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Associations between vitamins A and E and legacy POP levels in highly contaminated Greenland sharks (*Somniosus microcephalus*)

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HIGHLIGHTS

- POP levels in Greenland sharks are among the highest reported in Arctic animals.
- There were inverse relationships between plasma vitamin A and POP compounds.
- There were positive associations between plasma vitamin E and PCB congeners.
- The vitamin status and health of Svalbard Greenland sharks can be affected by POP.

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ABSTRACT

The Greenland shark (*Somniosus microcephalus*) is a top predator in Arctic marine ecosystems, and the species bioaccumulates high levels of biomagnifying persistent organic pollutants (POP). In teleost fish, as well as in marine mammals and seabirds, legacy POP have been shown to interfere with the vitamin A and vitamin E homeostasis. Thus, there is the potential for negative health effects from these legacy compounds in Greenland sharks. In the present study we examined associations among plasma levels of legacy POP and plasma vitamin A (retinol [RET], retinyl palmitate [RPA]) and vitamin E (α-tocopherol [α-TOC]) in Greenland sharks from Svalbard, Norway. Plasma levels of POP were on average higher than the hepatic levels previously reported in Greenland sharks from Iceland and Davis Strait, Canada. Levels were also higher than the plasma levels reported in Arctic marine mammals. DDTs (mean 8069 ng/g l.w., range: 900–59,707 ng/g l.w.), PCBs (mean 5766 ng/g l.w., range 1344–16,106 ng/g l.w.) and chlordanes (mean 1551 ng/g l.w., range: 323–5756 ng/g l.w.) had the highest concentrations among the POP groups studied. There were significant inverse relationships between RET concentrations and the concentrations of the dioxin-like compounds PCB-118 and PCB-156/171, and the non-dioxin-like compounds PCB-99 and PCB-128. There were also significant inverse relationships between RPA and 18 of the 38 POP compounds measured. Furthermore, there were significant positive associations between α-TOC and 13 of the 27 PCB congeners. The study suggests that these vitamin systems can be affected by the relatively high POP concentrations exhibited by Greenland sharks at Svalbard. However, the present study is correlative and thus the potential interplay between POP and vitamin dynamics of Greenland sharks must be interpreted cautiously, pending further research on this issue among elasmobranchs.

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1. Introduction

Persistent organic pollutants (POP) in the Arctic environment are of great concern (de Wit et al., 2004). They are lipophilic compounds known to accumulate in organisms and biomagnify through the food web, and can therefore reach high concentrations in top predators (Borga et al., 2004; Jenssen, 2006; Letcher et al., 2010). POP are released into the environment at southerly latitudes and transported into the Arctic via the atmosphere or ocean currents (Muir and de Wit, 2010; Wania and Mackay, 1993). Although legacy POP, those no longer produced or used, have declined in the Arctic environment since the late 1980s and early 1990s (Muir and de Wit, 2010), levels of these compounds are still high in many Arctic top predators (Bytingsvik et al., 2012; Letcher et al., 2010; Sonne, 2010). Organochlorines (OCs), such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes

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(DDTs), and chlordanes, are legacy POP that have been shown to cause adverse effects in Arctic top predators (Bustnes et al., 2003; de March et al., 1998; Letcher et al., 2010; Sonne, 2010; Sonne et al., 2006).

As compared to other Arctic animals, high levels of legacy POP have been reported in Greenland sharks (*Somniosus microcephalus*) from the Canadian Arctic (Baffin Island) and Iceland (Fisk et al., 2002; Strid et al., 2007), and there is concern about potentially adverse health effects on this species. Greenland sharks feed on a wide variety of prey species including invertebrates, fish and marine mammals (Compagno, 1984; Fisk et al., 2002; Lucas and Natanson, 2010; Yano et al., 2007). In some regions, such as Svalbard, a high prevalence of marine mammals in the stomachs of Greenland sharks strongly suggests that they commonly feed as top predators (Leclerc et al., 2012). Although conventional vertebral aging methods are not possible for Greenland sharks due to no apparent distinct calcification bands (MacNeil et al., 2012), studies based on tag-recaptures have suggested that the Greenland shark is long-lived, potentially to beyond 100 years of age (Hansen, 1963).

Several studies have documented that POP can affect the vitamin A balance of vertebrates, including fish (Boily et al., 2009; Jenssen et al., 2003; Murvoll et al., 2007; Palace et al., 1997). Retinol (RET) is the parent compound of vitamin A, and carotenoids are the precursors for vitamin A (Wolf, 1984). Retinol is diet-derived, and most is converted by lecithin:retinol acyltransferase (LRAT) to retinyl esters, such as retinyl palmitate (RPA) that if in excess are stored in liver stellate cells (Napoli, 1996, 1999; Simms et al., 2000). Upon mobilization from the liver, stored esters are hydrolyzed to RET by the enzyme retinyl ester hydrolase (REH) (McDowell, 2000a). Greenland sharks have high liver contents of vitamin A (Aure and Kløkstad, 1951), but the role of high hepatic vitamin A levels in sharks is currently unknown. In general, vitamin A is important for growth and development, vision, reproduction and immune function (McDowell, 2000a; Semba, 1998).

POP are also known to affect the homeostasis of vitamin E (α-tocopherol; α-TOC) in wildlife (Murvoll et al., 2005, 2007; Nyman et al., 2003). One of the most important functions of α-TOC is as an antioxidant (McDowell, 2000b). α-Tocopherol is the major lipid-soluble antioxidant responsible for protecting the polyunsaturated fatty acids (PUFAs) in membranes against lipid peroxidation by scavenging peroxyl radicals without reacting in further chain-propagating steps (Di Mascio et al., 1991).

Several field studies have revealed negative relationships between vitamins and POP in teleost fishes (Carballo et al., 2005; Nacci et al., 2001; Palace et al., 1997; Xu et al., 2002). However, there appears to be no knowledge on the effects of POP on vitamin status in cartilaginous fishes. The high levels of POP previously reported in Greenland shark from Iceland and the Canadian Arctic (Fisk et al., 2002; Strid et al., 2007), give cause for concern that this Arctic top predator may suffer from an imbalance in vitamin A and E homeostasis. Thus, the aim of the present study was to investigate levels of POP in Arctic Greenland sharks from Svalbard, Norway, and particularly to investigate associations between plasma concentrations of POP and RET, RPA, and α-TOC in Svalbard Greenland sharks.

2. Materials and methods

2.1. Sampling

Field work was conducted in the Kongsfjorden area, Svalbard, Norway, during June 2008 and June 2009. Greenland sharks were caught using longlines baited with seal blubber (bearded seal, *Erignathus barbatus*), from a fishing boat ("Viking Explorer") in 2008 and from a research vessel (RV "Lance") in 2009. A total of 43 sharks were sampled: 30 in 2008 (12 males and 18 females) and 13 in 2009 (4 males and 9 females). On board the vessels, a blood sample was collected from the caudal vein using a 50 mL syringe and transferred into vacutainers. The blood samples were centrifuged for 20 min at 3500 rpm and the supernatant fractions were transferred into cryovials, covered in aluminum foil, and frozen at −20 °C until analysis. The sharks were killed by cutting through the spinal cord immediately behind the head. The sex, body mass (BM, ±1 kg), and total body length (BL, ±1 cm) of all sharks were recorded. The liver was removed and weighed (LM, ±0.5 kg). A subsample of each liver was taken, wrapped in aluminum foil, and frozen at −20 °C until analysis. A condition factor (CF) was calculated according to the equation: $CF = (BM\ [g]/BL^3\ [cm]) * 100$ (Ricker, 1975). A hepatosomatic index (HSI) was calculated according to the equation: $HSI = (LM/BM) * 100$. Morphometric data of the sampled animals are presented in the Supplementary information. All animal handling procedures were performed in accordance with the guidelines of the Norwegian National Animal Research Authority.

2.2. Analysis for persistent organic pollutants (POP)

Samples were analyzed for POP at the Great Lakes Institute for Environmental Research (GLIER), University of Windsor, Canada, by gas chromatography electron capture detection (GC–ECD). PCBs, OCs and lipid percentage were analyzed using a micro-extraction technique (Daley et al., 2009), which uses a small sample mass (~1.0 g) and small amounts of solvents, but reflects a miniaturized version of the cold column extraction described in Lazar et al. (1992).

Plasma samples (~1 g) were weighed and homogenized with 15 g of activated sodium sulfate (ACS-grade, 10–60 mesh; activated by a muffle furnace at 450 °C overnight; Fisher Scientific; Ottawa, ON, Canada), and wet packed into a 20 mL glass syringe containing 15 mL of dichloromethane (DCM):hexane (1:1; pesticide grade; VWR, St Catherines, ON, Canada). The syringe was spiked with 50 ng/mL of PCB-30 (neat 2,4,6-trichlorobiphenyl, AccuStandard, New Haven, CT, USA) for use as a recovery standard. Another 10 mL of DCM:hexane was used to rinse the mortar and pestle and added to the syringe. The manifold valves were closed and the sample was extracted in the solvent for 1 h, after which the valve was opened and the column was eluted by gravity. An additional 15 mL of DCM:hexane was added to the column while the solvent was being eluted. Vacuum suction was used to draw any remaining solvent from the syringes and bedding into the reservoirs. The extracts were concentrated using a rotary-evaporator to a volume of 10 mL in volumetric flasks. One milliliter of the sample was removed for the gravimetric determination of neutral lipids (Drouillard et al., 2004). The remaining extract was concentrated to approximately 2 mL and sample clean up was performed by florisil chromatography as described by Lazar et al. (1992). Subsequently, extracts were concentrated to 1 mL using a rotary evaporator and placed in gas chromatography vials.

A Hewlett-Packard HP 6890 gas chromatograph coupled with a Waters GCT-premier time of flight (TOF) mass spectrometer was used for PBDE detection and analysis. The gas chromatograph was equipped with a DB5 column; 30 m × 0.25 mm ID × 0.25 μm — film thickness (J&W Scientific) and 7673 autosampler. Helium was used as the carrier gas (UHP) with a flow rate of 1 mL/min and a column head pressure of 69 psi. The sample (1 μL) was injected under splitless injection mode using an injection inlet temperature at 250 °C. The oven program began at 90 °C, which was held for 1 min, followed by a ramp up of 20 °C/min to 150 °C, followed by a second ramp up of 4.5 °C/min until a temperature of 280 °C was reached after which this temperature was held for 10 min. The GC–TOF was operated in EI mode at 70 eV following daily tuning and mass resolution calibration using a Metri (68.9952, 121.0014, 189.9966, 265.9965, 284.9949) calibration solution. The 284.9949 ion was used as the lock mass during sample runs.

For each batch of samples extracted, the sample injection sequences were set as follows: 5 external standard calibration curves for PBDEs (Wellington Laboratories certified PBDE native mixture), internal recovery standard, sample blank, internal reference homogenate (GLIER Detroit River Fish pool) and 6 samples. Post processing

of the high resolution-mass spectrometer detector (HR-MSD) output was performed using QuanLynx software. The three dominant ions for analytes (BDE-17, 28, 49, 47, 66, 100, 99, 85, 154, 153, 138 and 183) were extracted from the total ion chromatogram over a window of ±10 s from the expected analyte retention time. Peak areas were quantified using the analyte response relative to the external standard calibration curve. The limit of detection (LOD) for the compounds ranged from 0.001 to 0.038 ng/g. For each batch of six samples, a reference homogenate, method blank, external PCB standard (Quebec Ministry of Environment Congener Mix; AccuStandard, New Haven, CT, USA), OC standard, and PCB-30 recovery standard were analyzed. Recoveries of PCB 30 averaged (±standard error [SE]) 75.9±1.6%. Recoveries of individual PCB congeners in the in-house reference tissue, extracted with each batch of samples, were within two standard deviations of the mean laboratory database value derived from laboratory control charts. These standards as set by the GLIER organic analytical laboratory, which has Canadian Association for Environmental Analytical Laboratories accreditation and is ISO17025 certified, were established using standard cold column extraction techniques.

When studying the relationship between POP and dependent plasma variables in the blood, wet weight (w.w.) concentrations of POP were used, because w.w. concentrations, as opposed to lipid weight (l.w.) concentrations, are often preferred in toxicological studies. However, concentrations of POP were also presented on a l.w. basis to allow comparison with concentrations reported in other species.

2.3. Analysis of vitamins

Plasma concentrations of RET, RPA and α-TOC were analyzed using high performance liquid chromatography (HPLC, PerkinElmer 200 series, USA) at the Department of Biology, Norwegian University of Science and Technology (NTNU). Vitamins were extracted from plasma samples with a modified liquid–liquid extraction technique described by Siluk et al. (2007). Briefly, 200 μL of plasma was added to 20 μL of internal standard (IS; retinyl acetate [RAC], 25 μg/mL), and 200 μL of ultra pure water (Milli-Q). The solution was vortex-mixed for 10 s. After addition of ethanol (400 μL) with 0.04% BHT (2,6-di-tert-butyl-4-methylphenol, Sigma Aldrich, St. Louis, MO, USA) the solution was again vortex-mixed (10 s). For extraction of vitamins, hexane (800 μL) with BHT was added and then the mixture was mechanically shaken for 5 min (Vibramax 110, Heidolph, Germany). To facilitate phase separation the mixture was centrifuged for 3 min at 13,200 rpm (Eppendorf centrifuge 5415D, Eppendorf AG, Hamburg, Germany). The supernatant (ca. 700 μL) was transferred to a new micro tube (1.5 mL) and evaporated to dryness under a stream of nitrogen at 40 °C (Techne Sample Concentrator, Dri-Block DB-3D) for 10–15 min. The residue was resuspended in 200 μL of mobile phase (methanol–water, 98:2, v/v), mechanically shaken for 1 min and centrifuged for 3 min at 3000 rpm. Finally, aliquots of 150 μL were transferred to amber vials and 20 μL was injected into the HPLC system for analysis. Because light degrades RET, the whole extraction process was conducted under dim light conditions.

The chromatography was carried out using a HPLC instrument equipped with a fluorescence detector, an autosampler with a Peltier sample tray, a pump, vacuum degasser, and a column (Chrompack Intersil, ODS-3, 150×4.6 mm, 5 μm) from Varian, Inc. (Lake Forest, CA) connected to a guard column (ChromGuard SS 10×3 mm) also from Varian. Data were collected with Turbochrom Workstation version 6.1.2 software. Simple isocratic elution with a polar organic mobile phase composed of methanol (Sigma-Aldrich) and water (98:2, v/v) was applied as an eluent at a flow rate of 1.5 mL/min for 60 min at room temperature. RET and RAC were detected using an excitation wavelength of $\lambda_{ex}=325$ nm, and an emission wavelength of $\lambda_{em}=470$ nm. This setting was maintained from injection to 6.5 min into the process. At 6.5 min, the excitation wavelength was changed to $\lambda_{ex}=280$ nm and the emission wavelength to $\lambda_{em}=390$ nm in order to detect α-TOC. Finally, at 11 min, excitation and emission wavelengths were set to $\lambda_{ex}=325$ nm and $\lambda_{em}=490$ nm, respectively to detect RPA. The run was terminated after 60 min. Retinol, α-TOC, and RPA were quantified based on the peak area ratio of vitamin over the internal standard RAC, as obtained from calibration curves.

Standards consisted of RAC, RPA, α-TOC (Supelco, Bellefonte, PA, USA) and RET (Fluka Chemie, Bucs, Switzerland). All of the standards were dissolved individually in 100 mL of absolute ethanol, while retinyl acetate was dissolved in 100 mL of ethanol with an addition of 0.04% BHT. Standard solutions were stored at −70 °C and concentrations of these substances were corrected when necessary with a UV–vis spectrophotometer (Lambda 40, PerkinElmer, USA) according to the absorbance value E (1 cm/1%): RET (1835 at 325 nm); α-TOC (75.8 at 292 nm); and RPA (940 at 325 nm). Calibration curves consisted of a seven-point linear calibration line ($R^2>0.99$, each measured in duplicate) derived from a mixture of analytes prepared by diluting standard solutions in methanol: RET: 0.1–20 μg/mL; α-TOC 1–300 μg/mL; and RPA: 0.1–179 μg/mL. The final results were calculated as the mean of the concentrations determined for two replicate samples.

The performance of the method was examined by using the standard reference material (SRM) NIST 968 (fat-soluble vitamins, carotenoids, and cholesterol in human serum). The recoveries for RET were 102.9% (level I) and 108.6% (level II), and for α-TOC 93.0% (level I) and 105.1% (level II). These results were calculated by subsequent analysis (performed the same day) of two subsamples of each of the reference materials. Additionally, in each run aliquots of cow plasma were used as intra- and inter-assay controls. The coefficient of variation (CV) for 2 replicates of cow plasma was less than 10% in all analyses. Limits of detection (LOD) were calculated as signal (S) to noise (N) ratio, S/N = 3. For RET, α-TOC, and RPA the detection limits were 0.003, 0.424, and 0.179 μg/mL, respectively.

2.4. Statistical analyses

In the statistical analyses, only compounds that were detected in >60% of the samples were included. These were PCB-33, -44, -49, -70, -87, -95, -99, -101, -105/132, -110, -118, -128, -138, -149, -151/82, -153, -156/171, -158, -170, -177, -180, -183, -187, -194, -195/208, -201, and -206 (which constitute ∑PCBs); *p,p′*-DDT, *p′p′*-DDD, and *p,p′*-DDE (which constitute ∑DDTs); hexaclorobenzene (HCB); octachlorostyrene (OCS); *trans*-nonachlor, *cis*-nonachlor, *trans*-chlordane, *cis*-chlordane, and oxychlordane (which constitute ∑chlordanes), and mirex. The following contaminants were detected in less than 60% of the plasma samples, and were thus discarded from the statistical analysis: 1,2,4,5-TCB, 1,2,3,4-TCB, QCB, α-BHC, β-BHC, γ-BHC, PCB-18/17, PCB-31/28, PCB-52, PCB-191, PCB-205, PCB-209, PBDE-28, PBDE-47, PBDE-66, PBDE-85, PBDE-99, PBDE-100, PBDE-153, and PBDE-154.

Concentrations of POP and vitamins are presented as means, standard deviations (SD), medians, and ranges (Table 1 and text). Means, SD and medians were calculated using 2/3 of the detection limit for values <LOD for POP and vitamins. The contaminant concentrations were corrected for the internal standard PCB-30.

The statistical analyses were conducted using SigmaPlot version 11.0 (Systat Software Inc., Germany), Simca-P+ 12.0 (Umetrics, Umeå, Sweden), and PASW 18.0 (SPSS inc., Chicago, Illinois, USA). The variables lipids (%) and CF were normally distributed (Shapiro–Wilcoxon test). The variables RET, α-TOC and RPA were normally distributed after a square transformation, a square root transformation, and a reciprocal transformation, respectively. Except for mirex ($p=0.030$), PCB-49 ($p=0.038$), PCB-177 ($p=0.011$), and PCB-206 ($p=0.002$), all other variables were normally distributed following log transformation.

Table 1
Concentrations of individual contaminants (ng/g wet weight [w.w.] and ng/g lipid weight [l.w]) measured in the plasma of Greenland sharks collected at Svalbard, Norway, June 2008 and 2009. 43 samples were analyzed. "n" denotes how many of the samples were above the limit of detection. <LOD = below the limit of detection. When calculating mean and SD, values <LOD were defined as 2/3 of the LOD.

Compound	n	Wet weight				Lipid weight			
		Mean	SD	Median	Range	Mean	SD	Median	Range
Lipids (%)	43	–	–	–	–	0.59	0.31	0.51	0.15–1.47
HCB	43	1.30	1.21	0.83	0.13–4.79	210	149	143	36.2–670
OCS	38	0.08	0.06	0.07	<LOD–0.28	15.0	6.03	14.2	<LOD–37.3
trans-Nonachlor	43	6.42	8.50	3.40	0.33–44.1	946	776	689	190–3554
cis-Nonachlor	43	0.99	1.06	0.60	0.09–4.33	156	123	124	28.7–523
trans-Chlordane	43	0.23	0.22	0.16	0.06–1.41	41.1	26.2	35.6	12.4–155
cis-Chlordane	43	0.61	0.46	0.53	0.11–2.15	108	62.4	95.5	33.0–361
Oxychlordane	43	2.03	2.61	1.03	0.10–14.4	300	235	221	59.0–1164
∑Chlordanes	43	10.3	12.5	5.94	0.71–65.6	1551	1152	1178	323–5756
Mirex	42	0.22	0.27	0.13	<LOD–1.67	35.3	24.3	29.5	<LOD–135
p,p′-DDT	43	13.9	29.0	5.39	0.59–184	2159	3816	1110	189–24,497
p,p′-DDD	43	4.48	6.69	2.20	0.17–35.9	690	828	411	68.5–4772
p,p′-DDE	43	37.0	60.3	17.3	1.10–338	5220	5762	3235	642–30,438
∑DDTs	43	55.3	82.1	24.6	1.89–388	8069	8793	5163	900–59,707
PCB-33	30	0.13	0.12	0.12	<LOD–0.43	38.1	20.9	30.4	<LOD–82.6
PCB-44	43	0.35	0.24	0.28	0.09–1.04	64.1	37.9	55.2	19.8–223
PCB-49	43	1.78	1.18	1.32	0.22–5.51	338	195	319	33.1–1131
PCB-70	43	0.32	0.14	0.30	0.15–0.85	68.3	45.5	48.9	13.3–247
PCB-87	38	0.30	0.22	0.25	<LOD–0.92	70.4	40.5	59.1	<LOD–219
PCB-95	43	1.57	1.13	1.13	0.40–4.56	287	185	260	69.0–1108
PCB-99	43	2.40	2.66	1.51	0.27–13.6	379	251	308	114–1219
PCB-101	43	2.10	1.61	1.46	0.50–7.22	375	232	323	98.4–1423
PCB-105/132	43	1.06	0.92	0.61	0.19–4.10	181	124	146	50.2–683
PCB-110	43	1.38	1.09	0.96	0.33–5.62	251	164	224	54.4–907
PCB-118	43	2.54	2.21	1.80	0.40–11.4	427	257	324	145–1399
PCB-128	43	0.70	0.79	0.42	0.07–3.81	108	74.7	76.2	23.1–343
PCB-138	43	5.43	6.91	3.44	0.38–33.5	821	641	580	192–2848
PCB-149	43	2.29	2.29	1.44	0.24–9.84	373	283	254	79.5–1372
PCB-151/82	43	0.69	0.67	0.41	0.11–3.25	113	79.6	93.9	24.7–428
PCB-153	42	5.76	7.54	3.78	<LOD–38.9	885	679	672	<LOD–3133
PCB-156/171	40	0.50	0.57	0.34	<LOD–2.79	82.8	56.3	60.2	<LOD–250
PCB-158	43	0.27	0.31	0.15	0.03–1.49	43.2	31.3	32.2	7.81–134
PCB-170	43	0.75	1.02	0.50	0.04–5.47	114	95.9	80.8	22.2–441
PCB-177	40	0.34	0.45	0.19	<LOD–1.88	54.4	47.4	36.7	<LOD–216
PCB-180	43	2.12	2.83	1.45	0.15–15.0	321	262	249	64.9–1212
PCB-183	43	0.64	0.77	0.40	0.04–3.65	96.9	75.2	68.4	18.9–295
PCB-187	43	1.45	1.74	0.88	0.07–7.71	221	183	145	38.8–692
PCB-194	40	0.23	0.31	0.14	<LOD–1.68	37.9	29.8	26.6	<LOD–135
PCB-195/208	28	0.09	0.11	0.07	<LOD–0.56	18.3	11.9	14.7	<LOD–52.6
PCB-201	41	0.30	0.41	0.19	<LOD–2.08	48.4	39.5	32.0	<LOD–167
PCB-206	33	0.08	0.09	0.06	<LOD–0.46	15.5	8.03	13.6	<LOD–37.1
∑PCBs	43	35.6	36.3	24.6	4.77–169	5766	3716	4602	1344–16,106

A principal component analysis (PCA) was conducted using Simca-P+ to visualize the grouping of the variables. All POP compounds, and relevant biological variables were included in the PCA. For testing the correlations between variables indicated in the PCA plot, Pearson correlations were applied. Furthermore, for α-TOC and RET the PCA plot indicated relationships with both contaminants and biological variables. To investigate the relationships between the vitamins and the contaminants and the biological variables in more detail, multiple regression analysis and partial correlation tests were applied. The specific testing procedures are described when applied. Bonferroni correction was not applied when comparing associations between multiple variables because of the increased probability of producing false negatives (Morgan, 2003). The significance level in all statistical tests was set to $\alpha = 0.05$.

3. Results

The dominant group of POP in the Svalbard Greenland sharks was the ∑DDTs, followed by ∑PCBs and ∑chlordanes (Table 1). The most prevalent compounds were *p,p′*-DDE and *p,p′*-DDT. The major PCB congeners in plasma, ranked by their concentrations, were PCB-153 > PCB-138 > PCB-118 > PCB-99 > PCB-149, which in total contributed to 52% of the ∑PCB concentrations. Among the chlordanes, *trans*-nonachlor was the dominant compound. PBDEs were only detected in a few individuals, and were thus not included in further analyses.

The mean plasma concentration of RET in the sharks was 0.07 μg/mL (SD = 0.02, median = 0.07, range = 0.01–0.10, n = 43). The mean plasma RPA concentration in the sharks was 0.84 μg/mL (SD = 0.52, median = 0.91, range = <LOD–2.04, n > LOD = 31). The mean α-TOC concentration in the sharks was 9.41 μg/mL (SD = 9.71, median = 7.91, range = <LOD–34.2, n > LOD = 33).

A PCA was conducted to examine the relationships among the biological variables and contaminant variables. Since BM, BL, and LM were highly correlated with each other (Table 2), but not with any of the vitamins or the contaminants, these morphological variables were excluded from the PCA, and were represented by CF, which also correlated significantly with BM, BL and LM. The PCA resulted in two principal components, which explained 79.9% of the total variation in the data. PC1 and PC2 explained 74.7% and 5.2% of the variation, respectively. The PCA analysis indicated that there were no relationships between POP and sex (Fig. 1, loading plot).

The PCA indicated inverse associations among POP and RET (Fig. 1). Because the PCA also indicated positive relationships among RET, CF and HSI (Fig. 1), the influence of POP on RET was examined further using multiple regression analysis with HSI, CF, lipids (%) and the

Table 2
Significant correlations between morphometric variables and vitamins in plasma from Greenland sharks collected at Svalbard, Norway, June 2008 and 2009, using Pearson's correlation. (+) indicates positive correlation. α-TOC = α-tocopherol, BL = body length, BM = body mass, CF = condition factor, HSI = hepatosomatic index, LM = liver mass, RET = retinol, TL = liver mass. HSI = (liver mass/body mass) * 100. CF = (body mass/body length^3) * 100.

Body mass (kg)	(+) BL ($p<0.001$, $n=43$, $R^2=0.906$), (+) LM ($p<0.001$, $n=42$, $R^2=0.729$), (+) CF ($p=0.006$, $n=43$, $R^2=0.172$)
Body length (cm)	(+) BM ($p<0.001$, $n=43$, $R^2=0.906$), (+) LM ($p<0.001$, $n=42$, $R^2=0.577$)
Liver mass (kg)	(+) BM ($p<0.001$, $n=42$, $R^2=0.729$), (+) BL ($p<0.001$, $n=42$, $R^2=0.577$), (+) HSI ($p<0.001$, $n=42$, $R^2=0.419$), (+) CF ($p=0.001$, $n=42$, $R^2=0.236$)
HSI	(+) LM ($p<0.001$, $n=42$, $R^2=0.419$), (+) lipids ($p=0.022$, $n=42$, $R^2=0.125$), (+) RET ($p=0.020$, $n=42$, $R^2=0.127$), (+) CF ($p=0.014$, $n=42$, $R^2=0.143$)
Lipids (%)	(+) HSI ($p=0.022$, $n=42$, $R^2=0.125$), (+) α-TOC ($p=0.002$, $n=33$, $R^2=0.279$)
CF	(+) BM ($p=0.006$, $n=43$, $R^2=0.172$), (+) LM ($p=0.001$, $n=42$, $R^2=0.236$), (+) HSI ($p=0.014$, $n=42$, $R^2=0.143$)
Retinol (μg/mL)	(+) HSI ($p=0.022$, $n=42$, $R^2=0.127$)
α-Tocopherol (μg/mL)	(+) lipids ($p=0.002$, $n=33$, $R^2=0.279$)

individual POP compounds as covariates. This analysis identified significant inverse relationships between RET levels and the dioxin-like compounds PCB-118 and PCB-156/171, and the non-dioxin-like PCB-99 and PCB-128. Depending on the particular contaminant used in the model, the models explained 23–24% of the variation in plasma RET levels ($F_{4,35}>2.64$, $p<0.05$). The strongest model, PCB-99 ($F_{1,37}=4.61$, $p=0.038$), HSI ($F_{1,37}=1.90$, $p=0.176$), CF ($F_{1,37}=0.40$, $p=0.531$), and lipids (%) ($F_{1,37}=2.51$, $p=0.122$) explained 24% of the variation in plasma RET levels.

The PCA indicated inverse relationships between the POP and RPA (Fig. 1). This was investigated further using Pearson's correlation and significant inverse relationships were identified between RPA and 18 contaminants (Table 3). Furthermore, as indicated in the PCA, there was a significant positive relationship between RET and RPA ($r^2=0.249$, $p=0.001$).

The PCA indicated positive associations among the POP, α-TOC and lipids (%) (Fig. 1). Relationships between α-TOC and various contaminants were thus examined further using a partial correlation test (two-tailed) with lipids (%) as the controlling variable, and α-TOC and the various contaminants as variables. Significant positive relationships were found between α-TOC and 13 PCB congeners (Table 4).

4. Discussion

The mean l.w. plasma concentrations of ∑PCBs in the Svalbard sharks (Table 1) were 2.35 and 1.83 times higher than the l.w. hepatic concentrations previously reported for Greenland sharks from Arctic Canada (Cumberland Sound, Davis Strait) and from Iceland, respectively (Fisk et al., 2002; Strid et al., 2007). Although it is possible that the higher concentrations in the Svalbard Greenland sharks is due to the fact that different matrices were analyzed for POP in the studies (i.e. plasma versus liver), the differences between the populations may be linked to differences in dietary preferences and/or regional differences in contaminant content in their prey species. A high percentage (42.3%) of Svalbard Greenland sharks have remains of marine mammals in their stomachs (Leclerc et al., 2011; Leclerc et al., 2012). In contrast, only 14% of the Canadian Greenland sharks were reported to have remains of marine mammals in their stomachs (Fisk et al., 2002). Since POP are biomagnified, higher proportions of marine mammals in the diet of Svalbard Greenland sharks may explain their higher concentrations of ∑PCBs as compared to the Canadian and Icelandic Greenland sharks. With respect to regional contaminant differences in biota, levels of ∑PCBs have been shown to be ca. 2.20 times higher in the adipose tissue of Svalbard polar bears (*Ursus maritimus*) as compared to that in polar bears from South Baffin Island (where the Cumberland Sound is situated) (Verreault et al., 2005a). Furthermore, concentrations of ∑PCBs in blubber of white whales (*Delphinapterus leucas*) from Svalbard are approximately 3 times higher than in white whales from the Hudson Strait, which is relatively close to Cumberland Sound (Andersen et al., 2001; Kelly et al., 2008). A recent report shows that Greenland sharks tagged in Kongsfjorden, Svalbard, do not appear to migrate beyond the waters around Svalbard (Fisk et al., 2012). Thus, the high levels of ∑PCBs in the Svalbard Greenland sharks are in accordance with previous reports documenting that levels of POP generally are higher in biota from Svalbard (and East-Greenland) compared to other regions of the Arctic (Letcher et al., 2010; Sonne, 2010).

The l.w. plasma concentrations of ∑chlordanes and ∑DDTs in the Svalbard Greenland sharks were similar to the concentrations reported in the Canadian Greenland sharks (Fisk et al., 2002). This is consistent with the relative similar l.w. concentrations of ∑chlordanes and ∑DDTs reported in polar bear adipose tissue from Svalbard and Southern Baffin Island (Verreault et al., 2005a). It is assumed that Greenland sharks, as other fish, cannot form oxychlordane, and that the source of this compound is from consumption of marine mammals (Fisk et al., 2002). Thus, the high plasma concentrations of oxychlordane in the Svalbard Greenland sharks may be an indicator of seal and marine mammal consumption (Fisk et al., 2002).

It is noteworthy that PBDEs were only detected in the plasma of a few individual Svalbard Greenland sharks. PBDEs have been analyzed in muscle and liver tissues of Icelandic Greenland sharks, and of 12 analyzed PBDEs, 8 were present in all 10 individuals that were analyzed (Strid et al., 2010). In the North-Atlantic, levels of POP commonly decrease with latitude up toward the Arctic (Sobek and Gustafsson, 2004; Jasmin et al., 2010). Plasma concentrations of PBDEs have been reported to be relatively low (1–6 ng/g w.w.) in other Arctic top predators in Svalbard, such as ringed seals (*Pusa hispida*) and polar bears (Routti et al., 2009; Verreault et al., 2005b). Thus, as compared to PCBs and persistent pesticides, levels of PBDEs appear to be relatively low in Arctic top predators. This suggests that the low detection rate of PBDEs in the Svalbard Greenland sharks as compared to the Icelandic sharks is due to the belief that concentrations of PBDEs are lower in biota in Svalbard waters than in Icelandic waters.

The PCA analysis indicated that there were no relationships between POP levels and sex (Fig. 1, loading plot). This is in accordance with reports in Canadian and Icelandic Greenland sharks (Fisk et al., 2002; Strid et al., 2007). The PCA score plot (Fig. 1) indicates that the concentrations of most of the contaminant compounds were positively inter-correlated, similar to what Fisk et al. (2002) reported in Canadian Greenland sharks.

Plasma concentrations of POP in Svalbard Greenland sharks were 1.4 to 125 times higher than the values reported for plasma of other marine animals from Svalbard, including polar bears and white whales (Table 5). The relative differences in concentrations of the various POP groups among the marine animals in Svalbard (Bang et al., 2001; Bytingsvik et al., 2012; Gabrielsen et al., 2011; Villanger et al., 2011) have been attributed to their trophic position, different diets, their nutritional condition and species-specific differences in cytochrome P450 enzyme activities, which cause differences in the ability to biotransform POP among species (Borga et al., 2005; Fisk et al., 2005; Lydersen et al., 2002). The relatively high POP levels reported in polar bears and white whales, also from Arctic regions other than Svalbard, have been associated with a range of negative health effects (Letcher et al., 2010; Sonne, 2010; Villanger et al., 2011). Thus, the even higher POP levels reported in Svalbard Greenland sharks raise concern for the health effects of POP in these Greenland sharks.

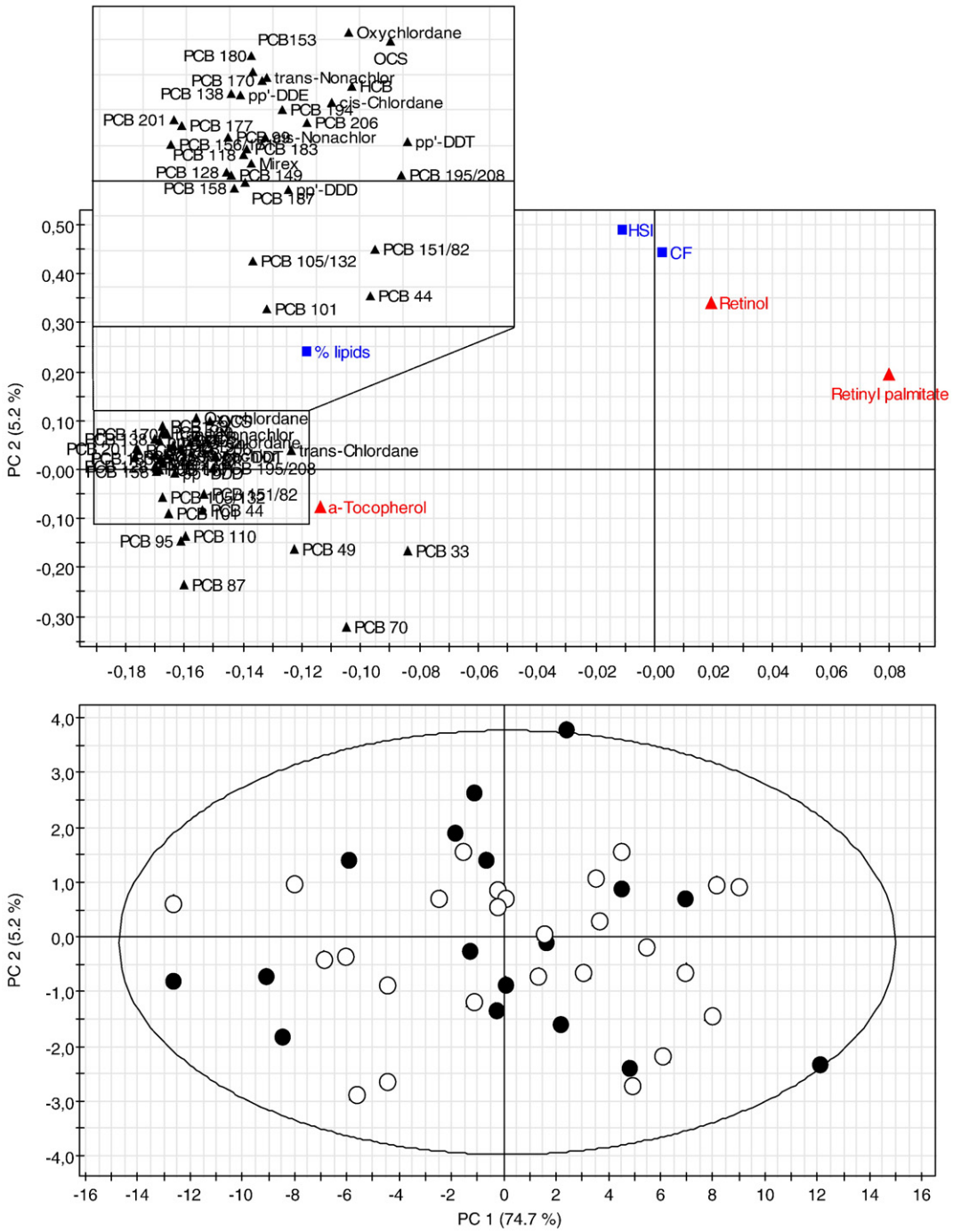

Fig. 1. PCA loading plot (top) and score plot (bottom) showing the relationship between contaminant and vitamin levels in the plasma of 43 Greenland sharks (*Somniosus microcephalus*) sampled in Svalbard, Norway, in June 2008 and 2009. Loading plot: solid squares = % lipids, hepatosomatic index (HSI), and condition factor (CF), red triangles = vitamins, solid triangles = contaminants. Score plot: solid dots = males, open dots = females. The PCA includes the variables % lipids, HSI, CF, retinol, α-tocopherol, retinyl palmitate, HCB, OCS, *trans*-nonachlor, *cis*-nonachlor, *trans*-chlordane, *cis*-chlordane, oxychlordane, mirex, *p,p′*-DDT, *p′p′*-DDD, *p,p′*-DDE, PCB-33, PCB-44, PCB-49, PCB-70, PCB-87, PCB-95, PCB-99, PCB-101, PCB-105/132, PCB-110, PCB-118, PCB-128, PCB-138, PCB-149, PCB-151/82, PCB-153, PCB-156/171, PCB-158, PCB-170, PCB-177, PCB-180, PCB-183, PCB-187, PCB-194, PCB-195/208, PCB-201 and PCB-206. HSI = (liver mass/body mass) * 100. CF = (body mass/body length^3) * 100.

Inverse relationships between POP and RET levels, or reduced RET levels in POP exposed populations, have been reported previously in several teleost species (Besselink et al., 1998; Nacci et al., 2001; Palace et al., 1997; Xu et al., 2002) and in other Arctic marine animals (Jenssen et al., 2003; Murvoll et al., 2006; Skaare et al., 2001). In the present study the weak dioxin-like compounds PCB-118, which was one of the most prevalent congeners, and PCB-156/171 were significantly negatively correlated with RET concentrations. This indicates that the aryl hydrocarbon receptor (AhR) could be involved in the lowered levels of RET in the Greenland shark. Cytochrome P450 1A (CYP1A) activity following exposure to beta-naphtoflavone, which is an AhR inducer, has been documented for cartilaginous fish (Hahn et al., 1998). In Atlantic salmon (*Salmo salar*) CYP1A1/2 and CYP2B have been described to be involved in the production of retinoic acid (RA) from RET (Arukwe and Nordbo, 2008). Furthermore, a study on lake trout (*Salvelinus namaycush*) indicated accelerated metabolism of RET with increasing PCB dose due to increased phase I or II enzyme activity (Palace et al., 1997). An induction of hepatic

Table 3
Significant negative correlations between retinyl palmitate (RPA) and contaminants in plasma from Greenland sharks collected at Svalbard, Norway, June 2008 and 2009, determined using Pearson's correlation.

Contaminant	Correlation with RPA	
	p-Value	R^2 value
OCS	0.023	0.178
trans-Nonachlor	0.050	0.126
trans-Chlordane	0.015	0.188
cis-Chlordane	0.020	0.173
Mirex	0.037	0.141
PCB-87	0.031	0.166
PCB-99	0.031	0.150
PCB-128	0.012	0.200
PCB-138	0.030	0.152
PCB-153	0.035	0.150
PCB-156/171	0.016	0.191
PCB-158	0.043	0.134
PCB-170	0.015	0.187
PCB-177	0.037	0.146
PCB-180	0.019	0.175
PCB-183	0.008	0.220
PCB-187	0.013	0.194
PCB-194	0.014	0.197

Table 4
Significant positive correlations from a partial correlation test (two-tailed) with lipids (%) as a controlling variable, and α-tocopherol (α-TOC) and individual contaminants as variables in plasma from Greenland sharks collected at Svalbard, Norway, June 2008 and 2009.

Contaminant	Correlations with α-TOC	
	p-Value	R^2 value
PCB-49	0.023	0.161
PCB-70	0.013	0.188
PCB-87	0.002	0.292
PCB-95	0.013	0.189
PCB-99	0.048	0.125
PCB-101	0.020	0.168
PCB-105/132	0.040	0.134
PCB-128	0.031	0.146
PCB-151/82	0.035	0.139
PCB-156/171	0.038	0.141
PCB-158	0.022	0.162
PCB-183	0.044	0.129
PCB-187	0.024	0.159

CYP1A1/2 activities by dioxin-like PCBs could thus lead to increased metabolism of RET to RA, and a resultant reduction in hepatic RET levels. It is thus possible that the inverse relationship between the PCBs and RET in plasma reflects effects that are manifested in the hepatic tissue.

Several RET disruptive mechanisms linked to POP exposure have been identified. These include increase of UDP (uridine diphosphate)-glucuronosyltransferase gene expression (Nishimura et al., 2005), modulation of CYP26, PXR (pregnenolon X receptor) dependent CYPs, RXR (retinoid X receptor) binding, and RAR (retinoic acid receptor) binding (Leiva-Presa et al., 2006; Lemaire et al., 2005; Nishimura et al., 2005; Schuetz et al., 1998). Furthermore, inhibition of REH may affect the conversion of stored retinol esters to RET and thus the mobilization of RET to the blood (Ndayibagira and Spear, 1999). PCBs have been shown to interfere with the plasma protein transport system for RET, the retinol binding protein (RBP)–transthyrethin (TTR)–thyroxin (T4) complex (RET–RBP–TTR–T4 complex) (Brouwer and van den Berg, 1986; Brouwer et al., 1986). However, in teleost fish and sharks the RET–RBP complex is not coupled to the TTR–T4 complex (Berni et al., 1992; Shidoji and Muto, 1977). A detailed review of the disruption of retinoid transport and metabolism by environmental pollutants is given by Novak et al. (2008). Unfortunately, very little information on these enzyme and receptors is available for elasmobranch species, and no information is available for Greenland sharks.

Plasma RET concentrations in Svalbard Greenland sharks (0.07 μg/mL) were lower than those reported for captive nurse sharks (*Ginglymostoma cirratum*) (0.09 μg/mL), bonnethead sharks (*Sphyrna tiburo*) (0.24 μg/mL), bull sharks (*Carcharhinus leucas*) (0.20 μg/mL), and lemon sharks (*Negaprion brevirostis*) (0.13 μg/mL) (Dierenfeld et al., 2003). However, it cannot be concluded that the low levels of RET in Svalbard Greenland shark are due to high exposure to POP; they may simply reflect species differences.

In the Svalbard Greenland sharks, RPA was detected in 31 of the 43 individuals. RPA is the storage form of vitamin A, and it is therefore not normally found in plasma (Nyman et al., 2003; Palace and Brown, 1994). In dogs (*Canis familiaris*), plasma levels of retinyl esters parallel the levels of vitamin A in the feed (Schweigert and Bok, 2000). Thus, the presence of RPA in the Greenland sharks could be due to feeding before capture, with the RPA being derived from their prey. Since data on the amount of gut content in the examined sharks recently have been published (Leclerc et al., 2012), it is possible to test if the vitamin concentrations correlated with the mass of their gut contents. This was not the case for RPA, nor for RET or α-TOC ($p > 0.55$). Furthermore, there were no association between the mass of marine mammal remains in their gut and the plasma vitamin concentrations, or between the mass of the gut contents and the blood lipid content ($p > 0.89$). The lack of the influence of the mass of the gut contents and the type of gut contents on plasma vitamin A concentrations could be due to a large variation in the RET content in the different prey items that constituted the diet of the individual sharks.

There was a significant positive relationship between plasma concentrations of RPA and RET. Since retinol is diet-derived, and converted by LRAT to RPA (Napoli, 1996; Napoli, 1999; Simms et al., 2000), and RPA is hydrolyzed back to RET by REH (McDowell, 2000a), the correlation between these two variables indicates that there is a regulation of the circulatory concentrations of these two vitamin A variables. Since there appears to be little knowledge regarding vitamin A physiology and its regulation in cartilaginous fish, the presence of RPA in the plasma of Greenland sharks may be normal.

Significant inverse relationships were identified between RPA and 18 contaminants (Table 3). In teleost fish and in mammals,

Table 5
Mean concentrations (ng/g l.w.) of PCBs, DDTs and chlordanes in plasma of adult marine animals from Svalbard.

Species	∑PCBs	∑DDTs	∑Chlordanes	Reference
Greenland shark (*Somniosus microcephalus*), 2008–09	8069	5766	1551	Present study
Polar bears (*Ursus maritimus*), females, 1997–98	5710	–	–	Bytingsvik et al. (2012)
Polar bears (*Ursus maritimus*), females, 2008	2559	–	–	Bytingsvik et al. (2012)
White whale (*Delphinapterus leucas*), both sexes, 1996–2001	3369	2965	370	Villanger et al. (2011)
Hooded seals (*Crystophora cristata*), females, 2008	561	259	115	Gabrielsen et al. (2011)
Bearded seals (*Erignathus barbatus*), males, 1994–1996	248	161	133	Bang et al. (2001)
Bearded seals (*Erignathus barbatus*), females, 1994–1996	159	46	58	Bang et al. (2001)
Ringed seals (*Phoca hispida*), males, 1994–1996	625	621	187	Bang et al. (2001)
Ringed seals (*Phoca hispida*), females, 1994–1996	337	165	114	Bang et al. (2001)

low hepatic levels of RPA have been associated with exposure to PCBs (Boyer et al., 2000; Kuzyk et al., 2003; Morse and Brouwer, 1995; Nyman et al., 2003). The reduction of the hepatic levels of RPA has been proposed to be due to decreased LRAT activity, which converts RET to RPA in the liver (Nilsson et al., 1996; Nilsson et al., 2000). Although this has not been investigated in sharks, it is possible that the inverse relationship between the PCBs and RPA in the plasma of the Svalbard Greenland sharks is manifested in their hepatic tissue.

In the plasma of Greenland sharks there were significant positive relationships between α-TOC and 13 PCB congeners (Table 4). Positive correlations between PCBs and α-TOC have previously been reported in the hepatic tissue of common eider hatchlings (*Somateria mollissima*) (Murvoll et al., 2007), and in plasma and blubber of ringed seals and gray seals (*Halichoerus grypus*) (Nyman et al., 2003). PCBs are known to be inducers of oxidative stress (Palace et al., 1996; Saito, 1990; Sridevi et al., 2007; Tharappel et al., 2002). α-Tocopherol functions to neutralize the free radicals in reactive oxygen species (ROS) (McDowell, 2000b), and has been found to counteract the negative effects of PCBs (Slim et al., 1999). Mobilization of α-TOC has also been observed after other types of oxidant exposure (Elsayed, 2001). However, increases in α-TOC require adequate amounts of stored α-TOC (Elsayed, 2001). Thus, although the positive association between PCBs and α-TOC in the Greenland sharks indicates a dose-dependent increase in oxidative stress, this positive association also indicates that Greenland sharks had adequate amounts of α-TOC in their blood to counteract the increased oxidative stress.

The levels of α-TOC in the Greenland shark (9.41 μg/mL) were lower than reported in captive bonnethead sharks (17.10 μg/mL), but higher than the levels reported in tiger sharks (*Galeocerdo cuvier*) (0.84 μg/mL) (Dierenfeld et al., 2003). However, since the physiology and regulation of α-TOC in sharks are unknown, the causes of this inter-species variation are not known.

In summary, plasma POP levels in Svalbard Greenland sharks were on average higher than the hepatic levels previously reported in Greenland sharks from Iceland and Canada, and also higher than the plasma POP levels reported in Arctic marine mammals. There were significant inverse relationships between RET concentrations and the concentrations of the dioxin-like compounds PCB-118 and PCB-156/171, and the non-dioxin-like compounds PCB-99 and PCB-128. There were also significant inverse relationships between RPA and several POP compounds, and significant positive associations between α-TOC and several PCB congeners.

5. Conclusions

The present study is the first to report relationships between retinoids and tocopherol and POP in Greenland sharks, which have high levels of POP as compared to other Arctic marine animals. Since vitamins, and in particular retinoids, play an important role in processes such as development, embryogenesis, reproduction, and immune function, there is cause for concern that the high levels of POP reported in Greenland sharks may have effects on their health, reproduction, and survival. However, further investigations are needed to draw conclusions on possible health effects of POP in Greenland sharks through disturbance of vitamin homeostasis.

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