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Research article

Testosterone and persistent organic pollutants in East Greenland male polar bears (*Ursus maritimus*)

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ABSTRACT

Legacy persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) are chemicals that undergo long-range transport to the Arctic. These chemicals possess endocrine disruptive properties raising concerns for development and reproduction. Here, we report the relationship between concentrations of testosterone (T) and persistent organic pollutant (POPs) in 40 East Greenland male polar bears (*Ursus maritimus*) sampled during January to September 1999–2001. The mean ± standard concentrations of blood T were 0.31 ± 0.49 (mean ± SD) ng/mL in juveniles/subadults (n = 22) and 3.58 ± 7.45 ng/mL in adults (n = 18). The ∑POP concentrations (mean ± SD) in adipose tissue were 8139 ± 2990 ng/g lipid weight (lw) in juveniles/subadults and 11,037 ± 3950 ng/g lw in adult males, respectively, of which Σpolychlorinated biphenyls (ΣPCBs) were found in highest concentrations. The variation in T concentrations explained by sampling date (season), biometrics and adipose tissue POP concentrations was explored using redundancy analysis (RDA). The results showed that age, body length, and adipose lipid content in adult males contributed (p = 0.02) to the variation in POP concentrations. However, although some significant relationships between individual organochlorine contaminants and T concentrations in both juveniles/subadults and adult polar bears were identified, no significant relationships (p = 0.32) between T and POP concentrations were identified by the RDAs. Our results suggest that confounders such as biometrics and reproductive status may mask the endocrine disruptive effects that POPs have on blood T levels in male polar bears, demonstrating why it can be difficult to detect effects on wildlife populations.

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1. Introduction

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) are chemicals that undergo long-range transport to the Arctic [1]. These compounds can be biomagnified in lipid-rich Arctic food chains and are generally found in high concentrations in apex predators such as polar bears (*Ursus maritimus*) and Arctic foxes (*Vulpes lagopus*) [2–7]. POPs are known to affect the health of Arctic apex predators [4,8–10]. The interaction between POPs and sex steroids, such as in previous reports on polar bears and Arctic foxes [11–15], might cause adverse effects on the reproductive system and thus lead to potential negative effects on population size.

In polar bears, changes in the reproductive rhythm are synchronized with sea-ice cover and accessability to prey, and mating is tightly regulated by the endocrine system [9,16]. Any interference with this system by POP exposure may consequently have detrimental effects on polar bear populations. Endocrine disrupting POPs in polar bears may therefore affect demographics and population size via a reduction in survival and recruitment [17,18]. It is therefore important to assess the possible harmful effects of POPs on circulating concentrations of reproductive hormones, which regulate mating and thus offspring production by male polar bears.

The negative effects of POPs on the male polar bears reproductive system are generally not taken into account when studying population level effects in wildlife [17]. Previous studies of polar bears and humans indicate that POPs may induce testicular and penile syndromes that influence semen quality and thus male fertility [19–21]. In humans, the so-called testicular dysgenesis syndrome (TDS) is well described and includes foetal-associated hypospadias, testicular malign neoplasms and cryptorchidism which, in adult life, may decrease testosterone (T) levels and semen quality [19,22,23]. Recent controlled studies on Arctic foxes suggest that POPs may affect circulating T levels in Arctic widlife in a way that may affect mating behaviour and even sperm quality [15]. Moreover, in American alligators inhabiting areas with high POP levels, penile and gonadal abnormalities including alterations to circulating testosterone concentrations has been reported [24]. Previous studies on polar bears also showed that POPs are likely to affect several steroids including sex steroids and cortisol [11,12,14,25]. For example, a study on the relationship between POPs and T levels in plasma of male Svalbard polar bears found that POPs in addition to body condition and season affected T concentrations [14]. In a recent study, a significant negative association between plasma concentrations of POPs and dihydrotestosterone (DHT) were reported in male polar bears in Svalbard [11]. In addition, the T levels appeared to be significantly confounded by biometrics such as body condition. Results from controlled studies of male Greenland sledge dogs (*Canis familiaris*) and farmed male Arctic foxes [13,15,25,26] suggest that POPs may also have effects on sex steroid concentrations in wild male polar bears [11,14]. Exposure to POPs may affect the steroidogenesis and steroid catabolism of male polar bears and is likely to occur via multiple endocrine pathways involving several organs and tissues [2,3,9,11,27]. Furthermore, during periods when stored body lipids are mobilised, lipophilic POPs are released from the adipose tissue and become bioavailable and remobilised in the blood [28] and subsequently to sensitive organs such as the thyroid glands, adrenals, gonads and central nervous system. Since the endocrine glands and the central nervous system are vital for reproduction, the starvation-related peaks in POP exposure during the reproductive season are of concern for Arctic wildlife [4,9,10].

The aim of the present study was to investigate the relationship between POPs levels in blubber (dense vascularized layer of fat beneath the skin thus, site of POP storage) and T levels in blood within and outside reproduction season as an indication of adverse effect on male reproductive endocrinology in East Greenland polar bears. This subpopulation is known to be among the most contaminated subpopulation in the Arctic [4,7,29,30], therefore, could represent the worst-case scenario of pollutant impact on reproduction system in wildlife. Moreover, the influence of age and biometrics as potential confounding factors on the POP-T relationship were investigated in a sample representing the reproductive annual cycle difficult to obtain from other subpopulation. Here we for the first time investigate the relationship between levels of POPs and T concentrations in East Greenland polar bears both within and outside the reproduction season, as an indication of an adverse effect on male reproductive endocrinology.

2. Materials and methods

2.1. Sampling and age estimation

Whole blood and subcutaneous adipose tissue samples were obtained from 22 juvenile/subadult male bears (0.5–5.5 years old) and 18 adult male polar bears (6–28 years) that were harvested by subsistence hunters between January 4th and September 27th during 1999–2001 from the Ittoqqortoormiit/Scoresby Sound area on the coast of central East Greenland between 69° 00′ N and 73° 30′ N. The study was approved by the Greenlandic (CITES export permits # 295/2000, 296/2000 and 297/2000) and Danish (CITES import permits # IM 0813–642/01 and IM 0312–080/01) authorities. Bears sampled between January 20th and June 20th were classified as within the reproduction season [31,32]. The samples were collected less than 12 h after the harvest and stored in separate polyethylene bags (adipose tissues) and lithium heparin vacutainers. Information on the animals were collected by the hunters in premade sampling sheets and included zoological body length and axial girth. Axial girth was measured as the circumference around the chest at the axilla. All samples were kept at −20 °C until adipose tissue samples were analyzed for POPs at the National Wildlife Research Centre at Carleton University, Canada and whole blood samples for T at Norwegian University of Science and Technology, Norway. Age estimation was determined by counting the annual dental cementum growth layer in lower right I_3 tooth taken from the sampled skulls [33].

2.2. Chemical analyses of POPs

Contaminant analyses were performed in adipose tissue from all 40 polar bear males sampled in 1999–2001. These 40 individuals

constitute a subsample of those bears in which POP concentrations previously were reported by Refs. [2,3]. The contaminant determination was conducted at the Organic Contaminant Research Laboratory at Environment and Climate Change Canada's National Wildlife Research Centre in Ottawa, Canada. Contaminants were extracted from polar bear adipose tissue and determined by gas chromatography-single quadrupole mass spectrometry (GC-MS) as described in Ref. [34] and more recently in Ref. [5]. Briefly, 0.5 g adipose tissue was homogenized with sodium sulfate and spiked with appropriate internal standards. The first fraction contained PCBs, OCPs (organochlorine pesticides) and PBDEs (polybrominated diphenyl ethers). Samples were monitored for 14 major PBDEs (BDE-17, -28, −47, −49, −66, −85, −99, −100, −138, −153, −154 (co-elutes with BB-153), −183, −190 and −209). Seventy-three PCBs were then analyzed, including CB-18, -17, −16/32, −31, −28, −33/20, −22, −52, −49, −47/48, −44, −42/59, −64/41, −74, −70/76, −66, −56/60, −95, −92, −101/90, −99, −97, −87, −85, −110, −118, −114, −105, −151, −149, −146, −153, −141, −130, −137, −138, −158, −128, −167, −156, −157, −179, −176, −178, −187/182, −183, −174, −177, −171, −172, −180, −170/190, −189, −202, −200, −199, −196/203, −208, −195, −207, −194, −206, and −201. Twenty organochlorine pesticides were analyzed, including chlorobenzenes (CBz), tetra- (TCBz), penta- (PCBz) and hexachlorobenzene (HCBz), α-, β- and γ-hexachlocyclohexanes (HCH), octachlorostyrene (OCS), mirex, six chlordanes (CHLs: heptachlor epoxide, oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor), DDTs (*p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT) and dieldrin. All concentrations were calculated and expressed as ng/g lipid weight (lw). Percent lipid was determined gravimetrically.

2.3. Analyses of testosterone

Testosterone concentrations in polar bear whole blood samples were analyzed with RIA (radioimmunoassay) diagnostic kit (Spectria® Testosterone RIA Coated Tube Radioimmunoassay) from Orion Diagnostica, Espoo, Finland. Due to logistical constrains, only whole blood was possible to collect in the field. Due to the freezing of whole blood and thus haemolysis, T was extracted with a modified method previously described by Ref. [35]. For final quantification gamma counter (Cobra Auto-Gamma; Packard Instruments Company, Dowers Grove, IL, USA) was used. All the samples were extracted in duplicates and each sample was analyzed in triplicate. Accuracy of the procedure was tested with intra-laboratory reference materials (extracted whole blood and bovine plasma) and standard reference material (BIO-RAD, Lyphochek® Immunoassay Plus Control, Level 2, SRM2, 11.8 nmol/L). The procedure (including quality assurance) is described in Supplementary Information.

2.4. Statistical analyses

Only contaminant variables with detection rates of more than 60% in all samples were used in the statistical analysis. For these contaminants and T, concentrations below the detection limits (n = 8 for T) were replaced with values calculated based on the robust regression on order statistics (robust ROS, [36]). Capture date was formatted to ordinal date (Julian day 1–366). Distance-weighted least-squares (DWLS) nonparametric curve fitting was used to visualize the relationship between T and capture date. Due to possible seasonal variation between age classes, T concentrations were compared with Kruskal-Wallis tests followed by multiple comparisons between juvenile, adults in reproduction and non-reproduction seasons. POP concentration differences between juvenile/subadults and adults, were tested with the Mann–Whitney *U* test. Associations between T and different biological (i.e., age, length, waist circumference, adipose tissue lipid content) and contaminant variables were tested using Spearman rank correlation test. Bonferroni correction was not applied when comparing associations between multiple variables because of the increased probability of producing false negatives [37]. As POP concentrations and biological variables typically intercorrelate with one another, principal component (PCA) and redundancy (RDA) analyses were conducted to determine whether POPs influenced T concentrations in polar bears (PCA), while accounting for the potential masking effect of relevant biological variables (RDA). Due to non-normal distribution of the data, T and POP concentrations were log-transformed ($\log_{10}$ x+1) to approximate normal distribution. The dataset was separated into juvenile/subadult and adult males, and the explanatory power of response variables (T concentration, sampling date, age, length, waist circumference and adipose lipid %) were incorporated into a stepwise partial RDA model. The explanatory power of each variable (R^2) was compared to the null model (i.e. PCA) by a permutation test. The variables with the greatest explanatory power were conditioned and forward selection was continued until no variables significantly accounted for the remaining variation within the pollutant dataset

Table 1
Capture date (Julian day of sampling from 1 to 366), age, biometric variables and adipose lipid content of adult (n = 18) and juvenile/subadult (n = 22) male polar bears sampled between 1999 and 2001 in the Ittoqqortoormiit/Scoresby Sound area on the coast of central East Greenland.

Variable	Class	Mean	SD	Median	Min	Max
Capture date (1–366)	Juvenile/subadult	156	107	227	4	270
	Adult	127	95	83	24	267
Age (years)	Juvenile/subadult	3.4	1.3	3.3	0.5	5.5
	Adult	10.3	6.6	8.0	6	28
Contour body length (cm)	Juvenile/subadult	197	36	200	122	260
	Adult	232	20	232	200	276
Axial girth (cm)	Juvenile/subadult	163	38	155	82	240
	Adult	182	29	187	128	250
Lipid content (%)	Juvenile/subadult	93.6	5.5	94.2	83.1	100.0
	Adult	81.4	10.5	82.6	53.6	99.0

[38]. Analyses were carried out in R v. 3.4.1 using the vegan package v. 2.4–4 [39,40] and Statistica version 13 (Dell Inc., 2016). The statistical significance was set to $\alpha = 0.05$.

3. Results

Details on ordinal date (sampling day of the year), age, biometric variables and adipose lipid content are presented in Table 1. As expected, adult males were larger than the juvenile/subadult male polar bears, although the percent lipid in adipose tissue was higher in juveniles/subadults than in adults (MW-U test, $p = 0.00008$). Concentrations of T in the juveniles/subadults ($n = 22$) and adult ($n = 18$) East Greenland male polar bears based on the reproductive season are presented in Table 2 and Fig. 1. T was found to be below the detection limit in six juvenile/subadults and two adult males. A seasonal pattern was found for adult males and differences were observed between adults in reproductive season and juvenile/subadults during polar bear reproductive season (Kruskal-Wallis test: $H = 19.0$, $p = 0.0001$). Mean T concentrations in juvenile/subadults ($n = 22$, 0.31 ng/mL, median = 0.137, SD = 0.49, range < limit of detection (LOD) - 2.16) were significantly lower (multiple comparison test) than in adults in reproductive season ($n = 11$, 5.62 ng/mL, median = 1.59, SD = 9.1, range 0.53–29.6, $p = 0.00006$), and adults in reproductive season differed significantly from adults in non-reproductive seasons ($n = 7$, 0.38 ng/mL, median = 0.29, SD = 0.40, range < LOD - 1.07, $p = 0.01$).

Mean concentrations of the analyzed POPs are illustrated in Fig. 2 and given in Table S1. $\sum PCB_{36}$ were found in the highest concentrations followed by $\sum CHL_6 > \sum DDT_3 > \sum HCH_3 >$ dieldrin $> \sum CBz_2 > \sum PBDE_{15} >$ OCS for both juvenile/subadult and adult male polar bears. Higher contaminant concentrations were found in adults (MW-U test, $p < 0.05$) for both the total concentration of all detected POPs, $\sum PCB_{36}$ and for the individual compounds (compounds in order of increasing p value): CB-206, CB-194, CB-170/190, CB-180, CB-200, CB-201, CB-172, CB-203/196, CB-195, BDE-153, CB-129/178, CB-153, β-HCH, BDE-100, CB-171/202/156, CB-183 (Table S1). Moreover, higher contaminant concentrations were found during the reproduction season for the total concentration of all detected POPs and $\sum PCB_{36}$ and for the individual compounds: CB-170/190, CB-180, CB-200, CB-194, CB-206, CB-153, CB-171/202/156, CB-99, CB-172, β-HCH (compounds in order of increasing p value). The only compound found in a lower concentration (MW-U test, $p = 0.04$) during the reproductions season, was the DDT metabolite *p,p'*-DDE.

Variation in concentrations of POPs among juveniles/subadults and adult males was analyzed by PCA (Fig. 3). There was a tendency for adults to be separated from juveniles/subadults along PC2 indicating higher concentrations of higher chlorinated PCBs in adult male bears than in juvenile/subadult male bears.

According to the RDA, the biological variables age, length, and adipose percent lipid contributed significantly to the total variation in pollutant concentrations in adult males ($R^2_{age} = 0.13$, $P = 0.03$; $R^2_{length} = 0.14$, $p = 0.02$; $R^2_{lipid\text{-}\%} = 0.16$, $p < 0.01$), but not to the variation in the T concentrations ($R^2_{testosterone} = 0.05$, $p = 0.35$). In juveniles/subadults, none of the biological variables contributed

Table 2
Testosterone concentrations (ng/mL) in adult ($n = 18$) and juvenile/subadult ($n = 22$) male polar bears (*Ursus maritimus*) in reproductive/non-reproductive season.

Class	reproductive season	n	Mean	SD	Median	Min	Max
Juvenile/subadult	yes	6	0.371	0.439	0.268	0.010	1.17
Juvenile/subadult	no	16	0.288	0.524	0.137	0.003	2.16
Adult	yes	11	5.62	9.09	1.59	0.527	29.6
Adult	no	7	0.377	0.398	0.290	0.006	1.07

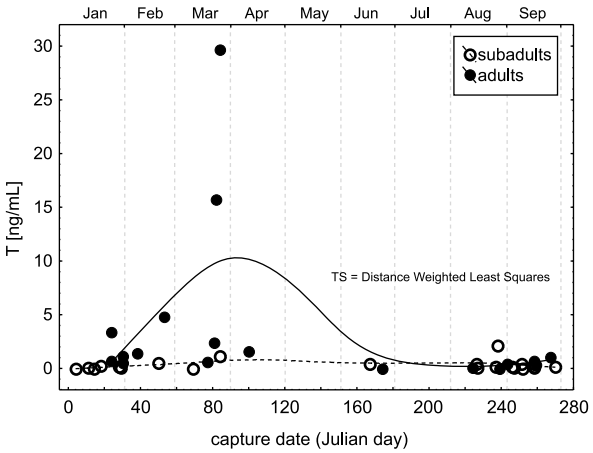

Fig. 1. Seasonal concentrations of mean testosterone (T mean ± SD) in juvenile/subadult ($n = 22$) and adult ($n = 18$) East Greenland male polar bears sampled 1999–2001. Distance-weighted least-squares (DWLS) nonparametric curve fitting was used to visualize the relationship between T and capture date (Julian day).

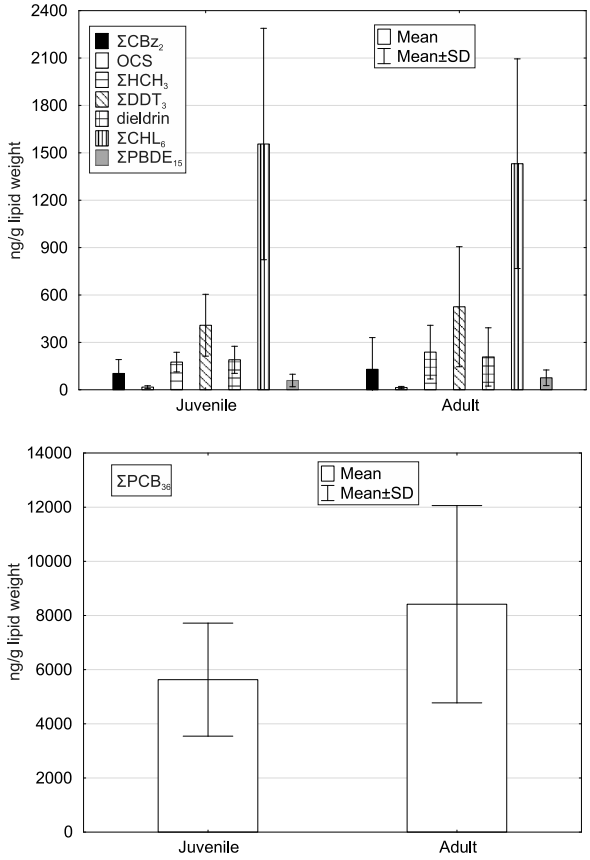

Fig. 2. Concentrations of POPs in juvenile/subadult (n = 22) and adult (n = 18) East Greenland male polar bears sampled 1999–2001. **Top:** six organochlorines and polybrominated diphenyl ethers. **Bottom:** sum of 36 PCB congeners.

significantly to the total variation in pollutants or T concentrations ($R^2_{testosterone} = 0.06$, $p = 0.22$, Table 3). However, when associations between the single adipose tissue contaminant concentrations and T in the adult and juvenile/subadult males were tested individually with Spearman rank correlations, CB-60 and *trans*-chlordane were both negatively associated with the testosterone concentration in adult males (Table 4). In the juvenile/subadult males, β-HCH, CB-118, and CB-74 were negatively associated with T (Table 4). In contrast, in adult males, Spearman rank correlations revealed positive associations for the congeners CB-194 and CB-206 during the reproduction season. In adult males, outside of the reproductive season, we found negative associations between CB-97 and ∑HCH, and T concentrations, while there was a positive correlation between adipose percent lipid and T concentrations (Table 5).

4. Discussion

4.1. Testosterone concentrations

The higher concentrations of T in adult males compared to juvenile/subadult males were expected due to sexual maturity and because T plays an important role in regulating reproductive physiology in post-pubertal males. This is in accordance with previous studies in male polar bears from Svalbard [11,14]. The T concentrations reported in all adults in the present study (n=18, 3.58 ng/mL) were similar to values previously reported in adult male Canadian polar bears (2.3 ± 0.4 ng/mL, n = 57 [41]. Plasma concentrations of up to 23 ng/mL in adult males from Svalbard have been reported during the mating season (between March and May) [14], whereas we observed a slightly higher maximum T concentration of 30 ng/mL during the same period. In another recent study from Svalbard [2,3], found that average T concentrations in serum of adult and juvenile/subadult male polar bears (April) were 13.6 (range: 1.17–32 ng/mL) and 0.8 (range: 0.01–2.4 ng/mL), respectively.

Overall, the results from the present study are in accordance with previous studies and demonstrate that T levels peak in adult male polar bears during mating, reaching levels up to approximately 30 ng/mL, while in the non-breeding season T levels in adult males are approximately 1.0 ng/mL, similar to levels found in juveniles [41,42]. In addition, the results indicate that the whole blood concentrations of T are comparable with serum/plasma T concentrations from other studies. This is in agreement with recent studies, where venous serum blood samples were compared with whole capillary blood with LC-MS/MS method in humans [43].

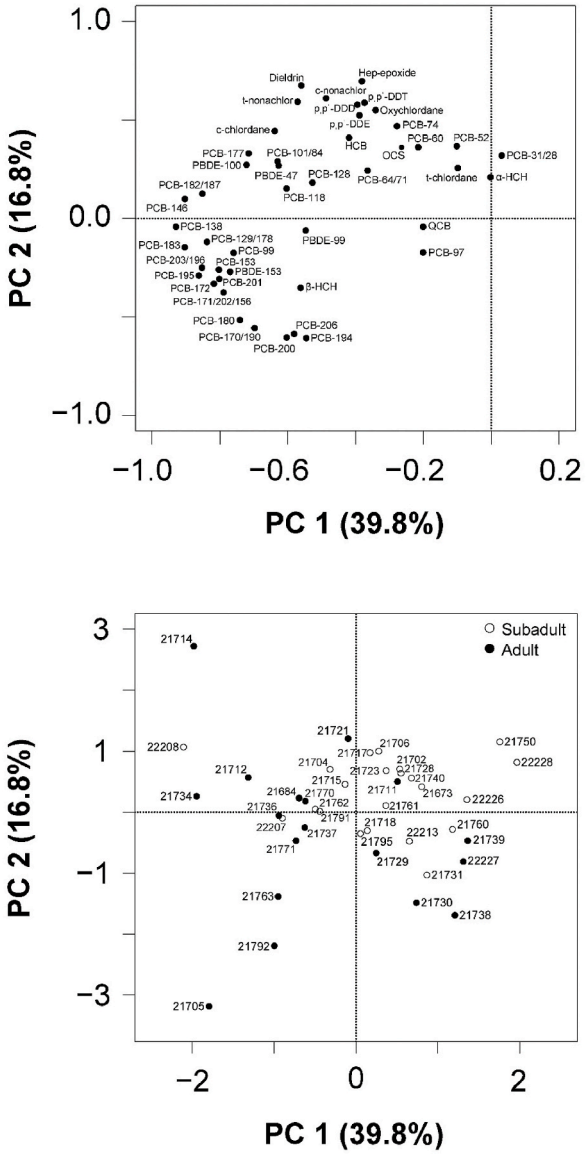

Fig. 3. Ordination diagram of PCA of POPs in the 40 East Greenland male polar bears. **Top:** variables (contaminants). **Bottom:** Individual (IdNos) juveniles/subadults and adult polar bears.

4.2. Testosterone and contaminants

Recent studies strongly indicate that the effects of POPs on circulating concentrations of steroid hormones in Arctic carnivores, including polar bears, are mainly driven by PCBs and their hydroxylated metabolites [2,3,11,13–15,25,44–46]. The present results further suggest that PCBs and other POPs may influence steroid hormones in male polar bears, as univariate statistical methods showed significant negative associations between T and the chemicals CB-60, CB-74, CB-118, *trans*-chlordane and β-HCH. Similar associations have been reported previously [14], where oxychlordane (metabolite of chlordanes), β-HCH and 9 of 16 individual PCB congeners (including CB-118) were found to be negatively related to T in male polar bears from Svalbard, Norway. In humans, serum testosterone levels were found to be negatively related to only more persistent PCBs (such as CB-74 and CB-118) in adolescent Akwesasne Mohawk males with documented toxicant exposure [22]. In adult male polar bears sampled in the non-reproductive season, CB-97 and ∑HCH were negatively associated with T concentration. In contrast, adult male polar bears sampled in the reproductive season showed positive associations of high chlorinated PCBs, such as CB-194 and CB-206 with T. This reflect the complex nature of the chemical activity in the bears' contaminant burden as well as reduced food intake during the reproductive season, which releases lipophilic PCBs from fat reserves leading to a difference in the CB-congeners association with T in the reproductive vs. non-reproductive season. Even though we found no significant relation between the adipose percent lipid in bears during the reproductive season, it was positively

Table 3
Summary of test statistics from the redundancy analysis (RDA) in 40 male polar bears from East Greenland. Only the PCA (null model) and models containing all relevant biological variables (full model) are shown. *P*-values for the models with the most significant explanatory variables from the forward selection process are listed in bold.

Model	Variance	Variation accounted (%)	F-score	*P*-value
Adult males (n = 18)				
PCA: null model (Pollutants)	46	37.6 (PC1); 22.5 (PC2)	–	–
RDA: full model (Testosterone, Ordinal date, Age, Axial girth, Body length, Lipid content)	22.39	48.7	1.74	**0.02**
Pollutants ~ Age	5.69	12.4	2.26	0.05
Pollutants ~ Lipid content + Condition (Age)	5.27	11.5	2.26	0.05
Pollutants ~ Body length + Condition (Age + Lipid content)	**6.19**	**13.5**	**3.01**	**0.02**
Pollutants ~ Testosterone + Condition (Age + Body length + Lipid content)	2.09	4.5	1.01	0.32
Pollutants ~ Age + Condition (Body length + Lipid content)	**5.82**	**12.6**	**2.82**	**0.04**
Pollutants ~ Bony length + Condition (Age + Lipid content)	**6.19**	**13.5**	**3.01**	**0.02**
Juvenile/subadults (n = 22)				
PCA: null model (Pollutants)	46	45.0 (PC1); 13.5 (PC2)	–	–
RDA: full model (Testosterone, Ordinal date, Age, Axial girth, Body length, Lipid content)	13.45	29.2	1.03	0.41
Pollutants ~ Testosterone	2.75	6.0	1.27	0.22

Table 4
Spearman Rank correlation analyses between the single predictor variables and testosterone in adult (n = 18) and juvenile/subadult (n = 22) male polar bears (*Ursus maritimus*).

Age group	Predictor	r	p
Adults	CB-60	−0.538	0.020
	t-chlordane	−0.488	0.040
Juvenile/subadults	β-HCH	−0.602	0.003
	CB-118	−0.531	0.011
	CB-74	−0.491	0.020
	Lipid-%	0.438	0.041

Table 5
Spearman Rank correlation analyses between the predictor variables and testosterone in adult male polar bears (*Ursus maritimus*) during the reproduction (n = 11) and non-reproduction (n = 7) seasons.

Season	Predictor	r	p
Reproductive	CB-194	0.691	0.019
	CB-206	0.627	0.039
Non-reproductive	CB-97	−0.857	0.014
	∑HCH	−0.821	0.023
	Lipid-%	0.821	0.023

related to T in bears during the non-reproductive season. In contrast it has been found, that percentage extractable plasma fat showed a significant reduction with increasing T concentrations for bears sampled in March, April, May and August [14]. This can be explained by the differences between the sampling periods, where in the present study the non-reproductive season was defined to be early January and during the entire summer season (June 20th-September 20th).

However, it is important to acknowledge that, in the present study, levels of most POPs were not correlated with T concentrations. This is in accordance with a previous study [11], in which T concentrations were strongly correlated with biometrics, in particular body condition index (BCI), but not with POP levels in adult males from Svalbard. Combined, that study [11] and the present study demonstrate that in polar bear males, T concentrations are independently associated with both biometric variables, such as the BCI, and the reproductive cycle. Thus, both these biological factors may serve as important confounding factors, masking any effects that POPs may have on male T levels in wild populations.

In controlled studies of relevant model species (sledge dogs and farmed Arctic foxes), in which diet, genetic variation and biometrics was at least partly controlled using brother siblings, long-term oral exposure to POPs negatively affected T concentrations in males even at lower burdens of POPs in fat (sledge dogs) and total body concentrations (Arctic foxes) [9,13,15,26]. If exposure to POPs reduces T production during the reproductive period, as indicated in farmed Arctic foxes [15], it may be critical since it is vital for semen production and reproductive health. Therefore, an attenuated T production is of concern and may indeed influence the breeding outcome of Arctic predators [6,15].

4.3. POPs effects on reproduction

That the current study found associations with certain POPs and T blood concentrations, adds to the weight of evidence that POP exposure may exert negative effects on male polar bears during the mating season [14], but confounding factors such as biometrics and reproductive status may mask these effects [11]. This is corroborated by studies on male farmed foxes [15,45]. A reduction in circulating concentrations of T at the time of mating caused by high body burdens of POPs may have negative effects on the reproductive success of male polar bears. Males with high POP concentrations and resultant low T levels may be less attracted to mating and pair bonding, and show less aggressive behaviour towards other males when competing for females. Thus, one hypothesis could be that high POP/low T males compete less for access to females compared to low POP/high T males. If large parts of the male reproductive population suffer from high POP levels, this may have a significant negative effect on population level due to possible changes in mating behaviour and reproductive success [17]. A physiologically-based pharmacokinetic (PBPK) model showed that POPs affect the reproduction in 11 polar bear subpopulations across Canada, Greenland and Svalbard [44]. In another meta-study analysing data from 14 polar bear subpopulations, it was shown that sub-population density was negatively associated with internal concentrations of certain POPs, including PCBs, dieldrin, DDT and PBDEs [47]. Temporal trend studies have shown that polar bears in East Greenland have experienced periods of elevated POP exposure prior to the present study, and hypothetically this may have had even more severe effects on T levels compared to present days [2,3,48,49].

Arctic-adapted species are specifically vulnerable to POP exposure because they experience seasonal periods where adipose tissue depots are mobilised and the accumulated POPs may be released to the blood stream and become bioavailable [4,9]. In this study, higher concentrations of POPs in adipose tissue of adult male polar bears than in juvenile/subadults were observed for both $\sum$POPs and $\sum PCB_{36}$ and for the individual compounds: CB-170/190, CB-180, CB-200, CB-194, CB-206, CB-153, CB-171/202/156, CB-99, CB-172, β-HCH. Moreover, in adult males, higher concentrations were found during the reproduction season than during the non-reproductive season for the total concentration of all detected POPs and $\sum CB_{36}$. When POPs are released from adipose cells to the general circulation, the endocrine disruption increases and e.g. T production may decrease which again may affect sperm quality in adults which mate during spring-peak [15]. Such effects on reproduction have previously been suggested for East Greenland polar bears using different physiological, statistical and individual-based modelling [17,44,46,48,50].

5. Conclusions

The concentrations of T in male juveniles/subadults and adults were similar to concentrations reported in other studies in polar bears from Canada and Svalbard. There were significant correlations between concentrations of T and age, biometrics and adipose tissue lipid content. Although significant inverse relationships between T and specific organochlorine contaminants were observed in both juvenile/subadult and adult male polar bears, overall concentrations of contaminants in adipose tissue could not explain the variation in the blood T concentrations in the bears. The present and previous studies therefore indicate that confounders such as biometrics and reproductive status exert such pronounced effects on male T concentrations that any effects of POPs on T levels may be difficult to identify in wild populations. However, meta-analyses combining POP and T data from a larger sample of bears across many regions over a longer period and accounting for reproductive status may provide insight on the overall effects that POPs may have on T levels in the future.

Author contribution statement

Tomasz M Ciesielski: Analyzed and interpreted the data; Wrote the paper.

Christian Sonne: Conceived and designed the experiments; Analyzed and interpreted the data.

Eli I Smette; Gro Dehli Villanger: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bjarne Styrishave; Daniel J Hitchcock: Analyzed and interpreted the data.

Robert Letcher: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Rune Dietz: Conceived and designed the experiments; Analyzed and interpreted the data.

Bjørn Munro Jenssen: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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