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Contaminants in Atlantic walruses in Svalbard part 1: Relationships between exposure, diet and pathogen prevalence☆

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ABSTRACT

This study investigated relationships between organohalogen compound (OHC) exposure, feeding habits, and pathogen exposure in a recovering population of Atlantic walruses (*Odobenus rosmarus rosmarus*) from the Svalbard Archipelago, Norway. Various samples were collected from 39 free-living, apparently healthy, adult male walruses immobilised at three sampling locations during the summers of 2014 and 2015. Concentrations of lipophilic compounds (polychlorinated biphenyls, organochlorine pesticides and polybrominated diphenyl ethers) were analysed in blubber samples, and concentrations of perfluoroalkylated substances (PFASs) were determined in plasma samples. Stable isotopes of carbon and nitrogen were measured in seven tissue types and surveys for three infectious pathogens were conducted. Despite an overall decline in lipophilic compound concentrations since this population was last studied (2006), the contaminant pattern was similar, including extremely large inter-individual variation. Stable isotope ratios of carbon and nitrogen showed that the variation in OHC concentrations could not be explained by some walruses consuming higher trophic level diets, since all animals were found to feed at a similar trophic level. Antibodies against the bacteria *Brucella* spp. and the parasite *Toxoplasma gondii* were detected in 26% and 15% of the walruses, respectively. Given the absence of seal-predation, *T. gondii* exposure likely took place via the consumption of contaminated bivalves. The source of exposure to *Brucella* spp. in walruses is still unknown. Parapoxvirus DNA was detected in a single individual, representing the first documented evidence of parapoxvirus in wild walruses. Antibody prevalence was not related to contaminant exposure. Despite this, dynamic relationships between diet composition, contaminant bioaccumulation and pathogen exposure warrant continuing attention given the likelihood of climate change induced habitat and food web changes, and consequently OHC exposure, for Svalbard walruses in the coming decades.

1. Introduction

Despite extensive bans on the use and production of numerous persistent organohalogen compounds (OHCs) (Stockholm Convention, 2001; 2009), concentrations of several OHCs are still high in many Arctic marine mammals such as polar bears (*Ursus maritimus*) (McKinney et al., 2010; Routti et al., 2017), walruses (Wolkers et al., 2006) and white whales (*Delphinapterus leucas*) (Villanger et al., 2011). This exposure is linked to the environmental persistence of these compounds and their capacity for long-range transport and deposition in the Arctic, as well as their biomagnification in food webs (Stockholm Convention, 2001). Top-predators of Arctic marine food webs are particularly vulnerable to the accumulation of high concentrations of lipophilic persistent

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organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) as well as perfluoroalkyl substances (PFASs). The main exposure route of these compounds in Arctic top-predators is diet and they tend to become concentrated at each step in food webs. Arctic top-predators' diets are lipid-rich and their need to accumulate large blubber stores to meet their thermoregulatory and energetic needs seasonally (Macdonald and Bewers, 1996; Welch et al., 1992) contributes to their high body burdens of lipophilic POPs. Dietary assessments can be achieved through non-terminal tissue sampling using stable isotopes (SI) (Hobson and Welch, 1992), since heavy and light isotopes of nitrogen and carbon exhibit recognizable cycles throughout the marine food web (Peterson and Fry, 1987). Ratios of ^{15}N to ^{14}N (expressed as $\delta^{15}N$) and ^{13}C to ^{12}C ($\delta^{13}C$) reflect the trophic position and carbon source (pelagic/benthic/sympagic) of the overall diet, respectively. Therefore, SIs can be used to quantify links between assimilated tissues in predators and their prey (Hobson and Welch, 1992). Walruses primarily consume benthic bivalves (Fay, 1982), however some individuals are known to hunt (or scavenge) pinnipeds, cetaceans or seabirds (Fay, 1982; Gjertz and Wiig, 1992). Increased feeding at higher trophic levels has been suggested to be a response to climate-change induced food web shifts in Pacific walruses (*O. r. divergens*) (Rausch et al., 2007; Seymour et al., 2014). In this region, benthic community production has been negatively impacted by sea ice retractions and other environmental change (Bluhm and Gradinger, 2008). Dietary variation has been correlated with concentrations of contaminants in walrus tissues; bivalve-consuming walruses have much lower levels of contamination than seal-eating individuals (Muir et al., 1995; Wolkers et al., 2006).

Whilst several studies have looked at the exposure of walruses to OHCs (Born et al., 1981; Muir et al., 1995; Wiig et al., 2000; Wolkers et al., 2006), none have investigated their potential effects on this species. Links between high concentrations of OHCs and adverse effects on Arctic marine mammals have been established using correlative field studies (Brown et al., 2014; Noël et al., 2014; Villanger et al., 2013; Villanger et al., 2011), *in vitro* investigations (e.g. De Guise, 1998; Levin et al., 2016) and extrapolations from surrogate species (Nymo et al., 2014; Ross et al., 1997). Common effects include disruption of the endocrine system (Letcher et al., 2010), but immunotoxic effects have also been suggested (reviewed by Desforges et al., 2016; Ross, 2002; Sonne, 2010). One example is immunosuppression in captive harbour seals (*Phoca vitulina*) following prolonged experimental exposure to contaminated fish (de Swart et al., 1994; de Swart et al., 1996). However, it is difficult to extrapolate results across species given interspecific differences in physiology and metabolism and excretion of contaminants (Macdonald and Bewers, 1996; Van Loveren et al., 2000).

If OHCs do impact the immune system of walruses, high concentrations of these compounds could pose disease risks (e.g. Jepson et al., 1999). However, the types of viral, bacterial and parasitic infections circulating in Atlantic walruses (*O. r. rosmarus*), remain relatively unknown. The Atlantic walrus is sympatric with other Arctic marine mammals known to host a variety of infectious agents. Screening studies using antibody detection techniques have revealed exposure to the bacteria *Brucella pinnipedialis* in several Arctic phocid seals from Svalbard and the Barents Sea (Nymo et al., 2013b; Tryland et al., 1999), as well as in walruses from a distinct population in Arctic Canada (Nielsen et al., 1997). *Brucella ceti* is thought to have negative consequences for reproduction in some cetaceans (Ewalt et al., 1994), however there is no evidence of associated pathology in pinnipeds (Nymo et al., 2011). Anti-*Toxoplasma gondii* antibodies have been detected in walruses and phocid seals from Svalbard (Jensen et al., 2010; Prestrud et al., 2007), though consequences for the health of these species are unknown (Tryland, 2000). Parapoxvirus has been detected in a single captive Pacific walrus (Melero et al., 2014) as well as in several Atlantic phocid seals (Becher et al., 2002; Nollens et al., 2006; Simpson et al., 1994; Tryland, 2011). Infection can result in the development of skin lesions in pinnipeds (Okada and Fujimoto, 1984) thought to be induced by immunosuppressive factors (Tryland, 2000), which may make the animal susceptible to secondary bacterial infection. Combining pathogen prevalence data, walrus feeding ecology and contaminant levels could potentially provide insight into exposure routes of contaminants and pathogens (e.g. inter-specific transfer including consumption of infected prey, intra-specific transfer and possible environmental reservoirs) as well as effects of contaminants (Fay, 1982; Lydersen and Kovacs, 2014; Rausch et al., 2007).

The aim of this study was to investigate current OHC exposure as well as potential relationships between OHC concentrations, diets and the presence of a variety of infectious agents, which may indicate compromised immunity. The hypothesis investigated was that individuals feeding at higher trophic levels will have higher concentrations of various OHCs and will therefore be more susceptible to infection by circulating pathogens.

2. Materials and methods

2.1. Ethics statement

All animal handling procedures were approved by the Norwegian Animal Care Authority (permit 2013/36153-2) and the Governor of Svalbard (permit 2014/00066-2 and 2015/00218).

2.2. Sampling

Thirty-nine apparently healthy walruses were immobilised from three haul-out sites in Svalbard, Norway between 9–15 July 2014 (n = 19 from Sletteøya, Fig. 1) and in the period 3–12 August 2015 (n = 4 from Sarstangen and n = 16 from Purchasneset, Fig. 1). The locations were selected according to where walruses haul out

Fig. 1. Map of the Svalbard Archipelago, Norway, showing the locations (red points) at which adult male Atlantic walruses were sampled 9–15 July 2014 (n = 19 from Sletteøya) and 3–12 August 2015 (n = 4 from Sarstangen and n = 16 from Purchasneset). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in Svalbard (Kovacs et al., 2014) and where the sampling was logistically possible. Individuals were approached on land and immobilised with an intramuscular injection of 7.8 mg of etorphine hydrochloride, which is a standard dose when the exact weight is unknown (Ølberg et al., 2017). Naltrexone (250 mg; intramuscular or intravenous, depending on the attainment of intravenous access) was used as a reversal agent. All animals recovered rapidly and fully. Tusk length and girth (proximal end) were used to calculate tusk volume following Skoglund et al. (2010), as a proxy for animal age (Fay, 1982). All sampled walruses were adult males (mean and standard deviation (SD) tusk volume: $403 \pm 175\ cm^3$; range: 135–894 cm^3; mean and SD standard body length 335 ± 28.5 cm, ranging from 226 to 390 cm).

Blood from all individuals was collected from the extradural vein in the lumbar-sacral region and transferred to serum and heparinised plasma vacuum tubes (Venoject, Terumo Corporation, Leuven, Belgium). Samples were kept cool and centrifuged (4000 rpm for 10 min) within a few hours. Serum, plasma and red blood cell (RBC) samples were frozen at −20 °C until analyses. Blubber biopsies (n = 38) were obtained as described in Wolkers et al. (2006), with the exception that the custom-made hollow stainless-steel corer was 8 mm in diameter and the samples including hair and dermis were kept frozen at −20 °C until analyses. Sterile cotton swabs were used to sample the mucosa of the eyes (conjunctiva) and nose from 19 individuals (2015). Swabs were placed in 1.8 mL cryotubes with 800 μL of Eagle's Minimum Essential Medium (EMEM, Nissui, Tokyo, Japan) containing antibiotics [10 000 U/ml penicillin, 10 mg/mL streptomycin, 1 mL/L of gentamicin (50 mg/mL) and 10 mL/L of amphotericin B (250 μg/mL); EMEMab 10 mL/L], stored in liquid nitrogen in the field and later transferred to a −80 °C freezer until analyses. Vibrissae from 38 individuals were clipped at the proximal end from the outer edge of the muzzle.

2.3. Determination of chlorinated and brominated compounds

Blubber was analysed for a selection of OHC compounds, comprising 17 OCPs, 24 PBDEs and 26 PCBs, (see Table S2). Detailed methodology regarding sample preparation, separation and quantification of targeted OHCs, analyses and quality assurance, can be found in the supporting information (see 'Determination of chlorinated and brominated compounds').

2.4. Determination of PFASs

PFASs targeted in walrus plasma were 4:2, 6:2 and 8:2 fluorinated telomere sulfonates, one (C_8) perfluoroalkane sulfonamide, $C_{4,\ 6\text{-}10}$ perfluoroalkyl sulfonates (PFSAs) and $C_{6\text{-}14}$ perfluoroalkyl carboxylates (PFCAs) (Table S3). Extraction and clean-up of targeted compounds as well as quantification of concentrations was conducted according to (Hanssen et al., 2013). Detailed methodology describing the separation and quantification of targeted PFASs, modifications and quality assurance can be found in the supporting information (see 'Determination of PFASs').

2.5. Stable isotope analysis

Sample preparation and stable isotope analyses were carried out following Marcoux et al. (2012). Briefly, RBCs, serum and dermis were homogenized and hair was cut into small pieces of <2 mm in length. All tissues excluding RBCs were lipid-extracted using a chloroform:methanol mixture. Specifically, vibrissae were washed in 5 mL of 2:1 chloroform:methanol using a sonic bath at 30 °C for 30 min, then at 40 °C for 10–15 min, before being dried by hand (using a kimwipe), placed in distilled water for sonification (10–15 min) and oven-dried overnight at 40 °C. Individual vibrissae were sectioned into inner, middle and outer sections (<2 mm per section), after which a dremel was used to crush each vibrissae section into a powder, which was then weighed. Analyses are described briefly in the supporting information (see 'Extended Methodology 3') along with standard reference materials and analytical and instrumentation accuracy.

2.6. Pathogen detection

2.6.1. Anti-Brucella spp. antibody detection

A Protein A/G Indirect Enzyme-Linked Immunosorbent Assay (iELISA), validated for the detection of anti-*Brucella* spp. antibodies in seals was performed as described previously (Nymo et al., 2013a). Each serum sample was tested in duplicate. Each sample was also tested for antibodies using the qualitative Rose Bengal Test (RBT; IDEXX Laboratories, Hoofddorp, The Netherlands).

2.6.2. Anti-Toxoplasma gondii antibody detection

An iELISA protocol was carried out on serum samples using the multi-species ID Screen® Toxoplasmosis Indirect kit (IDVET, Grabels, France), according to the manufacturer's instructions, using positive and negative controls provided in the kit. Plate wells were read using a spectrophotometer (Epoch Microplate Spectrophotometer, BioTek, Winooski, VT, USA; with software: Gen5TM, BioTek). The serological method used in this study is designed for multi-species testing and has been used for other marine mammals (Blanchet et al., 2014).

2.7. Parapoxvirus DNA detection and phylogenetic analysis

DNA was extracted from nasal and ocular swabs and purified using a Maxwell® 16 Buccal Swab LEV DNA Purification Kit (Madison, Wisconsin, USA). Optical density of eluates was measured spectrophotometrically (Nanodrop, NAN-ND-2000-uk, Fisher Scientific, Hampton, NH, USA) at 260 nm and 280 nm. DNA purity was deemed acceptable as the protein:DNA absorbency (A260/280) ratio was between 1.7 and 2.1 for 80% of the samples. The remaining samples fell between 1.4 and 1.7, indicating minor protein contamination, but they were deemed suitable for further testing.

Polymerase chain reaction (PCR) protocols were conducted with primers specifically targeting three parapoxvirus genes: the putative viral envelope antigen, *B2L;* the granulocyte-macrophage-colony-stimulating factor (and interleukin-2 inhibition factor), *GIF*; and the viral interleukin 10 orthologue, *IL10* (Klein and Tryland, 2005). PCRs were conducted as described previously (Klein and Tryland, 2005) using a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA) with the exception that Red Taq polymerase ('RedTaq', Sigma Aldrich, P-0982) replaced AmpliTaq Gold DNA polymerase, though PCR annealing temperatures were 57 °C (GIF and vIL-10) and 68 °C (B2L). Parapoxvirus isolates from sheep (*Ovis aries*) with the disease contagious ecthyma, caused by Orf Virus (ORFV, the prototype of the genus parapoxvirus) were used as a positive control. Consensus amplicon sequences were assembled using the Chromas pro software (version 1.7.7, Technelysium Pty Ltd, South Brisbane, QLD, Australia) and run through GenBank (NCBI, Bethesda, MD, USA) to search for homology with previously published sequences.

Phylogenetic analyses were performed to assess the evolutionary relationship between parapoxvirus DNA from this study and published sequences, using the Neighbour-Joining method (Saitou and Nei, 1987), where an optimal tree was generated (sum of branch lengths = 0.06368453). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was calculated (Felsenstein, 1985). Branch length

is equivalent to (same units) the evolutionary distances used to infer the tree. Evolutionary distances were calculated using the Tamura 3-parameter method (Tamura et al., 2011), where the units represent the number of base substitutions per site. Analyses involved nine nucleotide sequences and codon positions were 1st+2nd+3rd + Noncoding. Positions containing gaps and missing data were removed. The final dataset contained 331 positions. Phylogenetic analyses were conducted in MEGA7 (Kumar et al., 2016).

2.8. Data handling and statistical analysis

Concentrations of lipophilic POPs (PCBs, PBDEs and OCPs) were normalized to lipid weight (ng/g lw). Concentrations of PFASs were calculated as ng/g wet weight (ww). Only compounds present in more than 70% of individuals were used for calculation of summary statistics and further statistical analyses. For the compounds detected in more than 70% of individuals, concentrations less than the limit of detection (LOD) were replaced with half of the LOD (totalling 5% of the PFAS data and 6% of the data comprising PCBs, PBDEs and OCPs).

Statistical analyses were performed using R statistical software version 3.2.2 (R Core Development Team, 2015). Prior to analyses, structurally similar and correlated compounds were summed (Table 1), i.e. sum PCB (Σ_{18}PCB, correlation coefficient of summed variables [r] $\geq$0.59), sum chlordanes (Σ_2CHL, $r \geq 0.75$), sum HCH (Σ_3HCH, $r \geq 0.17$), sum PBDE (Σ_3PBDE, $r \geq 0.60$), sum PFASs (Σ_6PFAS, $r \geq 0.15$ with PFOA, $r \geq 0.32$ without PFOA) to reduce the number of variables. Due to lack of samples, stable isotope data was missing for hair and dermis for one individual, for vibrissae for another individual and for serum, plasma and blood for a third individual. Imputations for missing stable isotopes values were created using Fully Conditional Specification implemented by the MICE algorithm in R-package "mice" as described in (Buuren and Groothuis-Oudshoorn, 2011). Next, principal component analysis (PCA) derived from a correlation matrix was used to explore relationships among contaminants and between contaminant concentrations and stable isotopes. Σ_2CHL, mirex, Σ_3HCH, PeCB, *p,p*-DDE, Σ_{18}PCB and Σ_3PBDE were strongly correlated (Fig. S1; $r \geq 0.45$) and were therefore combined to produce the total concentration of lipophilic POPs (ΣPOP). Although HCB was not correlated with other lipophilic POPs it was also included in the ΣPOP due to its lipophilic nature (HCB contributed with <0.01% to ΣPOP). PCA (Fig. S1) suggested that only δ^{15}N values of RBC and the mid-vibrissal segment (Vb. Mid) were correlated with contaminant concentrations among all isotope data; these were thus retained in further analyses.

Linear regression models were constructed to investigate whether tusk volume (as a proxy for age), standard length, location and diet (δ^{15}N values of RBC and Vb. Mid, included as two separate variables) were significant predictors of ΣPOP and ΣPFAS concentrations. To prevent the inclusion of confounding predictor variables in the same model (Burnham et al., 2011), correlation coefficients were used to identify predictor variables that were highly correlated. A list of 11 candidate models were built for both ΣPOP and ΣPFAS as response variables (Table S4). The models were ranked according to Akaike's Information Criterion adjusted for small sample size (AICc; Burnham and Anderson, 2003). The R-package 'MuMIn' was used to make inferences from all candidate models (Barton, 2016). Averaged estimates were calculated for all predictor variables in the candidate model list weighted using AICc weights ($e^{(0.5(AICmin-AICi))}$; relative likelihood divided by the sum of

Table 1
Concentrations of a) lipophilic POPs extracted from the blubber and b) perfluoroalkyl substances extracted from the plasma of adult male walruses sampled in Svalbard in 2014 and 2015. Only compounds detected in ≥70% of the samples are reported. LOD = limit of detection.

a) Walrus blubber (n = 38; ng/g lipid weight)

	Median	Mean ± SD	Min.	Max.	Percent > LOD
Lipid percent	*79*	*77 ± 11*	*42*	*93*	
∑PCB[a]	923	3013 ± 5770	26.9	31617	100
∑PBDE[b]	1.68	11.1 ± 27.4	0.21	153	100
α- *hexachlorocyclohexane*	1.23	2.11 ± 3.35	0.582	18.8	100
β- *hexachlorocyclohexane*	24.4	43.2 ± 52.9	0.529	235	100
γ- *hexachlorocyclohexane*	0.387	0.429 ± 0.263	<0.1	1.36	95
Pentachlorobenzene	0.634	0.884 ± 1.07	<0.1	6.60	92
Hexachlorobenzene	0.418	0.607 ± 0.717	<0.22	3.46	76
Mirex	34.1	89.4 ± 160	2.99	760	100
Trans-nonachlor	2.55	22.0 ± 50.6	0.418	290	100
Oxychlordane	383	937 ± 1253	26.7	5715	100
p,p'-DDE	8.44	174 ± 479	0.759	2803	100
∑POP[c]	1484	4294 ± 7638	93.2	41554	

b) Walrus plasma (n = 39; ng/g wet weight)

	C_n	Median	Mean ± SD	Min.	Max.	% >LOD
Perfluoroalkyl sulfonates (PFSA)						
PFHxS	C_6	1.35	1.45 ± 0.750	0.259	3.82	100
PFOS[d]	C_8	2.22	2.89 ± 2.20	0.425	11.8	100
Perfluoroalkyl carboxylates (PFCA)						
PFOA	C_8	0.288	0.414 ± 0.405	0.057	2.46	100
PFNA	C_9	1.35	1.50 ± 0.845	0.403	3.48	100
PFDA	C_{10}	0.227	0.268 ± 0.201	<0.1	0.866	90
PFUnDA	C_{11}	0.269	0.320 ± 0.297	<0.1	1.59	80
∑PFAS[e]		5.40	6.84 ± 3.96	1.89	20.4	

[a] Sum of 18 detected PCBs (28/31, 47/49, 66, 74, 99, 101, 105, 118, 128, 138, 153, 156, 157, 170, 180, 183, 187, 194).
[b] Sum of detected PBDEs (47, 99, 153).
[c] Sum of all detected compounds.
[d] Sum of linear and branched PFOSs.
[e] Sum of detected PFASs (PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA).

all relative likelihoods).

A graphical exploration of diet (δ^{15}N and δ^{13}C in the seven tissue types), tusk volume, geographic location, ΣPOP and ΣPFAS was conducted to investigate whether these variables were related to the presence of *T. gondii* and *Brucella* spp. antibodies. If seropositive and seronegative individuals appeared to be different, these differences were tested using linear models. Parapoxvirus was detected in only one individual, so no further analyses were conducted on this virus.

Diagnostic plots of residuals from all models showed no violations of assumptions (primarily normal distribution of residuals), or the presence of outliers.

3. Results

3.1. Contaminant concentrations

Eighteen PCB congeners, three PBDE congeners, nine OCPs and six PFASs were detected in >70% of the walruses (Table 1). Concentration ranges for lipophilic POPs were in general large; for instance, ΣPCB ranged over three orders of magnitude (Table 1a). PCBs and oxychlordane accounted for 70% and 22% of ΣPOP in blubber, respectively. Among the PBDEs, PBDE 47 was the dominant congener, accounting for 72% of ΣPBDEs (Table S2b), whilst among PCBs, PCB 153 dominated, accounting for 59% of ΣPCBs (Table S2c).

ΣPFAS concentrations ranged over one order of magnitude (Table 1b). Perfluorooctanesulfonate (PFOS) was the most dominant PFAS, followed by perfluorononanoate (PFNA), perfluorohexanesulfonate (PFHxS) and perfluorononanoate (PFOA) (Table S3). ΣPOP concentrations were not correlated with ΣPFAS concentrations ($r = 0.20$, $p > 0.22$).

3.2. Stable isotopes

Variation in δ^{15}N and δ^{13}C values was generally low. The ranges for δ^{15}N and δ^{13}C values were approximately 2–3 and 2‰ units, respectively, for most of the tissue types analysed (Fig. 2). Mean values of δ^{15}N were highest in dermis > hair > vibrissae inner/vibrissae outer > vibrissae mid > serum > RBC (Fig. 2). A similar pattern occurred for δ^{13}C, though there was some variation in the order of the vibrissal segments. Lack of standard error bar overlap demonstrates that mean δ^{13}C values differed consistently between most tissue types, except for between vibrissal segments and hair (Fig. 2).

3.3. Relationships between contaminants and predictor variables

δ^{15}N values in the RBC and Vb. Mid were significant predictors of ΣPOP concentration (Table 2). Back-transformed model-averaged estimates (ε^{β}) demonstrated that contaminant concentrations increased by a factor of approximately 3 (95% confidence intervals [CI]: 1.5, 6.6) for every 1‰ increase in δ^{15}N Vb. Mid. This increase was greater, a factor of four (95% CI: 1.2, 15.6), for δ^{15}N values in the RBC. Top model estimates were consistent with their model-averaged counterparts, indicating model-averaging estimates were reliable.

Biological and isotopic predictor variables explained little of the variation in the ΣPFAS data (Table 2; Table S4). The exception was "Location (SL)", which indicated that walruses at Sletteøya had 32% lower ΣPFAS concentrations (95% CI: 2%, 52%) than those at Purchaseset (reference site; Table 2), however Location was not retained in the highest ranked model and did not explain a large proportion of the variation in the PFAS data (Table S4).

Table 2
Model-averaged estimates with 95% confidence intervals exploring relationships between ln-transformed concentrations of lipophilic POPs (ng/g lipid weight) in blubber and perfluoroalkyl substances (ng/g wet weight) in plasma according to tusk volume (as a proxy for age), standard body length, location (Sarstangen = SA; Slettøya = SL, Purchasneset = PN) and δ^{15}N ratios in the mid vibrissal segment (Vb. Mid) and δ^{15}N in red blood cells (RBC) in adult male walruses sampled on Svalbard. Bold values, where the 95% CI_A does not span 0, indicate significant model-averaged variables.

	ln(ΣPOP)	ln(ΣPFAS)
(Intercept)	−5.28 (−15.23, 4.67)	1.72 (−1, 4.45)
Tusk volume	0.000 (−0.003, 0.003)	−0.001 (−0.002, 0.000)
Length	−0.002 (−0.02, 0.016)	0.00 (−0.01, 0.01)
δ^{15}N Vb. Mid	**1.16 (0.43, 1.89)**	0.02 (−0.24, 0.29)
δ^{15}N RBC	**1.47 (0.18, 2.75)**	0.17 (−0.25, 0.59)
Location: SA vs PN	−0.41 (−2.3, 1.48)	−0.24 (−0.81, 0.33)
Location: SL vs PN	−0.5 (−1.66, 0.66)	**−0.38 (-0.74, -0.02)**

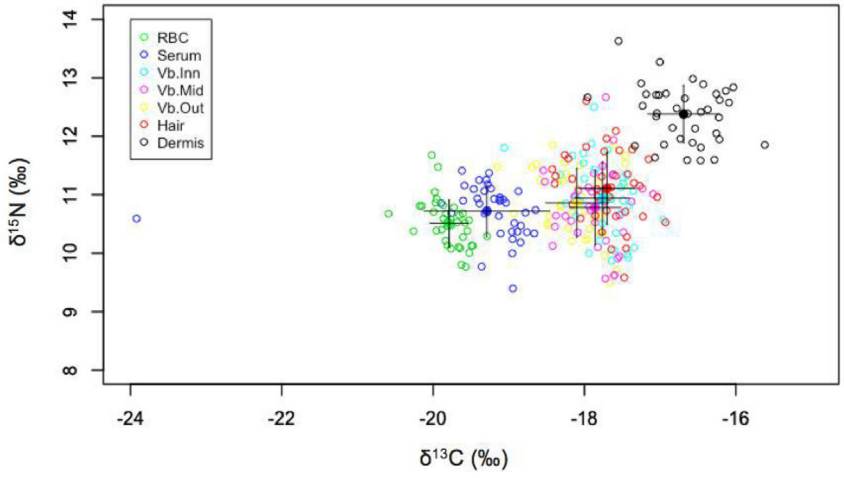

Fig. 2. Scatter plot of δ^{13}C values against δ^{15}N values for seven tissue types sampled from adult male walruses on Svalbard in 2014 and 2015 (n = 38). RBC = red blood cells; Vb. Inn = inner-vibrissal segment; Vb. Mid = mid-vibrissal segment; Vb. Out = outer-vibrissal segment. Open circles represent individual walruses whilst solid circles represent mean values. The latter are accompanied by error bars of standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4. Pathogen prevalence and relationships with predictor variables

For the sero-survey of anti-*Brucella* spp. antibodies, 26% of samples were classified as positive using the iELISA (10 of 39; Tables S5) and 23% with the RBT. The iELISA and the RBT produced similar results in 92% of cases. Fifteen % of samples (6 of 39) were positive for anti-*T. gondii* antibodies (Table S5).

The sequencing of the amplicons generated by the parapoxvirus PCR (GIF gene only) of a single nasal swab indicated the presence of a parapoxvirus (GenBank, accession number MF175205) (Table S5). However, there was no evidence of clinical symptoms resembling parapoxvirus infection in this animal. Neither of the remaining PCRs (B2L, IL10) provided positive samples. BLAST (NCBI) analysis demonstrated that forward and reverse sequences had highest homology with ORFV strains from sheep, as compared to a seal parapoxvirus isolate (Fig. S2).

Tusk volume (as a proxy for age) was significantly related to the presence of anti-*T. gondii* antibodies in the walruses (Fig. S3). Median tusk volume was larger (171 cm^3; 95% CI: 21, 320) in individuals that had anti-*T. gondii* antibodies compared to those in which the antibody was not present (Fig. S3). Dermis $\delta^{15}N$ values were significantly larger (0.46‰; 95% CI: 0.11, 0.79) in walruses that had anti-*Brucella* spp. antibodies compared to those in which the antibody was not present. No other parameters differed between the seropositive and seronegative individuals ($p \geq 0.08$; Fig. S3).

4. Discussion

This study provides new information on concentrations of persistent OHCs in adult male walruses from Svalbard, creating a second point in a time series (Wolkers et al., 2006). In addition, dietary information and prevalence of various pathogens has been surveyed, allowing for exploration of potential links between dietary exposure to OHCs and prevalence of pathogens.

Despite previous studies providing evidence of seal predation by walruses in Arctic Canada (Muir et al., 1995) and repeated observations of walruses in Svalbard eating seals (Marine Mammal Sightings Data Base (NPI) - photographic evidence, Wolkers et al., 2006), the range of $\delta^{15}N$ values identified in this study did not reflect consumption at differing trophic levels, since all individuals apparently fed at a low trophic level. $\delta^{15}N$ values in the walrus' mid-vibrissal segment varied between ratios of only 9.57 and 12.7‰, and those in the RBC between 9.77 and 11.7‰, values, which are representative of the consumption of benthic invertebrate prey. Bivalve muscle $\delta^{15}N$ in North Water Polynya is thought to range from 7.1 ± 0.1 to 11.8 ± 0.2‰ (Hobson et al., 2002) whilst reported $\delta^{15}N$ values of *Mya truncata* in Kongsfjorden, Svalbard, have been reported to be as low as 6.8 ± 0.5‰ (Vieweg et al., 2012). In contrast, the whiskers of ice-associated phocids such as the ringed seal from Svalbard (*Pusa hispida*); have $\delta^{15}N$ values between approximately 13 and 16‰ (Lowther et al., 2017), higher than $\delta^{15}N$ values detected in this study. Furthermore, comparative $\delta^{13}C$ data (Hobson and Welch, 1992) indicate that the walruses in this study ingested predominantly benthic dietary carbon sources, with $\delta^{13}C$ values very similar to those found in *M. truncata* (Vieweg et al., 2012). In addition, another study on walruses from Svalbard shows that fatty acid composition of the inner blubber resembled that of lipids in *M. truncata* and *Buccinum* spp. (Skoglund et al., 2010). Given that the formerly large Svalbard walrus population was decimated by 350 years of unregulated hunting (Kovacs et al., 2015), the benthic community in Svalbard likely has a carrying capacity that can support more individuals than occupy the region today. Nonetheless, climate-induced changes such as increased river run-off and reductions in sympagic nutrient inputs are likely to reduce benthic productivity in coastal and shelf areas in the coming decades, possibly forcing walruses to switch prey in the future (Bluhm and Gradinger, 2008; see also Seymour et al., 2014).

The specific turnover rates for walrus tissues are unknown (Seymour, 2014), though half-lives $\delta^{15}N$ and $\delta^{13}C$ in metabolically active tissues such as blood components range from a week to months in other large carnivores such as the polar bear (Rode et al., 2016). Hard tissues - such as hair and vibrissae - are metabolically inactive, therefore stable isotope signatures remain biochemically unchanged once deposited. However, the interpretation of stable isotope ratios in hard tissues is complex, as growth rates of specific tissues must be known. It is conceivable that walrus vibrissae exhibit an asymptotic growth curve, reaching almost full-length within a few months, as is seen in other pinnipeds (e.g. Greaves et al., 2004; McHuron et al., 2016), but this is not known with certainty. In addition, vibrissae are likely worn-down during intensive bottom-feeding, which is the norm for walruses.

Contaminant patterns in adult male walruses from Svalbard have remained quite consistent over the past two decades (Wiig et al., 2000; Wolkers et al., 2006; this study), with PCB 153, *p,p*-DDE, and oxychlordane remaining the most dominant compounds. However, comparable geometric means between walruses sampled in this study and from the same population sampled between 2002 and 2004 (Wolkers et al., 2006), indicate dramatic decreases in contaminant concentrations; e.g. *p,p*-DDE (124 ng/g lw, CI: 50–310 ng/g lw vs 13 ng/g lw, CI: 5.6–29 ng/g lw); oxychlordane (2047 ng/g lw, CI: 1036–4045 ng/g lw vs 421 ng/g lw, CI: 269–658 ng/g lw); ΣPCB (2160 ng/g lw, CI: 1165–4005 ng/g lw vs 814 ng/g lw, CI: 453–1461 ng/g lw).

Median PCB and oxychlordane concentrations were a few times higher in walruses compared to male harbour seals and ringed seals of both sexes sampled 2009–2010 (MOSJ, 2017; Routti et al., 2014). Conversely, ΣPCB and oxychlordane concentrations were approximately 20 and 50% of those found in female polar bears sampled in 2012 and 2013 (Tartu et al., 2017b), though ranges overlapped significantly. Plasma concentrations of the most dominant PFAS in Svalbard walruses were 5–6% of the values found in ringed seals and male harbour seals from the same region sampled in 2009–2010 (Routti et al., 2016; Routti et al., 2014). Median PFOS concentrations in walruses were only 1% of those found in polar bear females sampled in 2012–2013 (Tartu et al., 2017a). The relatively high PCB and oxychlordane concentrations, but low PFAS concentrations, in walruses compared to other marine mammals from Svalbard are likely related to spatial distribution of these compounds in the oceanic environment. Walruses feed mainly on benthos whereas ringed seals, harbour seals and polar bears are coupled primarily to pelagic food webs (Andersen et al., 2004; Derocher et al., 2002; Labansen et al., 2007; Skoglund et al., 2010; this study). A recent study shows that PFAS concentrations in the Northern Barents Sea are several times higher in the upper surface water column compared to deeper depths (Yeung et al., 2017). In contrast, sediments and deeper water masses are the main reservoirs for PCBs in the Arctic Ocean (Sobek and Gustafsson, 2014; Sun et al., 2016). When looking at PFAS pattern in the walruses, the concentration of PFOA relative to PFOS and ΣPFCAs was 4–35 times higher in walruses compared to ringed seals and harbour seals sampled from Svalbard (Routti et al., 2016; Routti et al., 2014). This is likely due to high concentrations of PFOA at depths where walruses consume filter-feeding organisms (Lowther et al., 2015; Yeung et al., 2017).

$\delta^{15}N$ values in the mid-vibrissal segment and RBCs were significant predictors of ΣPOP concentration in the walruses, despite the concurrent lack of evidence for consumption of high trophic level prey by walruses in this study. The extreme inter-individual variation in contaminant concentrations suggests that exposure of walruses feeding at the same, or similar, trophic level is highly

variable. The walruses might exhibit individual preference for benthic invertebrates of a specific species or size class, or they may be feeding in different areas. Tracking data has indicated that walruses from certain areas in Svalbard exhibit different migratory patterns, with some ranging more widely than others (Freitas et al., 2009; Lowther et al., 2015). Many travel to Franz Josef Land which is influenced by riverine inputs from the Russian mainland containing high concentrations of OHCs (Carroll et al., 2007). Additionally, concentrations of OHCs have been found to differ in beds of *M. truncata* at different locations around Svalbard, possibly as a result of the differential influx of Arctic vs Atlantic water (Vieweg et al., 2012). Though sampling location was not a consistent significant predictor of contaminant concentrations in this study (seen for mirex only), comparisons were limited by sample size and haulout locations in themselves might not necessarily provide a good indication of where the walruses fed. Contaminant concentrations also differ among various bivalve species found in Svalbard as shown for α-HCH as well as total chlordane concentration that were highest in *M. truncata* and lowest in *Serripes groenlandicus* (Vieweg et al., 2012). Consumption of different bivalve species at similar but non-identical trophic levels by individual walruses may explain the significant relationship between δ^{15}N values and ΣPOP concentrations. However, large inter-individual differences in contaminant concentrations may also be related to feeding habits in the past that are not detected by stable isotope analyses, amount of food ingested, body condition and/or biotransformation.

Walruses can live for up to 40 years (Kovacs and Lydersen, 2006) and males, unlike lactating females, do not offload contaminants (via nursing young). Thus, they accumulate high concentrations of contaminants and therefore age is commonly a reliable predictor of contaminant concentration (Born et al., 1981). However, in this study, as has been shown previously for this population (Wolkers et al., 2006), tusk volume (as a proxy for age) was not a significant predictor of contaminant concentration for either ΣPOP or ΣPFAS (Table 2). This may be either because there was insufficient variation in walrus age within the study group given the relatively small sample size and the bias toward sexually mature animals, or because tusk volume is not a precise predictor of age.

This study represents the first sero-survey for anti-*Brucella*-antibodies in Svalbard walruses. Seroprevalence was 26%, which is higher than that found in mixed sex groups of adult Atlantic walruses and ringed seals from Canada (Nielsen et al., 1997 $\leq$ 12% and $\leq$10%, respectively). Conversely, rates of up to 35% have been reported in mixed sex groups of young hooded seals (*Cystophora cristata*) from the West Ice, East Greenland (Tryland et al., 1999). In hooded seals, prevalence in pups and adults is low, with few seropositive individuals beyond the age of five years (Nymo et al., 2013b). This suggests that the bacteria are not passed from mother to young, and therefore that exposure is likely environmental (Nymo et al., 2013b). Terrestrial transmission pathways of *Brucella* bacteria (e.g. *Brucella abortus* and *Brucella suis*) are known to involve exposure to infected aborted material, where infection occurs via the ocular, respiratory or mucosal systems (Nymo et al., 2011) but given that female walruses move away from the herd for a few day when they are going to give birth (Fay, 1982), this is an unlikely path of transmission for walruses. Though *Brucella* spp. are not thought to cause complex pathological or clinical symptoms in adult phocids (Nymo et al., 2018), evidence indicates that the bacteria can cause abortions in dolphins (Ewalt et al., 1994). Effects in male livestock include epididymitis and orchitis (Corbel, 2006). However, since it is not known how this pathogen affects health and reproduction in walruses, nor how concurrent infection and contaminant exposure may affect disease development, *Brucella* spp. infection dynamics should be investigated further.

It is important to note that the use of serological techniques for the detection of antibodies can result in the generation of false positives due to cross-reactivity with bacterial strains presenting similar antigens. Furthermore, percentage corroboration between the iELISA and RBT tests may have been improved further by using a cleaning technique designed to remove excess lipid and debris from serum samples (Godfroid et al., 2016). In addition, the presence of anti-*Brucella* antibodies indicates only that the animal has at some point in time been infected, and does not confirm infection at the time of sampling, which could only be achieved by isolation of bacteria (Poester et al., 2010). Loss of circulating anti-*Brucella* antibodies can also result from chronicity of infection (Nymo et al., 2013b).

The seroprevalence of *T. gondii* in adult male walruses from Svalbard has been reported to be as low as 6% using the direct agglutination test (Prestrud et al., 2007), as compared to adult male polar bears which have demonstrated age-class dependent seroprevalences of 28–40% (Oksanen et al., 2009). Specific antibody prevalence for walruses in this study was 15%, which is similar to that found for male ringed seals (17%; age unspecified) but higher than that in adult male bearded seals (*Erignathus barbatus,* 8%) which are benthic foragers similar to walruses (Jensen et al., 2010).

The epidemiology of *T. gondii* in the Svalbard region is poorly understood, though mammals at Svalbard are thought to be infected by ingesting oocysts or parasitic stages present in animal tissue (Dubey et al., 2003). Walruses are likely exposed to infectious oocysts via their consumption of filter-feeding bivalves, since oocysts can be transported in fresh water run-off and ocean currents (Miller et al., 2002), and have been shown to concentrate in filter-feeding benthic molluscs (Lindsay et al., 2001). *T. gondii* DNA has also been recovered from bivalves inoculated with the pathogen experimentally (Arkush et al., 2003). Influx of increasingly warmer water masses may increase the viability of northward transported oocysts, resulting in increased exposure of this parasite to Arctic animals, including walruses (Jensen et al., 2010). Arctic foxes (*Vulpes lagopus*), phocids and migratory geese are intermediate hosts of the *T. gondii* parasite (Jensen et al., 2010; Prestrud et al., 2007), and walruses can encounter all of these animals in Svalbard. Clinical symptoms of *T. gondii* infection are not usually severe in marine mammals, with experimental infection of grey seals (*Halichoerus grypus*) resulting in only mild behavioural changes (Gajadhar et al., 2004). However, some fatal cases of associated encephalitis have been reported in sea otters (*Enhydra lutris*, Thomas and Cole, 1996) and pinnipeds (Migaki et al., 1977), whilst abortion and placentitis may result from infection in other mammals (Dubey, 2016). Immunosuppressed individuals can be particularly vulnerable to reactivation of *T. gondii* parasites (Miller et al., 2001).

Parapoxvirus was detected in a single walrus in this study using primers specific to the GIF gene; a virulence factor unique to the parapoxvirus and useful for rapid genus-identification (Klein and Tryland, 2005). This is the first detection of parapoxvirus in a wild walrus, where the detection of viral DNA from a swab sample obtained from the nasal mucosa demonstrates active viral shedding at the time of sampling. Phylogenetic analysis implicated that the amplified gene region was consistent with ORFV, a virus that is distributed world-wide in sheep and goats, although neither of these host species are found in Svalbard. Surprisingly, the amplified DNA sequences were more distantly related to parapox viruses isolated from seals, e.g. grey seals (Fig. S2), harbour seals and Weddell seals *Leptonychotes weddellii* compared to sheep (Klein and Tryland, 2005; Tryland et al., 2005). However, limited conclusions can be drawn without concurrent isolation and propagation of the virus. A common clinical symptom of infection with parapoxvirus is the presence of proliferative dermal lesions on the chest, flippers, neck and occasionally in the oral mucosa, which are

vulnerable to secondary bacterial infections (Tryland, 2011). No such clinical symptoms were noted when the sero-positive individual was sampled. Infection likely occurs when the virus enters lesions of the dermis and the oral mucosa, indicating a high risk of transmission for walruses that haul out in dense colonies.

The probability of walruses presenting anti-*T. gondii* and anti-*Brucella*-antibodies was not related to ΣPOP concentrations. However, natural killer cell function and T-lymphocyte cell function have been impaired in harbour seals contaminated with PCB concentrations >16000 ng/g lipid (de Swart et al., 1996). Though sample size of parapoxvirus positive individuals was limited in the present study, development of the clinical stage of this virus has been associated with immunosuppressive agents, both environmental (Parkinson et al., 2014) and viral, e.g. morbillivirus (Heide-Jørgensen et al., 1992). Any influence of contaminant-induced immunotoxicity on pathogen prevalence may become more apparent with increased exposure to pathogens resulting from climate change via changes in habitat suitability and therefore host range or migration routes (Jensen et al., 2010). Yet, the responses of parasites, viruses and bacteria to climate change are anticipated to be highly specific and their infection patterns in Svalbard walruses should be monitored closely into the future.

5. Conclusions

This study demonstrated an overall decrease in the concentration of lipophilic POPs in Svalbard walruses over the past decade. PFAS concentrations in walruses are low relative to other pinnipeds from this area. Large ranges in POP concentrations were found among individuals, despite the fact that all sampled individuals were adult males. Stable isotope analyses showed that this was not the result of seal predation by some walruses and it may instead be caused by spatial variation in contaminant concentrations among bivalves that occupy the same, or a similar, trophic level. In addition, new sero-surveys conducted for anti-*T. gondii* and anti-*Brucella* spp. antibodies provide an update of the prevalence of these pathogens circulating in Svalbard walruses, and PCRs revealed evidence of parapoxvirus infection. Though total contaminant load was not a significant predictor of antibody prevalence, more focused studies should investigate these relationships further, especially given that some individuals possessed exceptionally high OHC concentrations. This is especially pertinent given the influence of a changing climate on potential changes in pathogen prevalence, oceanic productivity and walrus habitat, which may result in dietary switching and consequentially differential contaminant and pathogen exposure in the future.

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Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.10.001.

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