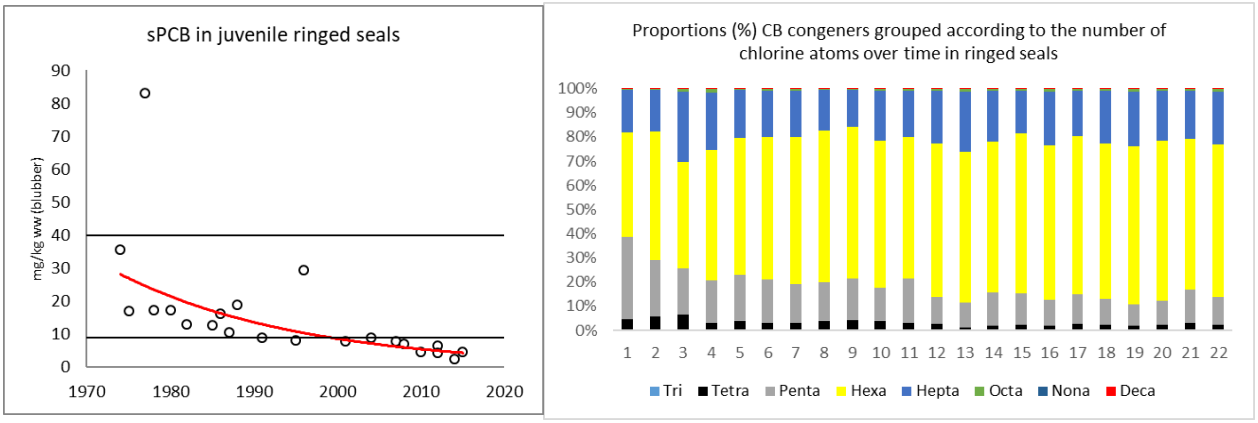

Figure 1. Left: concentrations of sPCB ww (21 congeners, mg/kg ww) in blubber from juvenile ringed seals during 1974-2015 (n=22 pooled samples from 69 seals). The red log-linear regression line was statistically significant (p<0.001). The lower line is the equivalent sPCBs concentrations threshold (9.0 mg/kg lw) for onset of physiological effects in experimental marine mammal studies and the upper line is the equivalent sPCB concentrations threshold for the highest PCB toxicity threshold for marine mammals based on marked reproductive impairment in ringed seals in the Baltic Sea [7]. Right: Proportions of CB congeners grouped according to the number of chlorine atoms from 1974-2015.

5.2 Chlorinated pesticides

Trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor, o,p-DDE, p,p-DDE, o,p-DDD, p,p'-DDD, o,p-DDT and mirex were analysed in the 22 blubber samples. All but DDT are presented in ng/g w.w. and DDTs as mg/kg. One extreme value was seen for each compound and it was a pooled sample of three individuals from 1977.

sDDT decreased with a rate of ca -8.9% yearly (p<0.001, Figure 2). The proportion of p,p'DDE increased over time as p,p'DDT decreased. Other DDT congeners were insignificant. Chlordanes and nonachlor also decreased over time (-4.6%, p<0.001, Figure 3). Trans-nonachlor was the most predominating compound (Figure 4). Finally, mirex decreased over time at a rate of approximately -2.3% annually (p<0.01).

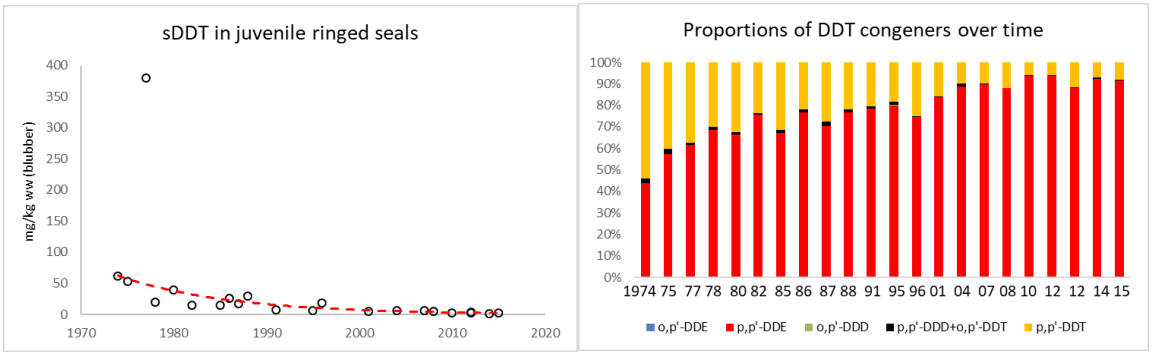

Figure 2. Left: sDDT decreased in juvenile ringed seals over time (mg/kg ww blubber). Right: The proportion of p,p'DDE increased over time as p,p'DDT decreased.

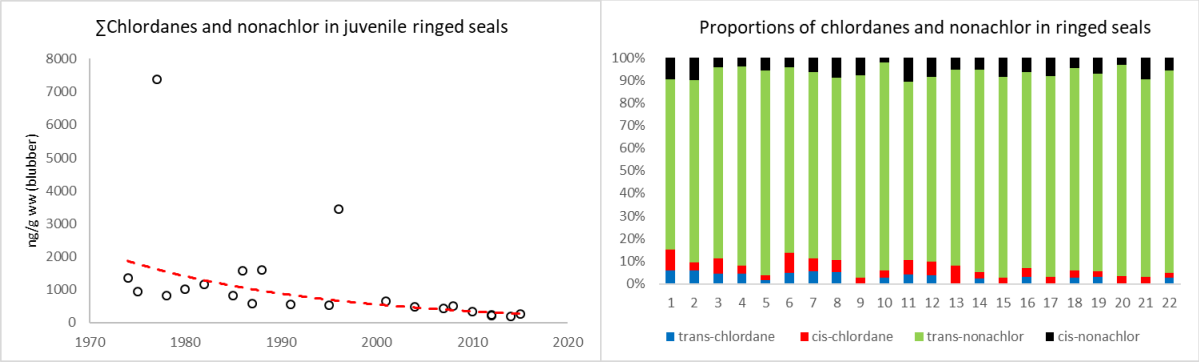

Figure 3. Left: ∑Chlordanes and nonachlor in juvenile ringed seals over time (ng/g ww blubber). Right: Proportions of Chlordanes and nonachlor. The majority consists of trans-nonachlor.

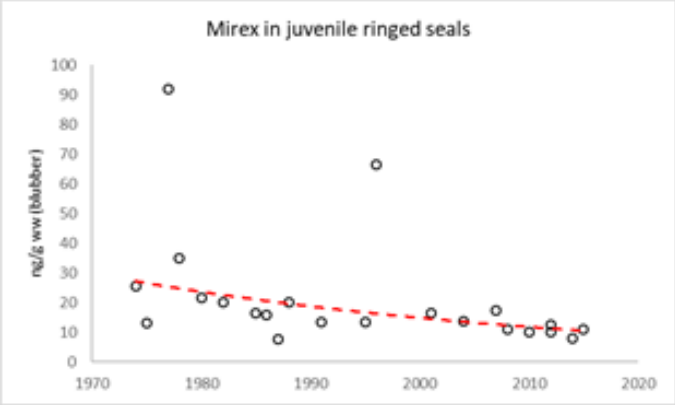

Figure 4. Concentrations of mirex (ng/g ww. blubber) in juvenile ringed seals have decreased over the study period.

5.3 Brominated flame retardants

Nine BDE congeners were analyzed in the juvenile ringed seals: BDE-28, -47, -66, -85, -100, -99, -154, -153 and -183 (Table 2). The concentrations of BDE-83 were below LOD in all 22 samples (<0.6 pg/g ww) and BDE-183 were below LOD (<11 pg/g ww) in 16 samples, therefore they were not included in sPBDE nor in the statistical analyses. BDE-66 was below LOD (0.37 pg/g ww) in only three samples and therefore could be included in the analyses.

The predominating BDE was BDE-47 (ca 70%) followed by BDE-100 and BDE-99 (Figure 5). Most BDE congeners peaked during the 1990s (see for example BDE-47 in Figure 5).

Table 2. Min, max and median concentrations of fat % and BDE concentrations (pg/g ww).

	Fat%	BDE#28	BDE#47	BDE#66	BDE#100	BDE#99	BDE#154	BDE#153	BDE#85	BDE#183	sPBDE (exl 183)
min	88	150	12709	0	1511	2119	39	137		<11	16675
max	101	1793	395441	408	46356	31102	3865	9422	<0,60	108	486749
median	94	674	87287	156	11541	7829	1554	1776		46	113930

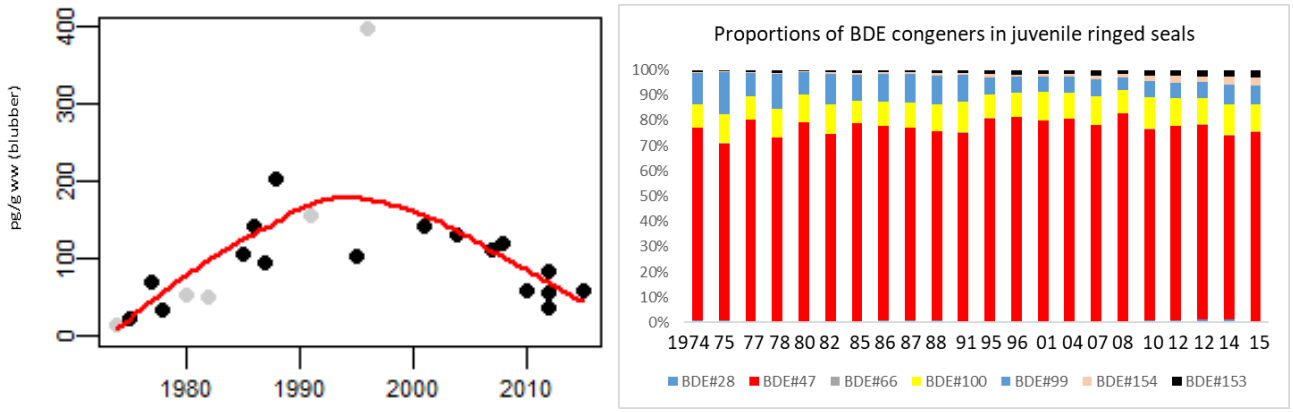

Figure 5. Concentrations of BDE-47 over time (pg/g ww in blubber) peaked in mid 1990s, as most PBDE congeners. Grey circles represent individual seals whereas black circles represent pooled samples (left). BDE-47 constituted approximately 70% of sPBDE in ringed seals and the proportions of BDE congeners were fairly stable over time (right).

5.4 Perfluorinated substances

Eleven perfluorinated substances were analysed in ringed seal liver: PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFPeDA, PFHsS, PFOS and the precursor FOSA.

PFHxA and PFHpA was below detection limits (<0.25 and 0.035 ng/g ww, respectively) in all but three samples and hence not included in the statistical analyses.

All but PFOSA showed increasing trends over the study period, with increasing rates of +5-9% annually. However, if only looking at the last 15 years the concentrations appeared stable (i.e. neither increasing nor decreasing). FOSA showed decreasing rates over the entire time period as well as the last 15 years (Table 3). PFOS was observed in the highest concentrations (range 9.4-400 ng/g ww) and was the predominant PFAS (Figure 6). PFNA was the predominating PFCAs followed by PFDA and PFUnDA (Figure 7).

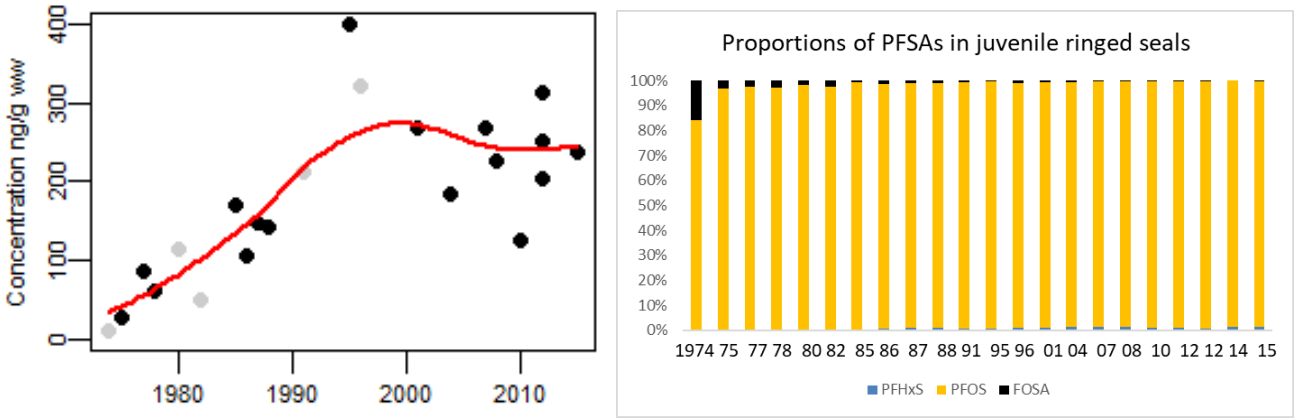

Figure 6. Left: concentrations of PFOS in juvenile ringed seals 1974-2015 over time (ng/g ww, liver). Grey circles are individual seals and black dots represent pooled samples. Right: Proportions of PFSA in juvenile ringed seals over the same time period.

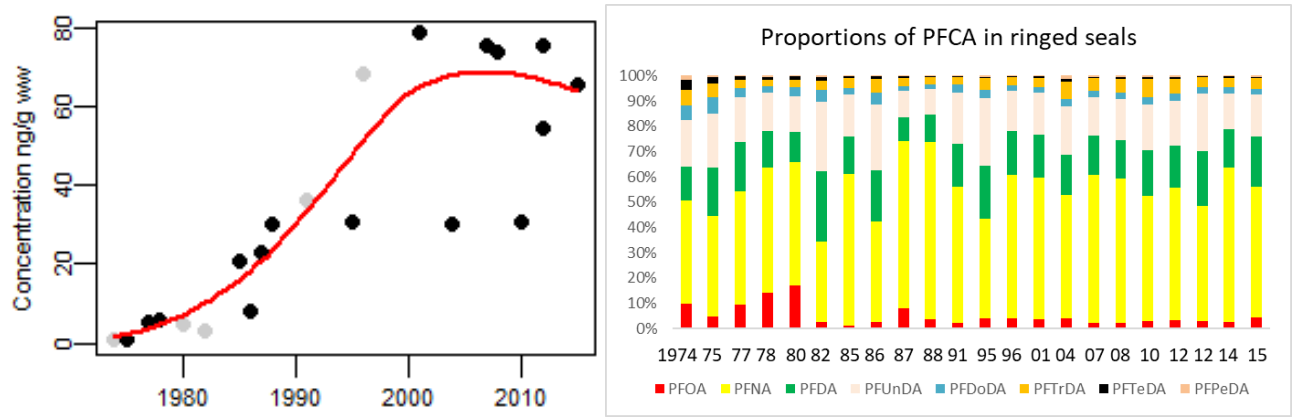

Figure 7. Left: concentrations of PFNA in juvenile ringed seals 1974-2015 over time (ng/g ww, liver). Grey circles are individual seals and black dots represent pooled samples. Right: Proportions of PFCA in juvenile ringed seals over the same time period.

Table 3. Annual changes in PFAS concentrations for the full period (1974-2015) as well as for the last fifteen years (right).

		1974-2015			2001-2015		
		n	% yearly change	p-value	n	% yearly change	p-value
Carboxylates	PFOA	22	5,5	p<0,001	9	ns	p<0,68
	PFNA	22	8,5	p<0,001	9	ns	p<0,71
	PFDA	22	8,3	p<0,001	9	ns	p<0,49
	PFUnDA	22	7,9	p<0,001	9	ns	p<0,66
	PFDoDA	22	6,6	p<0,001	9	ns	p<0,45
	PFTrDA	22	8,8	p<0,001	9	ns	p<0,78
	PFTeDA	22	4,8	p<0,001	9	ns	p<0,31
	PFPeDA	22	8,5	p<0,001	9	ns	p<0,53
Sulfonates	PFHxS	22	8,4	p<0,001	9	ns	p<0,95
	PFOS	22	4,6	p<0,001	9	ns	p<0,90
	FOSA	22	-3,1	p<0,001	9	-9,6	p<0,04

5.5. Metals and selenium

Arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), chromium (Cr), mercury (Hg), manganese (Mn), nickel (Ni), selenium (Se) and zinc (Zn) were analysed in 22 samples of liver from ringed seals (see Table 4). Cr was below detection limit in all samples but one, which was at detection limit. Ni was below detection limit in all samples (<0.02-0.03 mg/kg ww).

Co decreased in concentrations at a rate of ca -1.3% annually (p<0.04 mg/kg ww). Otherwise rather stable concentrations were seen. Concentrations of Pb was very low, and after 2000 below detection limit (<0.03 mg/kg ww) indicating decreasing concentrations.

Table 4. Range and median concentrations (mg/kg ww) in juvenile ringed seal.

	As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Se	Zn
min	0,06	0,02	0,01	<0,2	2,27	0,48	1,85	<0,2	<0,2	0,57	26
max	0,24	0,38	0,04	0,02	10,5	30,2	5,17	<0,3	0,13	8,94	42
median	0,13	0,06	0,02	<0,2	4,79	2,79	3,225		0,06	1,27	33

The concentrations of Hg were between 0.48-10.1 plus one extreme value at 30.2 mg/kg ww in 1974. The relationship Hg vs Se on a molar basis showed no trend over time. Generally, the ratio was slightly below 1, but in five samples it did exceeded 1 (mean ratio was 0.9) see Figure 8.

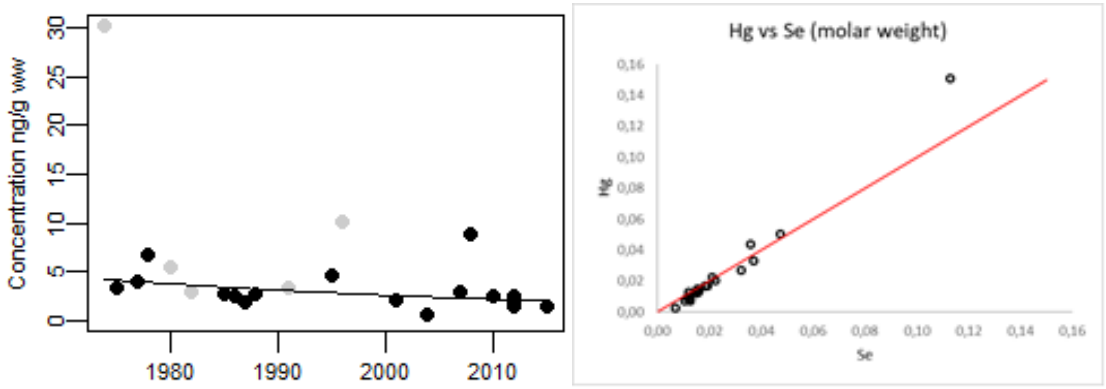

Figure 8. Left: concentrations of Hg in juvenile ringed seals 1974-2015 over time (ng/g ww, liver). Grey circles are individual seals and black dots represent pooled samples. Hg vs Se on molar weight basis. The red line indicate a 1:1 ratio.

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