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Relationships between POPs, biometrics and circulating steroids in male polar bears (*Ursus maritimus*) from Svalbard

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Abstract

The aim of this study was to determine the effect of persistent organic pollutants (POPs) and biometric variables on circulating levels of steroid hormones (androgens, estrogens and progestagens) in male polar bears (*Ursus maritimus*) from Svalbard, Norway (n=23). Levels of pregnenolone (PRE), progesterone (PRO), androstenedione (AN), dehydroepiandrosterone (DHEA), testosterone (TS), dihydrotestosterone (DHT), estrone (E1), 17α-estradiol (αE2) and 17β-estradiol (βE2) were quantified in polar bear serum by gas chromatography tandem mass spectrometry (GC-MS/MS), while POPs were measured in plasma. Subsequently, associations between hormone concentrations (9 steroids), POPs (21 polychlorinated biphenyls (PCBs), 8 OH-PCBs, 8 organochlorine pesticides (OCPs) and OCP metabolites, and 2 polybrominated diphenyl ethers (PBDEs)) and biological variables (age, head length, body mass, girth, body condition index), capture date, location (latitude and longitude), lipid content and cholesterol levels were examined using principal component analysis (PCA) and orthogonal projections to latent structures (OPLS) modelling. Average concentrations of androgens, estrogens and progestagens were in the range of 0.57-83.7 (0.57-12.4 for subadults, 1.02-83.7 for adults), 0.09-2.69 and 0.57-2.44 nmol/L, respectively. The steroid profiles suggest that sex steroids were mainly synthesized through the Δ-4 pathway in male polar bears. The ratio between androgens and estrogens significantly depended on sexual maturity with androgen/estrogen ratios being approximately 60 times higher in adult males than in subadult males. PCA plots and OPLS models indicated that TS was positively related to biometrics, such as body condition index in male polar bears. A negative relationship was also observed between POPs and DHT. Consequently, POPs and body condition may potentially affect the endocrinological function of steroids, including development of reproductive tissues and sex organs and the general condition of the male polar bears.

1. Introduction

The Arctic region is a pristine environment with few local pollution sources. Nevertheless, the ecosystems in this region are strongly affected by global pollution (Riget et al 2010; 2011; Letcher et al 2010). The contamination sources are most often located outside the Arctic region, and long-range atmospheric transport of pollutants delivers most of the persistent organic pollutants (POPs) to the region (Riget et al. 2010). Other major routes for contaminants are via north flowing rivers from mid-latitude areas (Wania and Mackay 1993; 1995), such as Northern Eurasia and North America, which are flowing into the Arctic oceans where they enter the Arctic food chain (Lohmann et al. 2007). Since most POPs are lipophilic they accumulate in lipid-rich tissues of living organisms, and thereby biomagnify in the food web (Letcher et al. 2010, Blais et al., 2005). POPs include many different contaminant groups such as organochlorine pesticides (OCPs), including dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs) used as flame-retardants, and industrial organochlorines (OCs) such as polychlorinated biphenyls (PCBs).

Polar bears (*Ursus maritimus*), as top predators, generally have high levels of POPs (Letcher et al. 2010). The Svalbard (Norway) and East Greenland polar bears are reported to have the highest PCB concentrations, while lower levels are found in North American sub-populations of polar bears (Haave et al, 2003; Verreault et al. 2005, Letcher et al. 2010). The PCB congeners 99, 118, 138, 153, 156, 170, 180 and 194 have been reported to be the most abundant PCBs in Svalbard polar bears (Bernhoft et al. 1997; Bytingsvik et al. 2012a). However, polar bears have an efficient cytochrome P450 system, and can metabolize POPs (Letcher et al 2009). This high metabolic capacity may lead to an accumulation of metabolites such as hydroxylated PCBs (OH-PCBs) (Verreault et al. 2005, Letcher et al. 2010; Bytingsvik et al 2012a), which in some cases may be more toxic to polar bears than the parent PCB congener (Therani and Van Aken 2014; Gustavson et al., 2015a). Most biotransformed OH-PCBs are easily excreted, while the remaining OH-PCBs are limited to 5-10 persistent single congeners and are mainly transformed from persistent penta-, hexa- and heptachlorinated PCBs (Letcher et al., 2000). The most commonly found OH-PCBs are the 4-OH-CB107, 4-OH-CB146, 4'-OH-CB172 and 4-OH-CB187 (Gebbink et al 2008; Bytingsvik et al 2012a; Gustavson et al 2015a).

Exposure to PCBs and OH-PCBs may potentially affect the endocrine system, and certain OH-PCBs have been suggested to affect steroid homeostasis in female polar bears from Svalbard (Gustavson et al 2015a). Pesticides such as DDT and HCB are also found in polar bears in high concentrations and may potentially interact with their reproductive hormones (Oskam et al., 2003; Gustavson et al

2015a). PBDEs have been reported to act as agonists by binding to estrogen receptors (ERs) *in vitro* (Meerts et al., 2001). In the Arctic food chain, most PBDEs have been found to biomagnify in the lower trophic levels. In the upper part of the food chain, however, biomagnification appears to be lower, perhaps due to higher metabolism. For example, Only BDE-153 was reported to biomagnify in polar bears (Sørmo et al., 2006), although other BDEs were also present.

Despite obvious potential interactions, not much is known about the relationship between steroids, and body size and condition, in male polar bears. In addition to controlling male characteristics such as reproductive organs, testosterone (TS) is also involved in the development of secondary male characteristics, and it is well established that both TS and dihydrotestosterone (DHT) have anabolic effects on the skeleton and muscles (Wiren 2005; Clarke and Khosla, 2009; Thakur 2016). The production and secretion of most sex hormones are controlled by neurons and negative hormone feedback regulation involving the hypothalamic–pituitary–gonadal axis (HPG axis) (Hill et al., 2008). This regulation of steroid hormones is linked to biological factors such as sexual maturity and body size, but toxic chemicals may impede the endocrine system (Klaassen, 2008). In mammals, TS is the primary androgenic steroid in males and is mainly secreted from the Leydig cells present in the testes. When TS has reached its target organ, it may be metabolized into (DHT), or bind directly to the androgen receptor (AR). Other androgens, such as androstenedione (AN) and dehydroepiandrosterone (DHEA), have lower androgenic potency and function mainly as precursors for TS (Nieschlag and Behre, 2004). Both TS and DHT elicits their effects through binding to the AR. However, DHT amplifies the effect of TS due to a stronger affinity for AR (Hill et al., 2008; Kovacs 2012). Testosterone and AN can be further metabolized into estrogens by the aromatase (Miller and Auchus, 2011). An overview of relevant steps in the gonadal steroidogenesis is illustrated in Supplement Material Figure S1.

The aim of the present study was to investigate interactions between biometrics, POPs and circulating steroid levels in male polar bears. We therefore analysed 9 circulating steroid hormones and the steroid precursor cholesterol, along with biometric variables. Furthermore, we analysed circulating concentrations of 8 OCPs, 21 PCBs, 8 OH-PCBs and 2 PBDEs in 23 male polar bears between 3 and 21 years of age. The relationships between biometrics, steroids and POPs were investigated using principal component analysis (PCA) and bivariate correlations. To identify the most potent variables explaining the variation in androgen concentrations, the effects of biometric and POP variables on steroid concentrations were modelled using orthogonal projections to latent structures (OPLS).

2. Materials and Methods

2.1. Sampling

The polar bear samples were collected at Svalbard in April 2008 as part of the International Polar Year project BearHealth. The sample location ranged from Vitovskybreen in the south (76.7 °N) to Waldenøya in the north (80.6 °N), and from Liefdefjorden in the west (12.6 °E) to Duvefjorden in the east (23.8 °E). Capture and handling procedures followed standard protocols (Derocher and Wiig, 2002; Stirling et al., 1989) and were approved by the National Animal Research Authority (NARA, Oslo, Norway). The bears were sedated for sampling by remote injection of a dart containing Zoletil (tiletamine/zolazepam, 200 mg/mL; Virbac Laboratories, Carros, France), fired from a helicopter. The rifle used was a Cap-Chur rifle with 5 or 7 ml metal darts and one barb needles of 20-42 mm in length, depending on the sex, age and condition of the bear. The date of each individual sampling event was recorded as the ordinal date (0-366). The age for each bear was estimated from the number of annual growth layer groups (GLGs) in a rudimentary premolar tooth for adults that had not been captured earlier (Christensen-Dalsgaard et al 2010). Alternatively, age was calculated based on recapture of the bears captured as juveniles, of which age was known.

The sampled polar bears were divided into two age classes, those from 5-21 years (n=17) were categorized as adults, while 3 and 4 year olds (n=6) were termed sub adults (Rosing-Asvid et al 2002). The straight-line body length (SLBL) was measured as the dorsal straight line from the nose tip to the caudal end of the last tail vertebrae. The contour body length (CBL) was measured as the distance from the tip of the nose to the tip of the tail along the contour of the spine, when the bear was aligned laterally. Axial girth was measured as the circumference around the chest at the axilla. The head length was recorded as the straight-line length between the upper middle incisors at the gum line, to the most posterior dorsal scull process of the sagittal crest. Zygomatic width was the maximum width between the zygomatic arches. Body mass (BM) was estimated based on the body length and axial girth using the equation given by Derocher and Wiig (2002): body mass = $0.00003377 \cdot \text{axial girth}^{1.7515} \cdot \text{body length}^{1.3678}$. To obtain an indication of the condition of the polar bears, we calculated the body condition index (BCI) according to Cattet et al. (2002) using the formula: $BCI = (\ln BM - 3.07 \cdot \ln SLBL + 10.76) / (0.17 + 0.009 \cdot \ln SLBL$

Approximately 60 ml (6 x 10 ml) of blood was collected from the bears using 40 mm needles (21G) and stored at 4 °C. The collected blood samples were centrifuged with anti-coagulant in heparinised Venoject tubes (10 ml, Thermo Electron Corporation, Belgium) and without anti-coagulant for

160 separation of plasma and serum, respectively. Centrifugation was performed within eight hours after
161 sampling. Serum samples were used for steroid hormone determination, and plasma was used for
162 POPs and cholesterol. The procedures related to the sampling are described more closely in
163 Bytingsvik et al (2012a,b).
164
165 **2.2. Determination of environmental contaminants**
166 Plasma samples were analysed for concentrations of OCPs and their metabolites, PCBs, OH-PCBs and
167 PBDEs using gas chromatography electron capture detection (GC-ECD) and gas chromatography
168 mass spectroscopy (GC-MS/MS). The multicomponent method with extraction and clean-up
169 methods, chromatographic separation, equipment and quality control is described in detail in
170 Bytingsvik et al. (2012a) and Sørmo et al. (2006). The following groups of contaminants were
171 analysed: 8 OCPs and metabolites (HCB, α-HCH, β-HCH, oxychlordane, trans-nonachlor, mirex, p,p'-
172 DDE, p,p'-DDT), 21 PCBs (PCB-47, PCB-74, PCB-99, PCB-101, PCB-128, PCB-137, PCB-138, PCB-153,
173 PCB-170, PCB-180, PCB-183, PCB-187, PCB-194, PCB-206, PCB-105, PCB-114, PCB-118, PCB-156, PCB-
174 157, PCB-167, PCB-189), 8 OH-PCBs (4'-OH-CB107, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-
175 OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187) and 2 PBDEs (BDE-47 and BDE-154).
176
177 **2.3. Steroid hormone analysis**
178 Serum samples were analysed by GC-MS/MS for determination of steroid hormone levels in
179 accordance with Hansen et al. (2011). The following nine steroids were quantified: pregnenolone
180 (PRE), progesterone (PRO), DHEA, AN, TS, DHT, estrone (E1), 17α-estradiol (αE2) and 17β-estradiol
181 (βE2). These hormones cover most of the testicular steroidogenesis. The analysis was done on a
182 Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA, USA) with a large volume
183 programmable temperature vaporizer (PTV) injector coupled to a Varian 1200 triple-quadrupole
184 mass spectrometry system (Varian Inc., Palo Alto, CA, USA), operated in the selective reaction
185 monitoring (SRM) mode. The column was a Zebron-5HT Inferno (30 m×0.25 mm, 0.25 µm,
186 Phenomenex Inc., Torrance, CA, USA) operated at a constant carrier gas flow of 1.0 mL/min. Method
187 limit of detection is steroid hormone specific and was determined in the plasma, and ranged
188 between 0.006 and 0.28 ng mL^{-1}. Internal standard absolute recoveries were between 69 and 94%
189 (Poulsen et al. 2015). Data were not recovery corrected. A detailed description of the analytical
190 procedure is described in Hansen et al. (2011).
191
192 **2.4. Lipid and cholesterol analysis**

The cholesterol concentrations in the plasma samples were measured with a clinical chemistry analyser, equipped with reagent carrier (test strip) and reflectance photometer (Reflotron® cholesterol strips, Roche Diagnostics, Mannheim, Germany) and lipid content was determined gravimetrically and expressed as percentage (%) of the total sample weight (Bytingsvik et al. 2012a).

2.5. Statistical analysis

In cases where the steroid and contaminant concentrations were under the detection limit, random numbers between 0 and LOD were inserted for the purpose of multivariate statistical analysis. However, random values were not used in the calculation of descriptive statistic parameters presented in Tables 2 and 3. Non-parametric statistical analysis, comparing two independent groups (Mann-Whitney U test) without random values, were conducted for the investigation of concentration differences of contaminants and hormones between the age classes (subadults and adults).

Prior to analysis, all variables were log transformed to approximate normality and unit variance (UV) scaling and mean centering was applied. Unit variance scaling gives each variable a variance of one and an equal chance of being represented in the models and mean centering improves the interpretability of the model.

For the multivariate analysis, the software Simca (Version 14.0, Umetrics, Umeå, Sweden) was used to perform PCA and OPLS. In these analyses, the following variables were included: sampling date, sampling location, age, BM (estimated), BCI, body length, axial girth, zygomatic width, cholesterol, lipid content, AN, DHEA, DHT, TS, E1, αE2, βE2, PRO, PRE, and the 8 OCPs and metabolites, 21 PCBs, 8 OH-PCBs and 2 PBDEs listed above.

The OPLS model, which is an extension of the partial least square (PLS) model, was applied to identify the explanatory X-variables (POPs and biometrical variables) which best explained the variation in the response Y variable (steroid hormones). OPLS has been designed to deal with multiple regression problems where the number of observations is limited and where collinearities among the independent variables exist. OPLS separates the systematic variation in the X space into two parts, where one part predicts the correlation between X and Y, while the other part is uncorrelated (orthogonal) to Y and expresses the systematic X variation (Trygg, 2004). For each OPLS model, a R^2X, R^2Y and a Q^2 value were calculated, where the R^2–values shows the goodness of fit (explained variation) for X- and Y-variables, respectively, and Q^2 shows the goodness of prediction

(predicted variation) based on cross-validation test of the model. Variable importance in projection (VIP) coefficients reflects the relative importance of each X variable in the prediction model. VIP allows classification of the X-variables according to their explanatory power of Y. The coefficient plot (CoeffCS) summarises the relationships between the Y variable and the X variables. Default jack-knifed confidence intervals in the coefficient plot, combined with the VIP plot, identifies important and significant variables in the model. Predictors with a large VIP, larger than 1, are the most relevant for explaining Y. The original models were optimized by removing VIP < 0.5, as they are considered to have to low of an importance for the model (Umetrics, 2008). Subsequently, the variables were deleted one-by-one until a significant model was achieved. If this was not achieved, the model was defined as non-significant. For significance testing of the OPLS prediction, ANOVA of the cross-validated residuals (CV-ANOVA) was applied. For further investigation of the results achieved from the PCA and OPLS models a Spearman two-tailed correlation analysis was applied (Statistica v. 13, Dell Inc., 2015).

3. Results

3.1. Biological variables

Details on biological variables (age, condition, body length, axial girth, head length, zygomatic width and estimated total body weight) and sampling locations for the 23 male polar bear individuals are presented in Table 1. The body condition index (BCI) varied from -1.174 to 1.284 with a mean of -0.185. Estimated body mass was 390 kg for adults, whereas the estimated body mass for subadults was 225 kg.

3.2. Environmental contaminant levels

The plasma concentrations of each of the contaminant groups, i.e. their respective summed (Σ) concentrations, are presented in Table 2. The levels for the individual compounds and congeners are presented in Supplement Material Table S1. The OH-PCBs were found to have the highest average concentrations, followed by PCBs, pesticides, and PBDEs. 4-OH-CB187 was the single contaminant with the highest concentrationz (21 -258 nmol/L) in both age groups. Amongst the PCBs, PCB-153 was found to have the highest concentration (15 – 142 nmol/L).

3.3. Steroid hormone levels

Serum concentrations of steroid hormones, cholesterol and lipid content are presented in Table 3. Androgens were found in the highest concentrations, in particular AN (6.97-215 nmol/L) and TS

(0.035-111 nmol/L). The two progestagens, PRE and PRO were found in concentrations around 1 nmol/L (however PRO was <LOD in sub adults), whereas the estrogens were the steroids found in the lowest concentrations, in particular βE2. Not all individuals had detectable levels of steroid hormones, especially regarding estrogens, where the levels were found to be low.

3.4. Relationships between steroid hormones, POPs, and biometrics

Initially, we conducted PCA analysis including both sub adult and adult male polar bears. This resulted in a model consisting of three significant principal components (R^2X cum.= 0.632, Q^2 cum.=0.377). From the loading plots in Figure 1A and B, generally, POPs and steroids, in particular, androgens (AN, DHEA, TS and DHT), were located distant from each other along the PC1, indicating an inverse relationship between POPs and androgens. However, the loading plots also indicated an inverse relationship between POPs and biometrics, and positive relationships between steroids and biometrics. In particular, BCI appears to be positively associated with androgens (Figure 1A). The feminising steroids (PRE, PRO, E1, αE2 and βE2) are located either on the left side of PC1 along with the contaminants, or more in the middle of the plot, suggesting either no correlation, or a weak positive correlation, with contaminants. Cholesterol and total lipids are associated with the environmental contaminants.

From the score plots in Figure 1C and D, a clear difference can be observed between the two age classes, sub adult and adult polar bears. This indicates that the relationships in the loading plots between POPs, androgens and biometrics may heavily rely on the age class. The distribution of contaminants between the age classes (adults – n_1 and subadults – n_2) was therefore tested. A significant difference (U = 13 - 22, n_1 = 15 - 17, n_2 = 5 - 6, $p < 0.05$) was found for oxychlordane, p,p´-DDT, 4-OH-CB146, 3´-OH-CB180, and BDE-47. The concentrations for these compounds were significantly higher in the sub adults compared to the adults, except for 3´-OH-CB180, which was significantly higher in adults than in sub adults.

We also investigated the levels of individual steroids in the two age classes. The two aromatase substrates, AN and TS were significantly higher (AN: U=14, n_1=17, n_2=6, p = 0.01; TS: U=2, n_1=14, n_2=6, p = 0.001) in adults than in sub adults. The opposite was the case for both estrogens, which are aromatase products, where βE2, levels were significantly higher (βE2: U=17: n_1=17, n_2=6, p = 0.019) in sub adults, compared to adults. For the other androgens, DHT was significantly higher in adults than in sub adults (DHT: U=15, n_1=16, n_2=6, p = 0.01), but DHEA was not statistically significant. Furthermore, there was a slightly higher level of PRE in adult males (PRE: U=22, n_1=17, n_2=6, p=0.046), while PRO was not detected above LOD in any sub adults but was detected in 7 adults. The

sum of the aromatase substrates (AN + TS) and the sum of the aromatase products (E1 + βE2) are shown in Figure 2. The concentration of substrates was approximately 6 times higher in adults than in sub adults (U=10, n_1=17, n_2=6, p = 0.005). The opposite was the case for the aromatase products, E1 and βE2, where the concentration was approximately 10 times higher in sub adults compared to adults (U=21, n_1=17, n_2=6, p = 0.039). Consequently, the aromatase substrates/products ratio was around 60 times higher in adults than in sub adults. This is likely to profoundly affect the PCA loadings (Figure 1 A, B).

To identify the most important variables in the PCA plots we conducted OPLS regressions with different reproductive steroids as the Y-variable, and all the contaminants and biometrics as X-variables. Only the TS model (Supplement Material Figure S2) as the response variable was found to be significant with a single orthogonal component (p = 0.030; R^2X=0.452, R^2Y=0.413, Q^2=0.299). Testosterone was negatively related to lipid content and to a total of 13 POPs, all with VIP > 1. However, the biometric parameters, BCI> girth>BM>zygomatic width were all positively associated with TS, and were more important for explaining variation in TS concentration than the POPs.

From these results, it may be suggested that the apparent negative relationships between TS and POPs may actually result from a positive relationship between TS and biometrics, in particular BCI, and negative relationships between BCI and POPs. We therefore analysed the relationships between BCI and concentrations of TS and DHT. Since TS concentrations heavily rely on age class, data are presented for both adults and sub adults (Figure 3). We observed a strong relationship between BCI and TS in adults, whereas no relationship was found in the sub adults. For DHT, no relationships were found for either adults or sub adults. This indicates that BCI, and not POPs, is the major driver for TS levels in male polar bears, particularly in adults.

We tested this hypothesis by conducting a new OPLS, this time only including the 17 adult males in the model, excluding the 6 sub adults, with reproductive steroids as Y-variables, and all contaminant and biometric variables as X-variables. Using this approach, the OPLS model for TS was no longer significant, which further indicates that the negative relationships in adult males between TS and POPs indicated in the PCA plot was confounded by BCI and age classes (Supplement Material Figure S2). A significant model was, however, obtained for DHT (R^2X=0.485, R^2=0.511, Q^2=0.344), including negative relationships with 19 different POPs, mainly PCBs. There were no other significant relationships with the biometric variables (Figure 4). These results indicated that a broad range of POPs exerted significant negative effects on circulating DHT levels in male adult polar bears. In contrast, circulating TS levels appeared to be mainly governed by biometrics.

Correlation tests were applied to confirm the OPLS relationships for TS and DHT. Biometric variables, location data, POPs and steroid hormones were found to be significantly correlated (Spearman correlation, $p < 0.05$) with TS and/or DHT levels (Table 4). In general, these results confirm the findings from the OPLS models. Testosterone levels were exclusively positively related to the biometric parameters with low p-values, whereas TS was exclusively negatively associated with POPs. Furthermore, TS was positively related to its steroidogenic precursors AN and DHEA, whereas it was negatively associated with the estrogens. For DHT, all the identified significant correlations were negative associations with POP levels, with only a single exception, a positive relationship between DHT and longitude. In fact, DHT was significantly negatively related with 23 different POPs, mainly PCBs including the PCB-153 and PCB-180, found in the highest concentrations. Furthermore, there were no significant relationships between DHT and biometrics. This is in accordance with the OPLS model (Figure 4).

Finally, we investigated the relationships between BCI and POP levels. Based on the analysis described above, we would expect negative relationships between BCI and POPs, mainly PCBs, in adults but essentially no relationships in the sub adults. Result for ΣPCBs as a function of BCI is shown in Figure 5 whereas relationships for individual POPs are shown in Supplement Material Table S2. As expected a negative relationship was observed between ΣPCBs and BCI ($p = 0.017$). In total, 9 POPs (7 PCBs) were negatively related to BCI in adults, whereas only a single PCB-congener was negatively related to BCI in the sub adults.

4. Discussion

4.1 Regulation of the polar bear life cycle by steroid hormones

The present study clearly demonstrates that important endocrinological processes take place when polar bears are growing into adulthood around the age of 5-6 years. At the onset of puberty, increased androgen action is responsible for the development of a sexual dimorphism such as differences in the skeleton and muscles between sexes (Tipton 2001; Wiren 2005; Bechshøft et al 2008; Clarke and Khosla, 2009; Thakur 2016). This may be caused by lower aromatase activity in males, thus changing the ratio between estrogens and androgens. This profoundly affects body growth. Interactions between androgens and biometrics such as body mass (BM) and body condition may therefore be expected. Saksena and Lau (1979) found serum estrogens to decrease in male rats when developing from sub adults to adults. However, DHT concentrations found in prepubertal male

rats did not change markedly after sexual maturation (Saksena and Lau, 1979), which is in accordance with the present study.

Testosterone and DHT may be produced by two different pathways, i.e. the Δ-4 pathway proceeding from PRO via AN to TS and DHT, and the Δ-5 pathway proceeding from PRE via DHEA to either of the two TS precursors ADIOL and AN. The "choice" of pathway appears to be species specific. For example, humans and dogs appear to be Δ-5 species (Nieschlag and Behre, 2004; Sonne et al. 2014), whereas the rat and perhaps also the hyena (*Crocota crocuta*) seem to be Δ-4 species (van Jaarsveld and Skinner, 1991; OECD 2011). In the present study, high levels of AN was found in the serum of polar bears. This is in accordance with previous studies on female polar bears from Svalbard (Gustavson et al. 2015 a,b) and East Greenland (Styrishave et al. 2016) and indicates that the polar bear is a Δ-4 species. Androstenedione is a weak androgen with low affinity for the AR and no known function in male mammals. Its major biological function is presumably a downstream conversion to the more potent steroids TS and DHT (Nieschlag and Behre, 2004). Female bears are assumed to have induced ovulation (Boone et al 1998; 2004) and polar bears have a seasonal breeding pattern. Consequently, high AN concentrations could potentially serve as a readily available reservoir for sex steroid production during encounter with the opposite sex during breeding season.

Several studies on polar bears have measured TS concentrations in circulating plasma. When presented on a ng/mL basis, the TS concentration in the adult polar bears sampled in April in the current study were around 14 ng/ml. A previous study found an average TS value in polar bears samples collected in April-May in Svalbard of 6.5 ng/ml (Ropstad et al. 2006). The current study is therefore in good accordance with concentrations reported in previous studies and provides a good indication of the TS levels in Svalbard male polar bears during the breeding season. This level is approximately one order of magnitude higher than the TS levels in male polar bears during autumn (September-November), which were generally below 1 ng/ml (Palmer et al., 1988), clearly indicating the seasonal breeding pattern of polar bears.

The average TS concentration in sub adult male polar bears was 0.80 ng/ml. This is in accordance with another study, which found average TS levels in sub adult polar bears from Svalbard to be 1.0 ng/ml in April-May (Oskam et al., 2003). The TS concentration in sub adults is similar to the average level (0.6-1.7 ng/ml) reported in adult male polar bears off breeding season in May-October (Howell-Skalla et al., 2002). The seasonal changes observed in adults do not occur in immature individuals, since the seasonal changes in TS concentrations are connected to the breeding season (Howell-Skalla et al., 2002).

The progestagens, and in particular the estrogens, were only found in low concentrations in male polar bears. This is not surprising since these steroids are feminising steroids, which are also found in low concentrations in other mammalian males, including humans (Merlotti et al 2011). However, estrogens may have some relevance in male sexual development. For instance, male fertility has been reported as being impaired in mice lacking the ER and the aromatase (O'Donnell et al., 2001). This includes loss of testicular germ cells, reduced number of sperm, and various degrees of general infertility.

4.2 Interactions between steroids and POPs

The levels of PCBs and OH-PCBs have been reported to decrease in Svalbard polar bears, but are still high enough to be of concern (Bytingsvik et al. 2012a). The negative relationship between ΣPCBs and BCI shown in Figure 5 indicates that these POPs may still have a negative impact on male polar bear health depending on their condition, and thus particularly in years with little sea ice, when the bears may have low access to food, and thus a low BCI. Furthermore, a recent study shows that emerging contaminants such as perfluoroalkyl substances may affect polar bear physiology (Pedersen et al., 2015) which may add to the negative consequences of climate changes and legacy POPs.

The mechanism by which PCBs and their hydroxylated metabolites affect steroid hormone production is not entirely known. Studies indicate an inhibition of the enzyme that performs the conversion of progestagens into androgens, i.e. the CYP17-hydroxylase. In female polar bears from Svalbard, Haave et al (2003) observed increased plasma progesterone levels with increasing ΣPCB concentrations. Also, possible inhibition of CYP17 was found in studies on male adult rats exposed to PCB mixtures (Andric et al., 2000; Murugesan et al., 2005, 2008). In addition, possible inhibitions of CYPscc, 3β-HSD and 17β-HSD by PCBs were reported (Andric et al., 2000; Murugesan et al., 2005, 2008). A study conducted by Han et al. (2010) found similar indications when exposing rats to PCB-126 and PCB-114. In that study, the transcription and translation levels of CYP17 and $P450_{scc}$ were significantly reduced in groups exposed to PCBs. In female polar bears from Svalbard, circulating steroid levels of PRE and AN were negatively associated with OH-PCBs (Gustavson et al 2015a) indicating that OH-PCBs may interfere either with CYP17 and/or $P450_{scc}$. It is therefore possible that PCBs may affect the top part of the steroidogenesis, thereby decreasing circulating sex steroids levels and, in turn, affecting basic physiology and condition.

Both BDE-47 and BDE-154 appeared to negatively affect DHT levels in male polar bears. However, both BDE-47 and BDE-154 has been banned from the European Union Marked since 2004 (European

Union, 2003), and concentrations are assumed to be on the decrease. Furthermore, in a study by Sørmo et al. (2006) neither BDE-47 nor BDE-154 was found to accumulate in polar bears, indicating that polar bears are able to metabolize most BDEs. This was supported by de Wit et al. (2010) where most PBDEs were found in lower concentrations in polar bears than in ringed seals, indicating biotransformation differences between the two species. Moreover, Ikonomou et al. (2005) reported stagnation and decreased PBDE levels from 2000 to 2003 in Canadian Arctic ringed seal blubber. In summary, this indicates that these PBDEs may presently be less problematic for male polar bear reproduction than the PCBs and their hydroxylated metabolites (Dietz et al., 2015).

Among the pesticides, HCB was found to be the most abundant contaminant in the sampled polar bears. This agrees with previous studies on Svalbard polar bears (Bernhoft et al., 1997). HCB was found to have a negative effect on the DHT concentration in the present study. Sonne et al. (2006) reported inverse relationships for baculum and testis size with HCB concentrations in male polar bears from East-Greenland. A decrease in testis size may further lead to reduce sperm quality due to testicular dysfunction (Sonne et al., 2006). Steroid hormone levels were not measured in the Sonne et al (2006) study, and a direct correlation between the reductions in reproductive organs and decreased steroid hormone concentrations can therefore not be made. However, both TS and DHT are produced in the testis and stimulate development of reproductive organs, such as penis, scrotum and prostate gland (Sundaram et al., 1995; Shabsigh, 1997; Hill et al., 2008). In addition, DHT has been found to be the active androgen in maintaining erectile function in the male rat penis (Shabsigh, 1997). A decrease in androgen levels, especially in TS and DHT concentrations, due to HCB exposure may therefore cause severe harm to the reproductive functions in male polar bears.

4.3 Are effects of body condition and POPs on androgens causing a flank attack on polar bears?

The present study demonstrates that TS is closely related to body size, body mass, and body condition in male polar bears. This is supported by Oskam et al (2003) who found axial girth to be the most important biological variable in male polar bears for explaining the variation in TS concentrations. Evidently, since TS stimulates the growth of non-reproductive tissue such as bones and muscles, this will increase body size parameters in male polar bears.

During summer (June-August) and early autumn, polar bears have limited access to sea ice and thus also limited access to their favourite prey, seals (Stirling and Derocher 2012; Derocher et al 2013). During this period, polar bears therefore rely on body reserves for energy (Cherry et al. 2009). Temperature increases, due to climate change, causes early sea ice breakup, thereby prolonging the

polar bears fasting period due to less access to hunting grounds (Gagnon and Gough, 2005; Stirling and Derocher, 2012; Jenssen et al 2015). The rate of sea ice loss has been particularly high in the Barents Sea area (Laidre et al. 2015). This prolonged starving period may, in turn, exert negative effects on body condition and thus perhaps also on steroid hormone homeostasis, since the results in the present study showed that TS was positively associated with body mass (BM) and BCI. This may have serious implications for polar bear growth and reproduction, but the individual and population consequences are not known. Thus, we speculate that increasing periods of starvation may decrease body condition and thereby TS levels. This may cause decreased growth in secondary male sex characteristics, such as bone mass, and may also affect fertility and reproduction. Future studies on polar bear body condition, in relation to environmental impacts and climate change, should therefore also include investigations into steroid hormone balance.

In target cells, including gonads, bone, muscle and brain, TS may undergo transformation into DHT by the 5α-reductase. Both TS and DHT exert their function in target tissues by binding to the AR, however DHT binds stronger than TS (Hill et al., 2008; Kovacs, 2012). In bone, DHT has been shown to stimulate osteoblast proliferation and plays a key role in sexual dimorphism and development and maintenance of male secondary sex characteristics (Clarke and Khosla, 2009). The possible negative relationships between DHT and POPs are therefore a cause for concern. The results indicate that in polar bears there may be combined, or interacting, effects of climate change induced reduction in body mass due to prolonged fasting and a consequent reduction of TS, which again may affect DHT concentrations.

A combined effect of several anthropogenic stressors, such as climate change and pollutant exposure has previously been suggested, and been referred to as an anthropogenic 'flank attack', indicating that there are multiple anthropogenic threats to polar bears and wildlife (Jenssen 2006; Jenssen et al. 2015). The combined effects of body condition and POPs on androgens, indicates that the polar bear 'flank attack' scenario suggested by Jenssen et al (2015) is not unrealistic. This hypothesis is further supported by the negative relationship between ΣPCBs and BCI shown in Figure 5. The negative relationship may result from PCBs being released to the blood stream during periods of starvation where the blubber is degraded. The effect of climate change may therefore be a poorer body condition and endocrine tissues being exposed to higher POP levels. Consequently, polar bear endocrinology should certainly be a part of future studies on the impact of POPs and climate changes on polar bear health.

5. Conclusion

The present study found significant interactions between POPs, steroid hormones, and biometry of male polar bears from Svalbard. In particular, the major androgen TS appeared to be influenced by biometrics such as body condition in adult polar bears. The other major masculinising steroid DHT was significantly negatively influenced by POPs. The study also shows that PCBs and the hydroxylated PCB metabolites are the chemicals exerting the greatest influence on polar bear endocrinology when compared to PBDEs, and OCPs. Based on these findings, the 'flank attack' hypothesis may be worth testing further, as climate change is assumed to continue. Further studies should include physiological and endocrinological end-points, focusing on determining how POPs and climate change affects the ability of polar bears to adapt to a changing environment.

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7. References

AMAP, 1998. Arctic Monitoring and Assessment Programme: AMAP assessment report- Arctic Pollution Issues

AMAP, 2002. Arctic Monitoring and Assessment Programme: AMAP assessment report - Persistent Organic Pollutants in the Arctic

AMAP, 2010. AMAP Strategic Framework 2010+. AMAP Report 2010:8. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway

513 Andric, S.A., Kostic, T.S., Stojilkovic, S.S., Kovacevic, R.Z., 2000. Inhibition of Rat Testicular
514 Androgenesis by a Polychlorinated Biphenyl Mixture Arochlor 1248. Biology of reproduction.
515 62:1882-1888

516 Bechshøft TØ, Sonne C, Rigét FF, Wiig Ø, Dietz R., 2008. Differences in growth, size and sexual
517 dimorphism in skulls of Eastern Greenland and Svalbard polar bears (Ursus maritimus). Polar Biol. 31,
518 945-958.

519 Bernhoft, A., Wiig, Ø., Skaare, J.U., 1997. Organochlorines in polar bears (*Ursus maritimus)* at
520 Svalbard. Environ. Poll. 95, 159-175.

521 Blais JM, Kimpe LE, McMahon D, Keatley BE, Mallory ML, Douglas MSV, Smol JP., 2005. Arctic
522 Seabirds Transport Marine-Derived Contaminants. Science 309, 445.

523 Boone, W.R., Catlin, J.C., Casey, K.J., Boone, E.T., Dye, P.S., Schuett, R.J., Rosenberg, J.O., Tsubota, T.,
524 Bahr, J.M., 1998. Bears as induced ovulators – a preliminary study. Ursus 10, 503-505.

525 Boone, W.R., Keck, B.B., Catlin, J.C., Casey, K.J., Boone, E.T., Dye, P.S., Schuett, R.J., Tsubota, T., Bahr,
526 J.M., 1998. Evidence that bears are induced ovulators. Theriogenology 61, 1163-1169.

527 Braathen, M., Derocher, A.E., Wiig, Ø., Sørmo, E.G., Lie, E., Skaare, J.U., Jenssen, B.M., 2004.
528 Relathionships between PCBs and thyroid Hormones and retinol in female and male polar bears.
529 Environ. Health Perspect. 112, 826-833.

530 Bytingsvik, J., Lie, E., Aars, J., Derocher, A.E., Wiig, Ø., Jenssen, B.M., 2012a. PCBs and OH-PCBs in
531 polar bear mother-cub pairs: A comparative study based on plasma levels in 1998 and 2008. Sci.
532 Total Environ. 417-418, 117-128.

533 Bytingsvik, J., van Leeuwen, S.P.J., Hamers, T., Swart, K., Aars, J., Lie, E., Nilsen, E.M.E., Wiig, O.,
534 Derocher, A.E., Jenssen, B.M., 2012b. Perfluoroalkyl substances in polar bear mother-cub pairs: A
535 comparative study based on plasma levels from 1998 and 2008. Environ. Int. 49, 92-99. doi:
536 10.1016/j.envint.2012.08.004.

537 Cattet, M.R.L., Caulkett, N.A., Obbard, M.E., Stenhouse, G.B., 2002. A body-condition index for
538 ursids. Can. J. Zool. 80, 1156-1161.

539 Cherry, S.G., Derocher, A.E., Stirling, I., Richardson, E.S., 2009. Fasting physiology of polar bears in
540 relation to environmental change and breeding behavior in the Beaufort Sea. *Polar Biol.* 32, 383–
541 391. doi: 10.1007/s00300-008-0530-0

542 Christensen-Dalsgaard, S.N., Aars, J., Andersen, M., Lockyer, C., Yoccoz, N.G., 2010. Accuracy and
543 precision in estimation of age of Norwegian Arctic polar bears (*Ursus maritimus*) using dental
544 cementum layers from known age individuals, Polar Biology, 33, 589-597.

545 Clarke, B.L., Khosla, S., 2009. Androgens and bone. Steroids 74, 296-305.

546 Derocher, A.E., Stirling, I., 1998. Geographic variation in growth of polar bears (Ursus maritimus). J.
547 Zool. 245, 65–72.

548 Derocher, A.E., Wiig, Ø., 2002. Postnatal growth in body length and mass of polar bear (Ursus
549 maritimus) at Svalbard. J Zool. Lond. 256, 343-349.

550 Derocher, A.E., Aars, J., Amstrup, S.C., Cutting, A., Lunn, N.J., Molnar, P.K., Obbard, M.E., Stirling, I.,
551 Thiemann, G.W., Vongraven, D., Wiig, O., York, G., 2013. Rapid ecosystem change and polar bear
552 conservation. Conservation Lett. 6, 368-375.

553 Dietz. R., Gustavson, K., Sonne, C., Desforges, J.-P., Rigét, F.F., Pavlova, V., McKinney, M.A., Letcher,
554 R.J., 2015. Physiologically-based pharmacokinetic modelling of immune, reproductive and
555 carcinogenic effects from contaminant exposure in polar bears (Ursus maritimus) across the Arctic.
556 Environ. Res. 140, 45-55.

557 European Union., 2003. Directive 2003/11/EC of the European Parliament and of the Council of 6
558 February 2003. Official Journal of the European Union. L42/45-46

559 Gagnon, A.S., Gough, W.A., 2005. Trends in the dates of ice freeze-up and breakup over Hudson Bay,
560 Canada. *Arctic* 58, 370–382. doi: 10.14430/arctic451

561 Gustavson, L., Ciesielski, T.M., Bytingsvik, J., Styrishave, B., Hansen, M., Lie, E., Aars, J., Jenssen, B.M.,
562 2015a. Hydroxylated polychlorinated biphenyls decrease circulating steroids in female polar bears
563 (*Ursus maritimus*). Environ. Res. 138, 191-201.

564 Gustavson, L., Jenssen, B.M., Bytingsvik, J., Styrishave, B., Hansen, M., Aars, J., Eggen, G.S., Ciesielski,
565 T.M., 2015b. Steroid hormone profile in female polar bears (*Ursus maritimus*). Polar Biol. 38, 1183-
566 1194.

567 Haave, M., Ropstad, E., Derocher, A.E., Lie, E., Dahl, E., Wiig, Ø., et al., 2003. Polychlorinated
568 biphenyls and reproductive hormones in female polar bears at Svalbard. Environ. Health Perspect.
569 111, 431–436.

570 Han, D.-Y., Kang, S.-R., Park, O.-S., Cho, J.-H., Won, C.-K., Park, H.-S., Park, K.-I., Kim, E.-H., Kim, G.-S.
571 2010. Polychlorinated biphenyls have inhibitory effects on testicular steroidogenesis by
572 downregulation of P45017α and P450scc. Toxicol. Industrial Health 26, 287-296.

573 Hansen, M., Jacobsen, N., Nielsen, F.K., Björklund, E., Styrishave, B., Halling-Sørensen, B., 2011.
574 Determination of steroid hormones in blood by GC-MS/MS. Anal. Bioanal. Chem. 400, 3409-3417.

575 Hill, R.W., Wyse, G.A., Anderson, M. 2008. Animal physiology. Second edition. Massachusetts.
576 Sinauer.

577 Howell-Skalla, L.A., Chattet, M.R.L., Ramsay, M.A., Bahr, J.M., 2002. Seasonal changes in testicular
578 size and serum LH, prolactin and testosterone concentrations in male polar bears (*Ursus maritimus*).
579 Reproduction 123, 729-733.

580 Jenssen, B.M., Villanger, G.D., Gabrielsen, K.M., Bytingsvik, J., Bechshoft, T., Ciesielski, T.M., Sonne,
581 C. Dietz, R., 2015 Anthropogenic flank attack on polar bears: interacting consequences of climate
582 warming and pollutant exposure. Front. Ecol. Evol. 3, 16. doi: 10.3389/fevo.2015.00016

583 Klaassen, C.D., 2008. Casarett and Doull″s Toxicology: The basic science of poisons. 7th edition. The
584 McGraw-Hill companies

585 Kovacs, W.J., 2011. Male reproductive function. In: Kovacs WJ and Ojeda SR, eds. Textbook of
586 Endocrine Physiology (6th Edition). Cary, NC, USA: Oxford University Press, USA

587 Letcher, R.J., Klaason-Wehler, E., Bergman, Å., 2000. Methyl sulfone and hydroxylated metabolites of
588 polychlorinated biphenyls. Handbook of Environmental Chemistry. Part K. 3, 315-359.
589
590 Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jorgensen, E.H., Sonne, C., Verreault, J., Vijayan,
591 M.M., Gabrielsen, G.G., 2010. Exposure and effects assessment of persistent organohalogen
592 contaminants in arctic wildlife and fish. Sci. Total. Environ. 408, 2995–3043. doi:
593 10.1016/j.scitotenv.2009.10.038

594 Løken, K.B., Lie, E., Lundanes, E., Skaare, J.U., 2006. Extension of a multicomponent method to
595 include the determination of OH-PCBs and OH-PBDEs in biological matrices. Organohalogen
596 Compounds 68, 2430-2433.

597 Meerts, I.A.T.M., Letcher, R.L., Hoving, S., March, G., Bergman, Å., Lemmen, J.G., van der Burg, B.,
598 Brouwer, A., 2001. *In Vitro* estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs,
599 and polybrominated bisphenol A compounds. Environ. Health Perspect. 109, 339-407.

Merlotti, D., Gennari, L., Stolakis, K., Nuti, R., 2011. Aromatase activity and bone loss in men. J. Osteoporosis. Article ID 230671, 11 pages, doi:10.4061/2011/230671

Miller WL, Auchus RJ. 2011. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrine reviews 32:81-151

Murugesan, P., Kanagaraj, P., Yuvaraj, S., Balasubramanian, K., Aruldhas, M.M., Arunakaran, J. 2005. The inhibitory effect of polychlorinated biphenyl Aroclor 1254 on Leydig cell LH receptors, steroidogenic enzymes and antioxidant enzymes in adult rats. Reprod. Toxicol. 20, 117-126.

Murugesan, P., Muthusamy, T., Balasubramanian, K., Arunakaran, J., 2008. Polychlorinated biphenyl (Aroclor 1254) inhibits testosterone biosynthesis and antioxidant enzymes in cultured rat Leydig cells. Reprod. Toxicol. 25, 447-454.

Nieschlag, E., Behre, H.M., 2004. Testosterone. Action, deficiency, substitution. 3rd edition. Cambridge University Press, Cambridge, UK

O´Donnell, L., Robertson, K.M., Jones, M.E., Simpson, E.R., 2001. Estrogen and spermatogenesis. Endocrine Reviews 22, 289-318.

OECD, 2011. Test No. 456: H295R Steroidogenesis Assay. OECD Publishing

Oskam, I.C., Ropstad, E., Dahl, E., Lie, E., Derocher, A.E., Wiig, O., Larsen, S., Wiger, R., Skaare, J.U., 2003.) Organochlorines affect the major androgenic hormone, testosterone, in male polar bears (Ursus maritimus) at Svalbard. J. Tox. Environ. Health A 66, 2119-2139.

Palmer, S.S., Nelson, R.A., Ramsay, M.A., Stirling, I., Bahr, J.M., 1988. Annual changes in serum sex steroids in male and female black (Ursus americanus) and polar (Ursus maritimus) bears. Biol. Reprod. 38, 1044–1050.

Pedersen, K.E., Basu, N., Letcher, R., Greaves, A.K., Sonne, C., Dietz, R., Styrishave, B., 2015. Brain region-specific perfluoroalkylated sulfonate (PFSA) and carboxylic acid (PFCA) accumulation and neurochemical biomarker responses in East Greenland polar bears (Ursus maritimus). Environ. Res. 138, 22-31.

Poulsen, R., Luong, X., Hansen, M., Styrishave, B., Hayes, T., 2015. Tebuconazole disrupts steroidogenesis in *Xenopus laevis*. Aquat. Tox. 168, 28-37.

627 Ropstad, E., Oskram, I.C., Lyche, J.L., Larsen, H.J., Lie, E., Haave, M., Dahl, E., Wiger, R., Skaare, J.U.,
628 2006. Endocrine disruption induced by organochlorines (OCs): Field studies and experimental
629 models. J Tox Environ Health A 69:53-76

630 Rosing-Asvid, A., Born, E., Kingsley, M., 2002. Age at sexual maturity of males and timing of the
631 mating season of polar bears (Ursus maritimus) in Greenland. Polar Biol. 25, 878–883.

632 Saksena, S.K., Lau, I.F.,1979. Variations in serum androgens, estrogens, progestins, gonadotropins
633 and prolactin levels in male rats from prepubertal to advanced age. Exp Aging Res 5, 179-194.

634 Sonne, C., Leifsson, P.S., Dietz, R., Born, E.W., Letcher, R.J., Hyldstrup, L., Riget, F.F., Kirkegard, M.,
635 Muir, D.C.G., 2006. Xenoendocrine pollutants may reduce size of sexual organs in East Greenland
636 polar bears (Ursus maritimus). Environ. Sci. Technol. 40, 5668-5674.

637 Sonne, C., Dietz, R., Letcher, R.J., Pedersen, K.M., Rigét, F.F., Styrishave, B., 2014. Steroid hormones
638 in blood plasma from Greenland sledge dogs (*Canis familiaris*) dietary exposed to organohalogen
639 polluted minke whale (*Balaenoptera acuterostrata*) blubber. Toxicol. Environ. Chem. 96, 273-286.

640 Stirling, I., Spencer, C., Andriashek, D., 1989. Immobilization of polar bears (Ursus maritimus) with
641 telazol in the Canadian Arctic. J Wildlife Diseases 25, 159-168.

642 Stirling, I., Derocher, A.E., 2012. Effects of climate warming on polar bears: a review of the evidence.
643 Glob. Change Biol. 18, 2694–2706. doi: 10.1111/j.1365-2486.2012.02753.x

644 Styrishave, B., Pedersen, K.E., Clarke, O., Hansen, M., Björklund, E., Sonne, S., Dietz, R., 2017. Steroid
645 hormones in multiple tissues of East Greenland polar bears (*Ursus maritimus*). Polar Biol. 40, 37-49.

646 Sørmo, E.G., Salmer, M.P., Jenssen, B.M., Hop, H., Baek, K., Kovacs, K.M., Lydersen, C., Falk-Petersen,
647 S., Gabrielsen, G.W., Lie, E., Skaare, J.U., 2006. Biomagnification of polybrominated diphenyl ether
648 and hexabromocyclododecane flame retardants in the polar beat food chain in Svalbard, Norway.
649 Environ. Tox. Chem. 25, 2502-2511.

650 Tehrani, R., Van Aken, B., 2014. Hydroxylated polychlorinated biphenyls in the environment: sources,
651 fate, and toxicities. Environ Sci. Pollut. Res. 21, 6334-6345.

652 Thakur, M.K., 2016. Molecular mechanism of androgen action. In: Singh SK, Ed. Mammalian
653 endocrinology and male reproductive biology. CRC PressTaylor & Francis Group, Boca Raton, FL
654 33487-2742.

655 Tipton, K.D., 2001. Gender differences in protein metabolism. Curr. Opin. Clin. Nutr. Metab. Care.
656 2001, 493-498.

657 Trygg, J., 2004. Prediction and spectral profile estimation in multivariate calibration. J. Chemometr.
658 18, 166-172.

659 van Jaarsveld, A.S., Skinner, J.D., 1991. Plasma androgens in spotted hyaenas (Crocuta crocuta)—
660 influence of social and reproductive development. J. Reprod. Fertil. 93, 195–201.

661 Verreault, J., Muir, D.C.G., Norstrom, R.J., Stirling, I., Fisk, A.T., Gabrielsen, G.W., Derocher, A.E.,
662 Evans, T.J., Dietz, R., Sonne, C., Sandala, G.M., Gebbink, W., Riget, F.F., Born, E.W., Taylor, M.K.,
663 Nagy, J., Letcher, R.J. 2005. Chlorinated hydrocarbon contaminants and metabolites in polar bears
664 (*Ursus maritimus*) from Alaska, Canada, East Greenland, and Svalbard: 1996-2002. Sci. Total Environ.
665 351-352, 369-390.

666 Wiren, M.W., 2005. Androgens and bone growth: It´s location, location, location. Curr. Opin.
667 Pharmacol. 5, 626-632.

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Table 1. Ordinal date (day of sampling from1-366), latitude, longitude, age and biometric variables of adult (n=17) and subadult (n=6) male polar bears from Svalbard sampled in April 2008.

Variable		Mean	SD	Median	Min	Max
Ordinal date (1-366)	Adults	107	5.6	104	98	115
	Subadults	102	4.8	100	98	111
Latitude (°N)	Adults	78.73	1.18	79.01	76.72	80.62
	Subadults	79.62	1.29	80.19	77.02	80.27
Longitude (°E)	Adults	17.48	3.26	18.03	12.60	23.70
	Subadults	19.08	2.60	18.00	16.60	23.81
Age (years)	Adults	13	4.2	13	6	21
	Subadults	3	0.5	3	3	4
Contour body length (cm)	Adults	248	11.5	245	231	266
	Subadults	210	26.6	202	179	257
Straight body length (cm)	Adults	232	11.1	232	214	252
	Subadults	198	26.6	191	170	246
Axial girth (cm)	Adults	152	15.4	153	127	176
	Subadults	123	17.9	116	110	157
Head length (mm)	Adults	399	20.0	399	358	439
	Subadults	356	29.5	349	321	403
Zygomatic width (mm)	Adults	254	21.0	257	215	288
	Subadults	203	34.0	197	174	267
Estimated body mass (kg)	Adults	390	86.2	408	255	539
	Subadults	225	111	179	145	442
Body condition index	Adults	-0.057	0.651	-0.032	-1.174	1.284
	Subadults	-0.546	0.345	-0.533	-0.997	-0.136

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672 Table 2. The sums of organochlorine pesticides, PCBs (also presented as poly-ortho PCBs and mono-
673 ortho PCBs separately), OH-PCBs, PBDEs and the total load (total sum of all the contaminant
674 concentrations) in plasma samples of adult (total n=17) and subadult (total n=6) male polar bears
675 from Svalbard sampled in April 2008. The number of individual contaminants is given in brackets.
676 Differences between adults and subadults were tested with Mann–Whitney U test; *<0.05.

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	Mean (nmol/L)		SD (nmol/L)		Median (nmol/L)		Min (nmol/L)		Max (nmol/L)	
Variables	Adult	Subadult	Adult	Subadult	Adult	Subadult	Adult	Subadult	Adult	Subadult
ΣOCPs and OCP metabolites* (8)	13.75	23.49	8.96	10.02	9.98	25.19	5.06	10.40	36.8	37.8
Σpoly-ortho PCBs (13)	120.6	144.7	72.37	72.29	96.07	112.9	42.2	83.1	340.9	244.9
Σmono-ortho PCBs (8)	7.89	7.08	4.12	2.77	6.71	6.18	3.85	4.84	18.3	12.3
ΣPCB (21)	128.5	151.8	74.36	74.66	100.4	118.7	53.35	88.30	355.5	257.2
ΣOH-PCBs (8)	208.2	294.0	107.3	140.9	186.4	241.6	85.01	142.2	432.0	475.9
ΣPBDEs (2)	0.366	0.568	0.189	0.260	0.288	0.499	0.158	0.213	0.819	0.928
Total load (39)	338.5	420.8	170.7	223.0	305.2	404.2	97.7	142.8	660.2	701.6

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Table 3. Lipid and cholesterol content in plasma and steroid hormone levels in serum, respectively, of adult (total n=17) and subadult (total n=6) male polar bears from Svalbard. Only detectable values were included in the calculations. d: detection frequency (number of individuals with detectable levels). Differences between adults and subadults were tested with Mann–Whitney U test; *<0.05, **<0.01.

		d	mean	sd	median	min	max
Lipid content (%)	Adults	17	0.844	0.181	0.83	0.56	1.22
	Subadults	6	1.11	0.314	1.11	0.74	1.5
Cholesterol (mmol/L)	Adults	17	6.47	1.21	6.5	3.9	8.3
	Subadults	6	7.85	2.36	7.35	5.1	11.5
Androstenedione** (nmol/L)	Adults	17	76.5	63.8	50.5	11	215
	Subadults	6	18.3	15.4	12.6	6.97	47.8
Dehydroepiandrosterone (nmol/L)	Adults	16	2.17	2.32	1.28	0.337	9.07
	Subadults	6	0.715	0.237	0.711	0.476	1.11
Dihydrotestosterone (nmol/L)*	Adults	16	0.922	0.537	0.868	0.181	2.31
	Subadults	6	0.408	0.272	0.444	0.076	0.725
Testosterone** (nmol/L)	Adults	14	47.3	35.3	46.7	4.07	111
	Subadults	6	2.8	3.44	1.33	0.035	8.38
Estrone (nmol/L)	Adults	11	0.435	0.356	0.317	0.081	1.05
	Subadults	5	2.85	3.36	0.48	0.324	6.86
α-Estradiol (nmol/L)	Adults	7	0.18	0.141	0.112	0.082	0.453
	Subadults	3	0.305	0.283	0.193	0.096	0.626
β-Estradiol (nmol/L)*	Adults	17	0.056	0.041	0.045	0.011	0.166
	Subadults	6	0.98	1.89	0.184	0.051	4.82
Pregnenolone* (nmol/L)	Adults	17	1.22	0.527	1.14	0.672	3.09
	Subadults	6	1.97	0.829	1.85	1.08	3.02
Progesterone (nmol/L)	Adults	7	1.37	1.73	1.23	0.133	5.08
	Subadults	0					

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688 **Table 4:** Significant correlations (Spearman's rank correlation test) between TS and DHT and
689 contaminants, other steroid hormones and biometry in adult and subadult male polar bears (n=23)
690 from Svalbard.

Steroid	Correlation group	Correlated variables	Correlation coefficient (r)	Significance level (p)
TS	**Biometrics**	Age	0.789	0.000
		Girth	0.721	0.000
		BCI	0.687	0.000
		BM	0.618	0.002
		Zygomatic width	0.595	0.003
		Head length	0.421	0.045
	POPs	4-OH-CB146	-0.588	0.005
		Mirex	-0.561	0.005
		PCB-137	-0.490	0.018
		PCB-183	-0.489	0.018
		p,p'-DDT	-0.489	0.018
		PCB-180	-0.483	0.020
		Oxychlordane	-0.452	0.031
		PCB-187	-0.450	0.031
		4-OH-CB187	-0.462	0.035
		BDE-154	-0.414	0.050
	Steroids	AN	0.653	0.001
		DHEA	0.567	0.005
		E1	-0.527	0.010
		aE2	-0.480	0.020
DHT	**POPs**	PCB-167mo	-0.688	0.000
		PCB-47	-0.637	0.001
		PCB-187	-0.597	0.003
		PCB-137	-0.593	0.005
		PCB-99	-0.570	0.005
		PCB-183	-0.568	0.005
		PCB-138	-0.557	0.006
		PCB-101	-0.546	0.007
		PCB-114mo	-0.540	0.008
		HCB	-0.540	0.008
		PCB-153	-0.533	0.009
		PCB-105mo	-0.517	0.012
		BDE-47	-0.516	0.012
		mirex	-0.512	0.013
		4-OH-CB146	-0.530	0.013
		BDE-154	-0.507	0.014
		PCB-128	-0.506	0.014
		PCB-180	-0.484	0.019
		oxychlordane	-0.481	0.020
		PCB-170	-0.477	0.021
		p,p'-DDE	-0.474	0.022
		4'-OH-CB130	-0.481	0.027
		4-OH-CB107	-0.449	0.041
	Location	longitude	0.436	0.037

Figure 1. PCA loading plots (A and B) and score plots (C and D) based on contaminant levels in plasma (X's), biometrics (X's) and steroid hormone concentrations in serum of adult and subadult male polar bears from Svalbard (Y's). A: Loadings in PC1/PC2 dimension. B: Loadings in PC1/PC3 dimension. C: Scores for PC1/PC2 dimension. D: PC1/PC3 dimension. Symbols in A and B: Black, POPs. Open circles, geographical position and capture date. Green, biometrics. Yellow, lipids and cholesterol. Red, estrogens and progestagens. Blue, androgens.

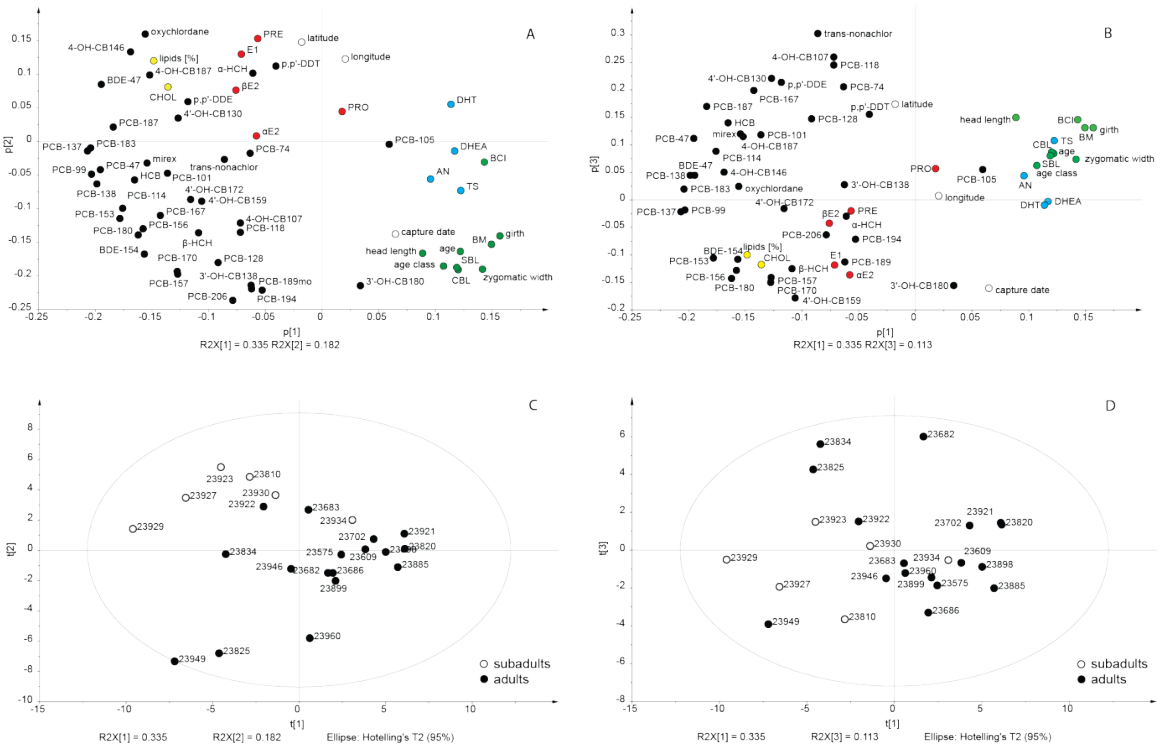

Figure 2: Aromatase substrates (AN + TS: dark blue bars) and aromatase products (E1 + βE2: light blue bars) in adult (n = 17) and subadult (n = 6) male polar bears from Svalbard sampled in 2008. Ages of subadults were 3-4 years, whereas the age of the adults ranged from 6-21 years. Enzymatic reactions catalysed by the aromatase are shown with black arrows.

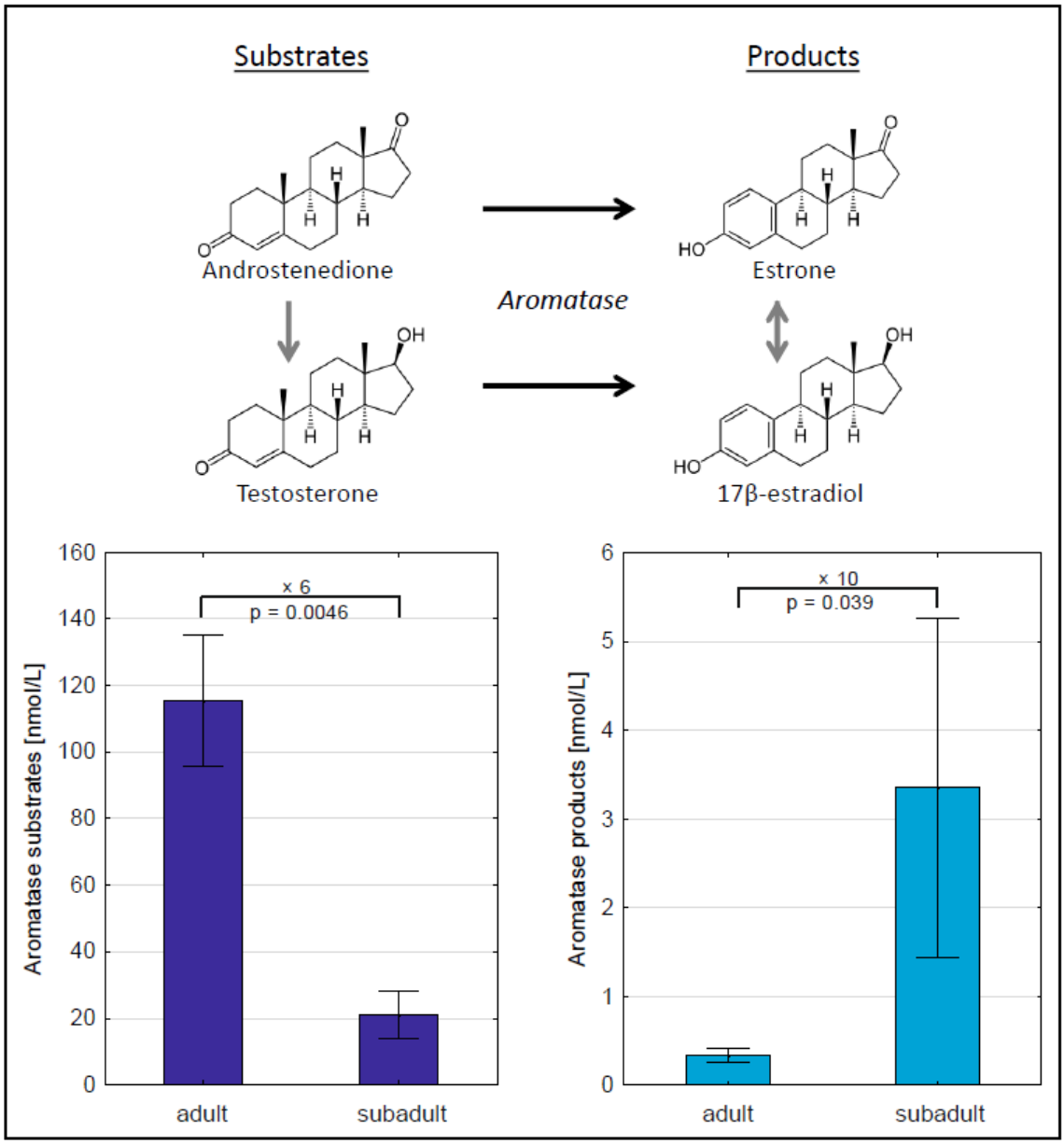

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Figure 3: Serum TS and DHT levels (nmol/L) as a function of BCI in adult (n = 17) and subadult (n = 6) male polar bears from Svalbard. Closed symbols: adults. Open symbols: subadults.

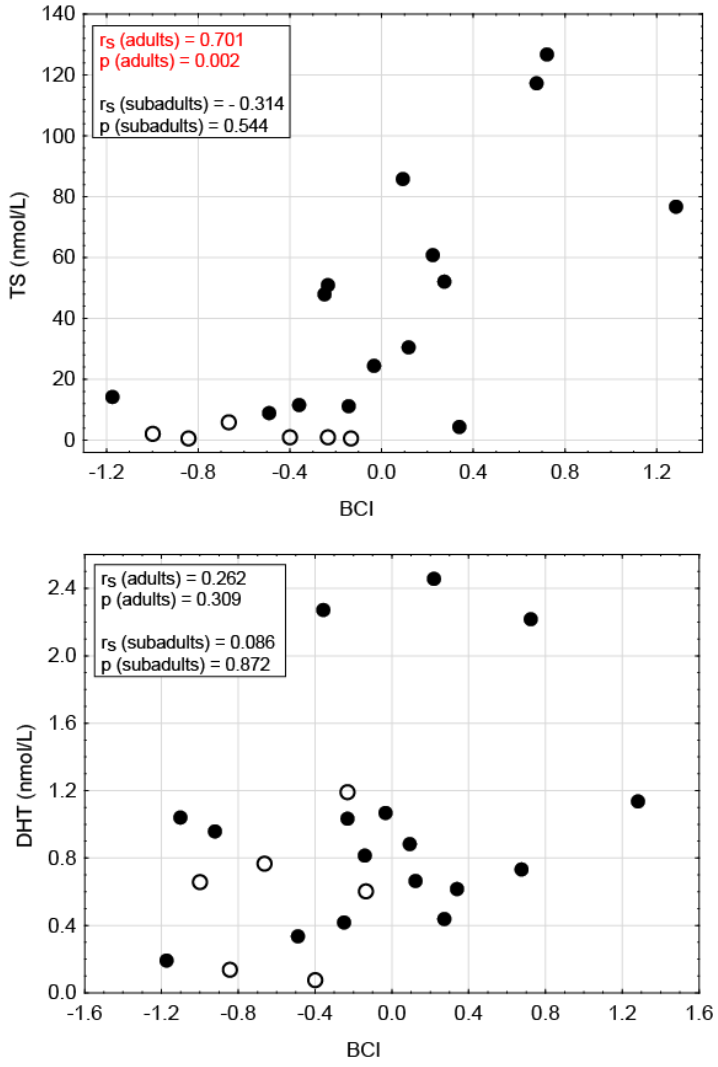

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Figure 4. OPLS regression coefficient plot summarizing the relationships between X-variables and DHT for adult male polar bears (n = 17). The whiskers represent the 95% confidence interval, and crossing of the zero-line indicates lack of significance. Variables with VIP > 1 are presented in grey.

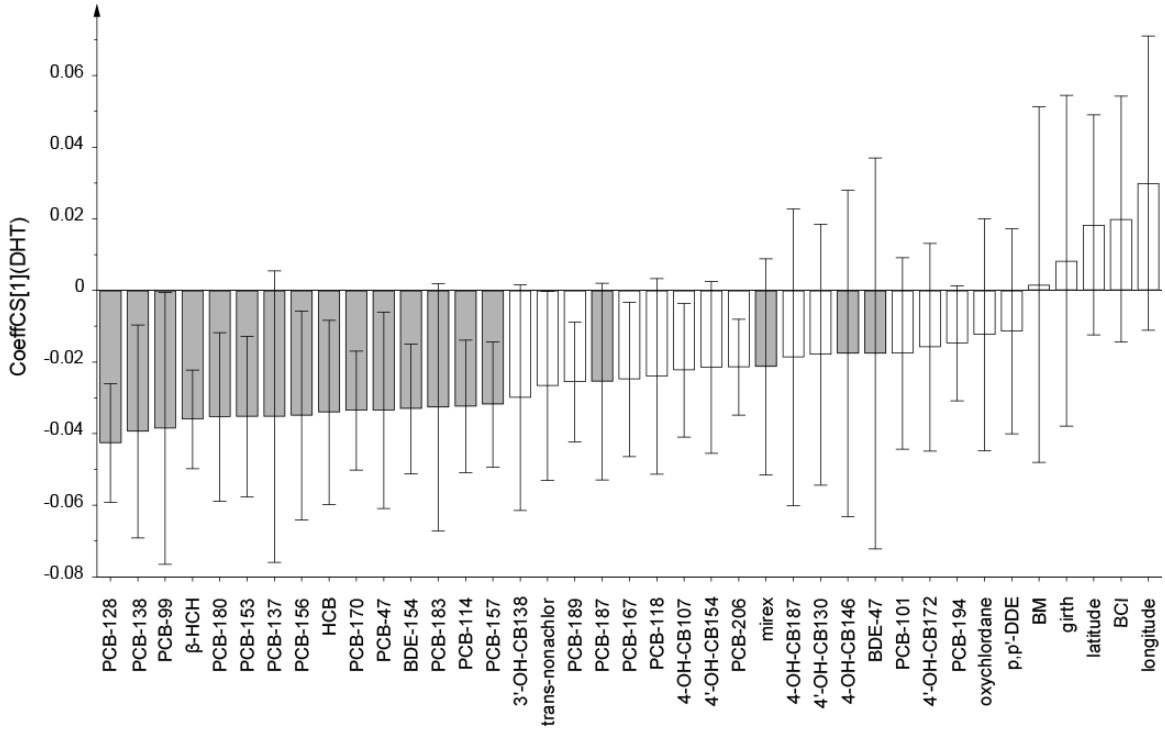

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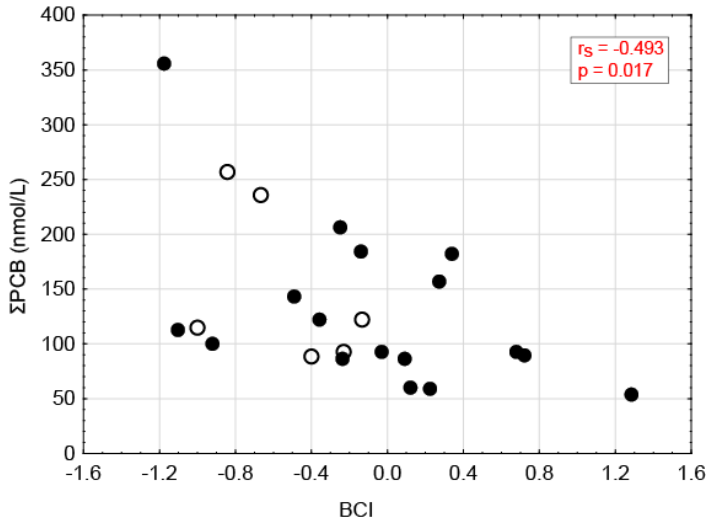
Figure 5: ΣPCBs (nmol/L) as a function of body condition index (BCI) in male polar bears from Svalbard (n = 23). Closed symbols: adults. Open symbols: subadults.



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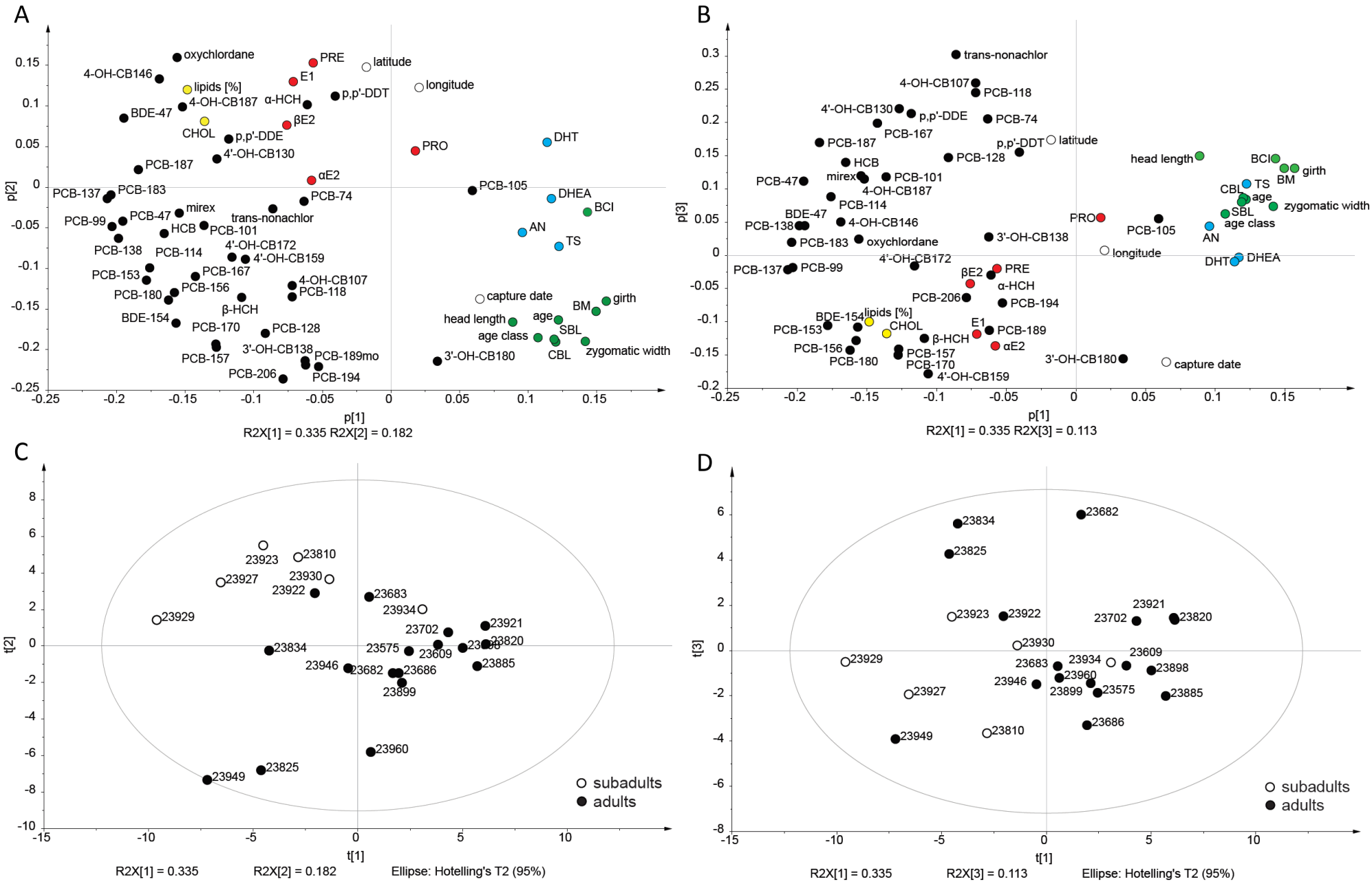
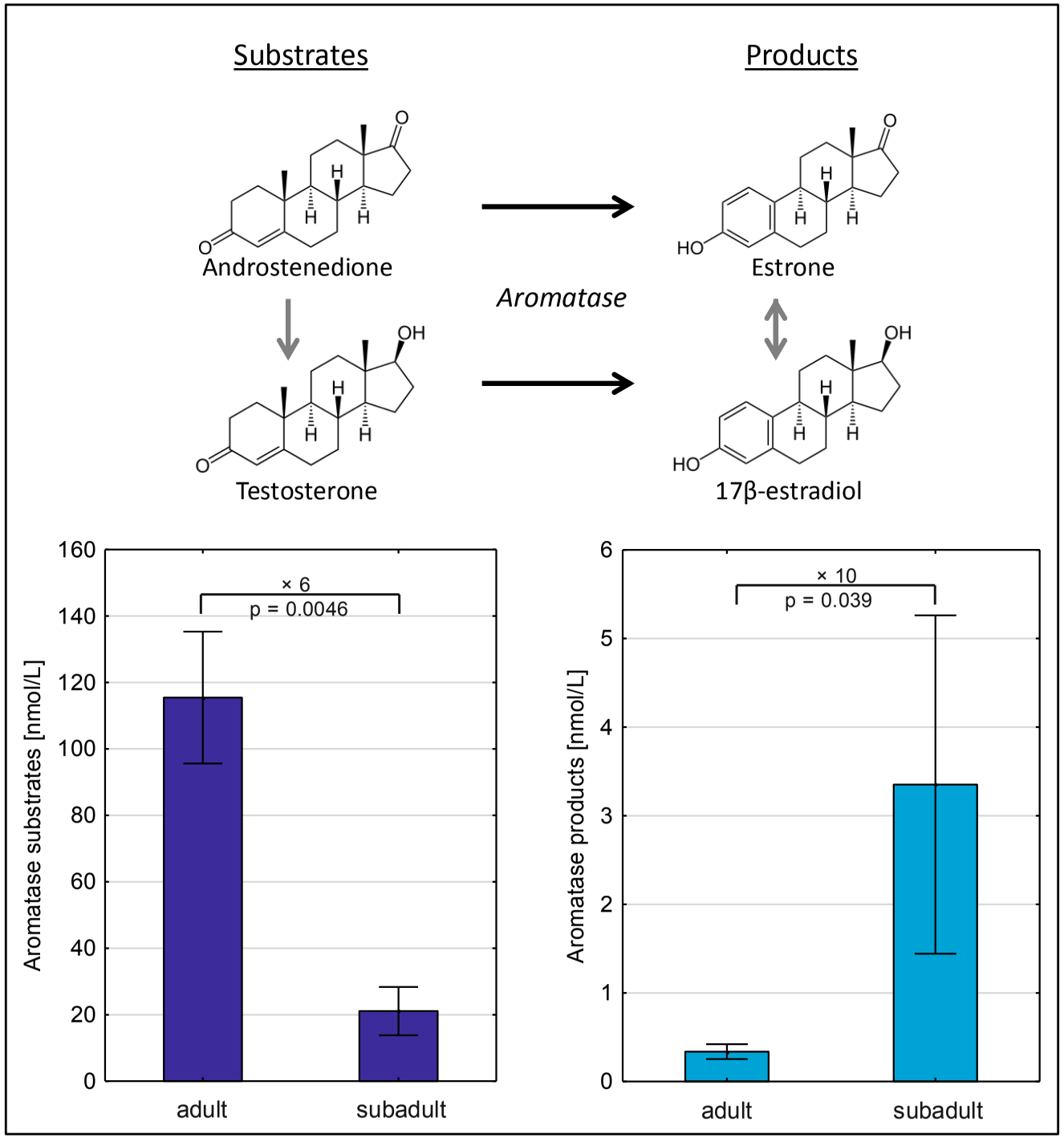
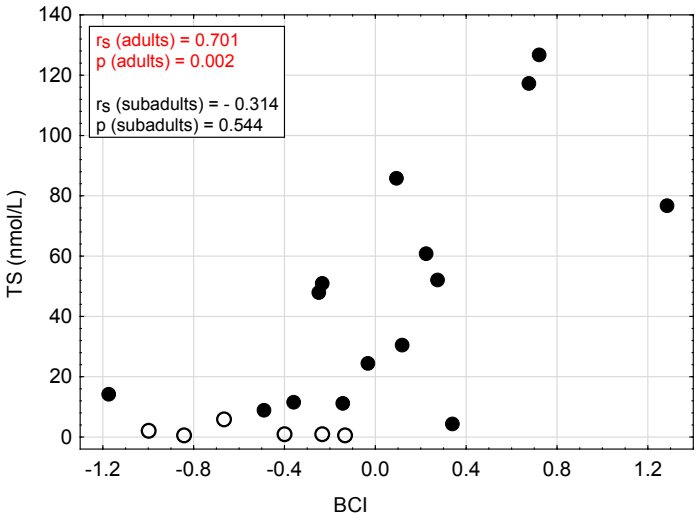
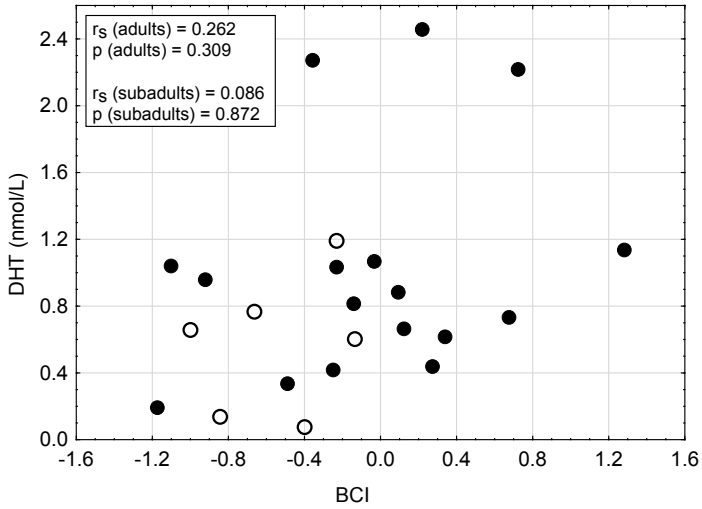
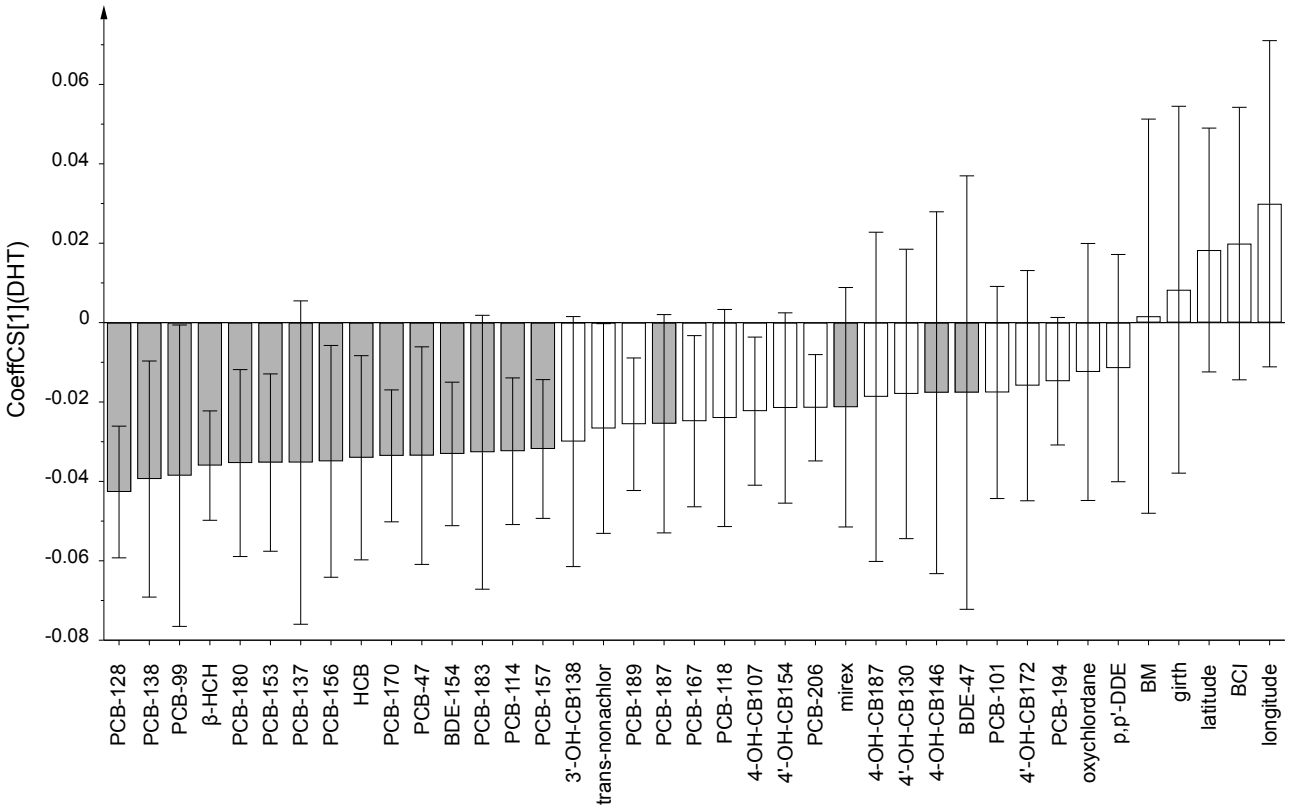
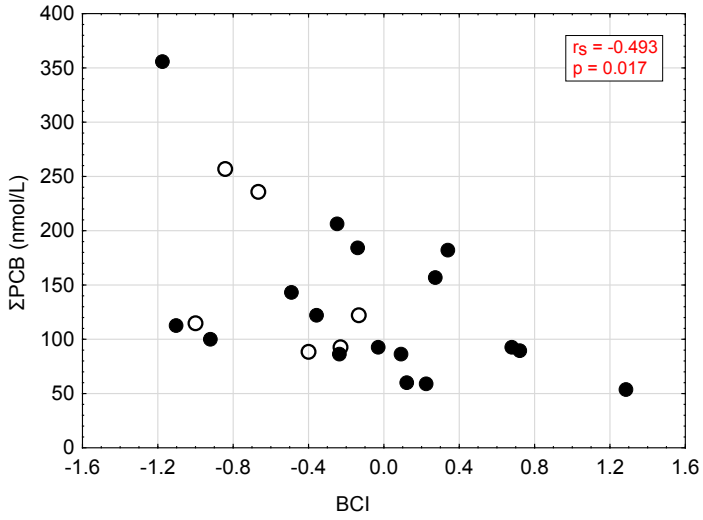

A
oxychlordane
PRE
latitude
4-OH-CB146
E1
lipids [%]
longitude
4-OH-CB187
α-HCH
p,p'-DDT
BDE-47
βE2
CHOL
p,p'-DDE
PRO
DHT
4'-OH-CB130
PCB-187
αE2
PCB-105
PCB-137
PCB-183
DHEA
PCB-74
mirex
BCI
PCB-99
PCB-47
trans-nonachlor
AN
HCB
PCB-101
TS
4'-OH-CB172
PCB-138
PCB-114
4'-OH-CB159
PCB-153
PCB-167
4-OH-CB107
PCB-180
PCB-156
PCB-118
capture date
girth
β-HCH
BM
BDE-154
head length
age
PCB-170
PCB-128
SBL
age class
3'-OH-CB138
zygomatic width
PCB-157
PCB-189mo
3'-OH-CB180
CBL
PCB-206
PCB-194
p[1]
p[2]
R2X[1] = 0.335 R2X[2] = 0.182
B
trans-nonachlor
4-OH-CB107
PCB-118
4'-OH-CB130
p,p'-DDE
PCB-74
PCB-167
PCB-187
p,p'-DDT
latitude
PCB-128
head length
BCI
HCB
girth
PCB-47
mirex
PCB-101
BM
TS
4-OH-CB187
CBL
PCB-114
age
BDE-47
zygomatic width
PRO
4-OH-CB146
SBL
PCB-138
PCB-105
age class
3'-OH-CB138
AN
PCB-183
oxychlordane
longitude
4'-OH-CB172
DHT
DHEA
PCB-137
PCB-99
βE2
PRE
α-HCH
PCB-206
PCB-194
BDE-154
lipids [%]
PCB-153
CHOL
E1
PCB-189
β-HCH
αE2
PCB-156
PCB-180
PCB-157
3'-OH-CB180
PCB-170
capture date
4'-OH-CB159
p[1]
p[3]
R2X[1] = 0.335 R2X[3] = 0.113
C
23923
23810
23927
23930
23922
23683
23929
23934
23921
23702
23820
23834
23575
23609
23946
23682
23686
23885
23899
23960
23825
23949
subadults
adults
t[1]
t[2]
R2X[1] = 0.335
R2X[2] = 0.182
Ellipse: Hotelling's T2 (95%)
D
23834
23682
23825
23921
23923
23922
23702
23820
23930
23929
23609
23683
23934
23898
23946
23960
23927
23899
23575
23885
23686
23949
23810
subadults
adults
t[1]
t[3]
R2X[1] = 0.335
R2X[3] = 0.113
Ellipse: Hotelling's T2 (95%)


Substrates
Products
Androstenedione
Estrone
Aromatase
Testosterone
17β-estradiol
Aromatase substrates [nmol/L]
× 6
p = 0.0046
adult
subadult
Aromatase products [nmol/L]
× 10
p = 0.039
adult
subadult


rS (adults) = 0.701
p (adults) = 0.002
rS (subadults) = - 0.314
p (subadults) = 0.544
TS (nmol/L)
BCI
140
120
100
80
60
40
20
0
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2


rS (adults) = 0.262
p (adults) = 0.309
rS (subadults) = 0.086
p (subadults) = 0.872
DHT (nmol/L)
BCI
2.4
2.0
1.6
1.2
0.8
0.4
0.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6


CoeffCS[1](DHT)
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
PCB-128
PCB-138
PCB-99
β-HCH
PCB-180
PCB-153
PCB-137
PCB-156
HCB
PCB-170
PCB-47
BDE-154
PCB-183
PCB-114
PCB-157
3'-OH-CB138
trans-nonachlor
PCB-189
PCB-187
PCB-167
PCB-118
4-OH-CB107
4'-OH-CB154
PCB-206
mirex
4-OH-CB187
4'-OH-CB130
4-OH-CB146
BDE-47
PCB-101
4'-OH-CB172
PCB-194
oxychlordane
p,p'-DDE
BM
girth
latitude
BCI
longitude


rs = -0.493
p = 0.017
ΣPCB (nmol/L)
BCI
400
350
300
250
200
150
100
50
0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6

Relations between steroids and POPs in blood and biometrics were investigated in male polar bears

The androgen/estrogen ratio was approximately 60 times higher in adult than in subadult males

Testosterone was positively related to biometrics such as body condition index

Dihydrotestosterone was negatively related to POP levels

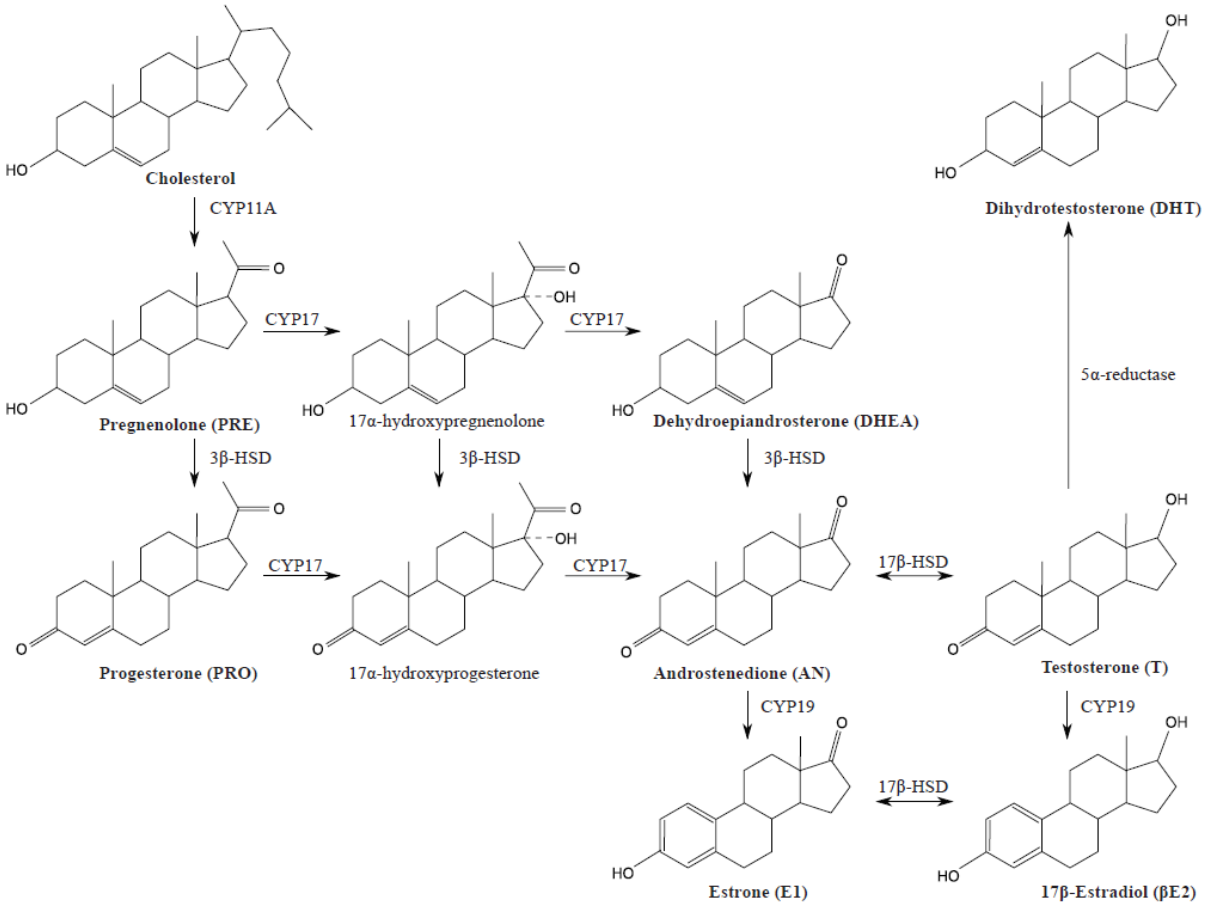
Body condition and POPs significantly affect blood steroid levels in male polar bears

Supplement Material to the manuscript:

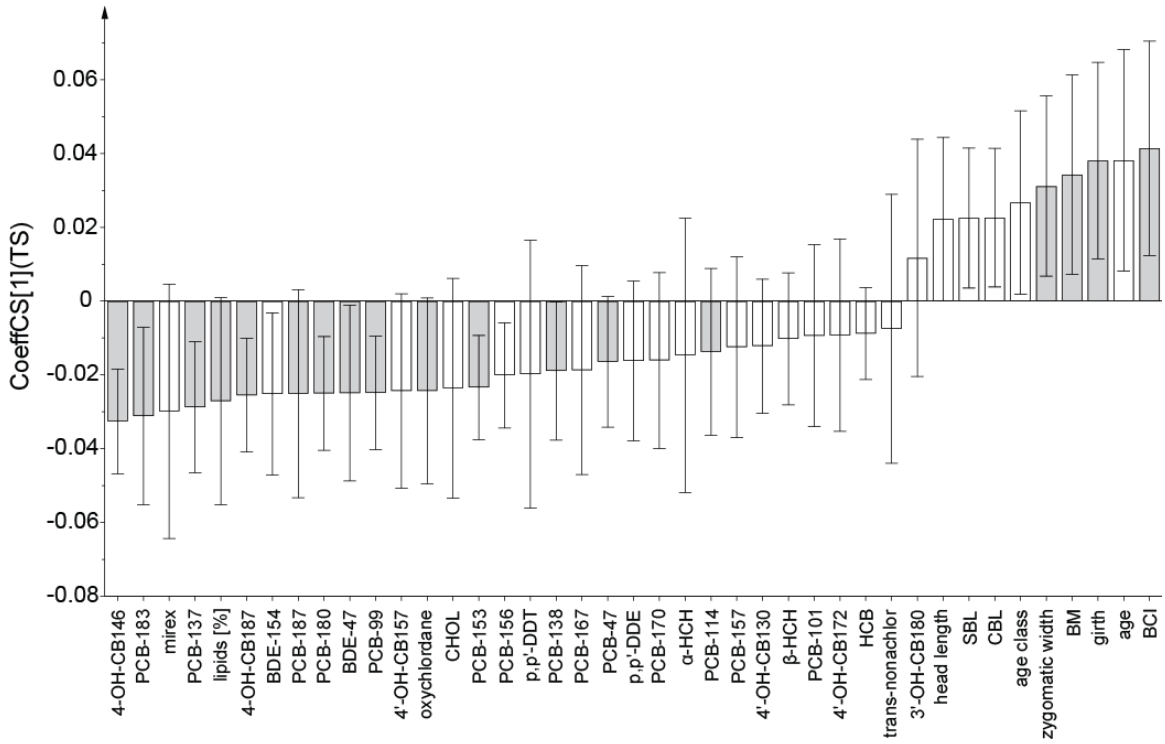
Relationships between POPs, biometrics and circulating steroids in male polar bears (*Ursus maritimus*) from Svalbard

Tomasz M Ciesielski, Ingunn Tjelta Hansen, Jenny Bytingsvik, Martin Hansen, Elisabeth Lie, Jon Aars, Bjørn M Jenssen, Bjarne Styrishave

Supplement Figure S1: Overview of the relevant steps in the steroidogenesis



Supplemental material Figure S2: OPLS regression coefficient plot summarizing the relationship between X-variables and TS for all males (17 adults and 6 subadults). The whiskers represent the 95% confidence interval, and crossing of the zero-line indicates lack of significance. Variables with VIP > 1 are presented in grey.



Supplement Material table S1: PCB, OH-PCB, pesticide and PBDE concentrations in plasma from adult (total n = 17) and subadult (total n = 6) male polar bears from Svalbard. SD: Standard deviation.

	Mean (nmol/L)		SD (nmol/L)		Median (nmol/L)		Minimum (nmol/L)		Maximum (nmol/L)		N	
Variable	Adult	Subadult	Adult	Subadult	Adult	Subadult	Adult	Subadult	Adult	Subadult	Adult	Subadult
PCBs												
PCB-47	0.56	0.89	0.49	0.63	0.38	0.79	0.12	0.29	1.69	2.01	17	6
PCB-74	0.37	0.36	0.12	0.02	0.35	0.36	<0.22	0.33	0.66	0.40	15	6
PCB-99	8.11	12.11	5.02	7.09	6.99	10.71	2.15	5.25	20.81	23.23	17	6
PCB-101	0.27	0.29	0.08	0.07	0.25	0.27	<0.19	<0.19	0.39	0.38	10	5
PCB-105	1.20	0.31	3.57	0.04	0.29	0.31	0.10	0.25	15.02	0.36	17	6
PCB-114	0.07	0.08	0.02	0.01	0.07	0.08	0.05	0.06	0.13	0.09	17	6
PCB-118	1.38	1.13	0.79	0.07	1.12	1.12	0.76	1.04	3.85	1.25	17	6
PCB-128	0.18	0.09	0.08	0.04	0.17	0.09	0.07	<0.03	0.29	0.13	11	4
PCB-137	0.73	1.20	0.44	0.74	0.60	1.06	0.24	0.40	1.81	2.26	17	6
PCB-138	8.41	11.65	5.17	7.70	7.77	9.54	2.84	4.30	19.74	23.76	17	6
PCB-153	47.79	60.52	31.06	34.19	38.47	48.01	14.58	26.65	141.66	108.05	17	6
PCB-156	2.16	2.49	1.34	1.87	1.88	1.89	0.42	0.92	5.95	6.03	17	6
PCB-157	2.11	2.16	1.14	0.80	1.83	2.00	0.87	1.23	5.04	3.52	17	6
PCB-167	0.09	0.08	0.04	0.02	0.08	0.07	<0.06	<0.06	0.18	0.11	7	4
PCB-170	15.09	15.20	9.96	5.41	11.75	12.44	5.64	10.55	44.95	22.82	17	6
PCB-180	28.17	33.24	18.73	16.71	22.41	24.74	9.46	19.36	88.40	58.10	17	6
PCB-183	0.60	1.00	0.39	0.71	0.46	0.81	0.15	0.29	1.35	2.06	17	6
PCB-187	0.16	0.29	0.11	0.17	0.12	0.31	0.04	0.10	0.43	0.56	17	6
PCB-189	0.57	0.48	0.39	0.18	0.45	0.45	0.19	0.25	1.43	0.79	17	6
PCB-194	9.25	7.29	4.74	2.51	7.17	6.82	4.11	4.55	20.43	11.36	17	6
PCB-206	1.39	1.01	0.63	0.33	1.15	0.96	0.86	0.64	2.85	1.52	17	6
OH-PCBs												
4-OH-CB107	14.61	12.15	8.99	6.49	10.78	10.86	6.03	5.00	37.95	20.44	16	5
4´-OH-CB130	0.50	0.62	0.26	0.27	0.49	0.59	<0.19	<0.23	1.06	0.98	11	4

3´-OH-CB138	3.23	2.11	1.39	0.76	2.97	2.29	1.84	1.27	6.27	3.08	16	5
4-OH-CB146	33.51	66.66	23.66	35.17	24.44	60.94	13.40	18.18	87.06	108.84	16	5
4´-OH-CB159	0.74	0.77	0.37	0.43	0.69	0.67	0.28	0.36	1.64	1.50	16	5
4´-OH-CB172	62.49	68.31	20.87	14.25	71.50	69.89	16.02	51.49	87.34	85.77	16	5
3´-OH-CB180	5.94	2.82	3.45	0.56	4.74	2.63	2.51	2.18	31.80	3.64	16	5
4-OH-CB187	87.24	140.65	70.48	93.04	55.63	111.51	21.09	44.28	248.66	257.91	16	5
Pesticides												
HCB	5.81	7.37	5.17	6.09	4.16	4.81	0.97	1.57	19.98	18.07	17	6
α-HCH	0.09	0.12	0.02	0.06	0.09	0.08	<0.05	0.07	0.14	0.21	14	6
β-HCH	1.04	1.04	0.50	0.48	0.90	0.92	0.35	0.60	2.29	1.63	17	6
Oxychlordane	4.25	11.29	3.04	5.52	3.13	11.58	1.29	3.04	12.02	18.67	17	6
Trans-nonachlor	1.91	1.20	0.88	0.32	0.97	1.17	0.34	0.88	3.29	1.78	17	6
Mirex	0.14	0.17	0.05	0.05	0.15	0.18	0.04	0.10	0.26	0.23	17	6
p,p´-DDE	0.91	1.65	0.76	0.95	0.50	1.50	0.25	0.54	2.78	2.87	17	6
PBDEs												
BDE-47	0.20	0.39	0.13	0.20	0.15	0.36	0.06	0.10	0.45	0.66	17	6
BDE-154	0.17	0.18	0.10	0.06	0.15	0.14	0.07	0.11	0.44	0.27	17	6

Supplement Material Table S2: Significant correlations (Spearman's rank correlation test) between body condition index and POPs in adult (n = 17) and subadult (n = 6) male polar bears from Svalbard.

Age class	Correlated variables	Correlation coefficient (r)	Significance level (p)
Adults	Mirex	-0.657	0.004
	PCB-183	-0.642	0.005
	PCB-137	-0.583	0.014
	BDE-154	-0.547	0.023
	PCB-156mo	-0.517	0.034
	4-OH-CB146	-0.529	0.035
	PCB-99	-0.500	0.041
	PCB-153	-0.498	0.042
	PCB-138	-0.495	0.043
Subadults	Oxychlordane	-0.828	0.042