Potentiation of ecological factors on the disruption of thyroid hormones by organo-halogenated contaminants in female polar bears (*Ursus maritimus*) from the Barents Sea

Sophie Bourgeon [a,b], Astrid Kolind Riemer [c], Sabrina Tartu [b], Jon Aars [b], Anuschka Polder [d], Bjørn Munro Jenssen [c], and Heli Routti [b]

[a] *The Arctic University of Norway, Department of Arctic and Marine Biology, 9037 Tromsø, Norway (sophie.bourgeon@uit.no)*

[b] *Norwegian Polar Institute, Fram Centre, 9296 Tromsø, Norway (sabrina.tartu@npolar.no; jon.aars@npolar.no; heli.routti@npolar.no)*

[c] *Norwegian University of Science and Technology, Department of Biology, 7491 Trondheim, Norway (bjorn.munro.jenssen@bio.ntnu.no; astridkriemer@gmail.com)*

[d] *Norwegian University of Life Science, Campus Adamstua, Oslo, Norway (anuschka.polder@nmbu.no)*

*Corresponding author: Sophie Bourgeon

The Arctic University of Norway

Department of Arctic and Marine Biology

9037 Tromsø, Norway

E-mail : sophie.bourgeon@uit.no

Phone : +47-77-64-60-33

ABSTRACT

As apex predators, polar bears (*Ursus maritimus*) are among the most heavily polluted organisms in the Arctic. In addition to this anthropogenic stressor, climate warming has been shown to negatively affect their body condition, reproductive output and survival. Among potential underlying physiological mechanisms, thyroid hormones (THs), which control thermoregulation, metabolism and reproduction, can be affected by a variety of both natural and anthropogenic factors. While THs have been extensively used as proxies for pollution exposure in mammals, including polar bears, there is a lack of knowledge of their natural variations. In this context, we examined seasonal variations in body condition and circulating TH concentrations in free-ranging female polar bears. Females with variable reproductive status (i.e., solitary, with cubs of the year or with yearlings) were sampled from locations with contrasted sea ice conditions. Furthermore, we studied THs in relation to levels of organo-halogenated contaminants. As predicted, solitary females were in better condition than females caring for offspring, especially in spring. In addition, TH levels were lower in autumn compared to spring, although this seasonal effect was mainly observed in solitary females. Finally, the negative relationships between organochlorine and perfluoroalkyl substances and some THs suggest a possible alteration of homeostasis of THs. Since the latter relationships were only observed during spring, we emphasize the importance of considering the ecological factors when using THs as proxies for pollution exposure. Yet, the combined effects of natural and anthropogenic stressors on THs might impair the ability of polar bears to adapt to ongoing climate changes.

Key-words: Breeding status; Climate change; Fasting; Organochlorines; Perfluoroalkyl substances.

FUNDING SOURCES

This study was supported by the Research Council of Norway (grant 216568/E10 to H.R) and by the Norwegian Polar Institute. Fieldwork in Svalbard was carried out under permit from the Governor of Svalbard.

1. INTRODUCTION

As Arctic top predators, polar bears (*Ursus maritimus*) show among the highest concentrations of organo-halogenated contaminants (OHCs) (Letcher et al., 2010). Although levels of polychlorinated biphenyls (PCBs) and organic chlorinated pesticides (OCPs) generally have decreased in the Arctic biota over the past decades, brominated flame retardants (BFRs) (e.g. polybrominated diphenyl ethers, PBDEs) show variable trends in Arctic wildlife populations (Dietz et al., 2013a, 2013b; Muir et al., 2013; Andersen et al., 2015). Among the perfluoroalkyl substances (PFAS), which are quantitatively the major contaminant group in polar bear plasma, concentrations of perfluorooctane sulfonate (PFOS) have decreased during recent decades whereas trends for perfluoroalkyl carboxylates (PFCAs) are more variable (Muir et al., 2013; Riget et al., 2013). Overall, subpopulations of polar bears from the European Arctic are among the most contaminated polar bear subpopulations within the circumpolar Arctic (Andersen et al., 2001; Verreault et al., 2005; Muir et al., 2006; McKinney et al., 2011). In addition to a high OHC exposure, polar bears are also amongst the most vulnerable species to climate change (Laidre et al., 2008; Kovacs et al., 2011). Indeed, the Arctic sea ice, which provides them a platform to hunt seals (Derocher et al., 2004), mate and reach denning areas, has been substantially declining over the past decades (Kinnard et al., 2011; Stroeve and Notz, 2015). Climate warming, through earlier spring sea ice break up and extended duration of ice-free periods, is therefore expected to present energetic challenges to polar bears by either restraining them to land (i.e., limiting their access to seals) or forcing energy costly migrations to find ice (Durner et al., 2009). In particular, the Barents Sea subpopulation is subject to more pronounced loss of habitat compared to most other subpopulations (Durner et al., 2009; Laidre et al., 2015; Stern and Laidre, 2016). This trend is

predicted to continue over the next decades and lead to up to a 50% loss of optimal habitat for this subpopulation by the end of the 21st century (Durner et al., 2009).

Several OHCs are known to have endocrine disruptive properties (Gore et al., 2015) and thyroid hormones (THs) have been widely used as biomarkers of pollutant exposure in marine mammals (Jenssen, 2006; Routti et al., 2008) and polar bears in particular (Braathen et al., 2004; Knott et al., 2011; Villanger et al., 2011a; Gabrielsen et al., 2015). THs have ubiquitous roles, controlling thermoregulation, metabolism and reproduction (McNabb, 1995). They are synthesized in the thyroid gland and thyroxine (T4), the predominant form of THs, is transformed to tri-iodothyronine (T3), the most bioactive form of THs, by deiodinases in peripheral tissues (McNabb, 1995). THs are transported by carrier proteins in the plasma and act via TH receptors (Hulbert, 2000). Given the multiple functions of THs, early-life exposure to TH disrupting chemicals may lead to neurocognitive deficits (Porterfield 1994; Brouwer et al., 1998; Zoeller et al., 2002). These irreversible changes can have long-term health effects at the individual level and ultimately at the population level through reduced survival and reproductive success (Jenssen 2006).

As outlined by Rosa et al. (2007) and references therein, TH variability within a species can be triggered by a variety of both extrinsic (e.g. season, contaminant load) and intrinsic factors (such as nutritional status, reproductive state, health condition). Paradoxically, very little is known about natural seasonal variations in TH levels of polar bears. To our knowledge, only one study has investigated seasonal variation of THs in polar bears (Leatherland and Ronald, 1981), a study performed in captivity. THs likely vary seasonally in free-ranging polar bears as they accumulate and lose massive fat depots following the fluctuations in accessibility of their prey throughout their life cycle (Ramsay and Stirling, 1988). For most bears, the peak feeding period occurs in spring and fasting begins in summer when sea ice has retreated. Polar bears therefore spontaneously undergo

fasting periods that can be sustained for up to 8 months in pregnant females whose fast is concomitant with gestation and lactation (Polischuk et al., 2001). Nevertheless, data on the condition of polar bears during ice-free periods are still scarce. Studies examining relationships between OHCs and TH levels in polar bears have often been restricted to one single sampling season. Namely, while free-ranging polar bears were mostly sampled during the spring season (Braathen et al., 2004; Knott et al., 2011), samples for a study using harvested individuals were collected during the winter season (Gabrielsen et al., 2015). There is a clear lack of studies investigating the role of ecological factors on the disruptive potential of OHCs on THs. The combined effects of natural and anthropogenic stressors (i.e., climate change and endocrine disruptors, respectively) (Jenssen, 2006) on the homeostasis of THs remain therefore to be documented.

In this context, the current study aimed at examining the seasonal variations in plasma THs in relation to body condition, fasting state (using plasma urea to creatinine ratio, UCR (Derocher et al., 1990; Cattet, 2000)) and plasma OHC concentrations (PCBs, hydroxy (OH)-PCBs, OCPs, PBDEs and PFAS) in adult female polar bears from the Barents Sea. We restricted our sampling effort to catching sexually mature free-ranging females to avoid gender-specific differences in physiology and/or behaviour; for example, sex and age differences in TH levels were reported in polar bears (Braathen et al., 2004; Knott et al., 2011). We sampled females with variable reproductive status (i.e., solitary, with cubs of the year or with yearlings) over two seasons (spring and autumn) and two years (2012 and 2013). Polar bears usually mate from March to May, but the implantation is delayed until October (Derocher et al., 1992). In Svalbard, pregnant females go into dens, give birth at the end of December/early January but do not emerge from the den before early April (Lønø, 1970). We have recently shown, using the same polar bears, that temporal and spatial retreat of sea ice was related to lower body condition and consequently higher OHC concentrations (Tartu et al., 2017). In the present

study, we further investigated factors affecting body condition. We hypothesize that solitary females are in better condition compared to females with offspring and that the seasonal difference is particularly pronounced in females with cubs of the year that undergo the most extended fast during winter. We also expected seasonal variations in plasma THs and UCR with lower levels in autumn, reflecting a lower metabolism and a fasting state, respectively. Finally, based on the knowledge that plasma levels of lipophilic OHCs measured in the same females were overall lower in autumn compared to spring (Tartu et al., 2017), we anticipated seasonal variations in thyroid disrupting effects of OHCs. Our results are further discussed in the context of the relevance of using THs as biomarkers of pollution exposure in fasting marine mammals.

2. MATERIAL AND METHODS

2.1 Field sampling

This study was restricted to female polar bears from the Barents Sea subpopulation that were sampled in April and September 2012 and 2013. Females were individually marked with ear tags and tattoos so they could be identified upon recaptures. The 112 samples collected (N=33 in April 2012, N=24 in September 2012, N=29 in April 2013 and N=26 in September 2013) represented 78 females with 26 of them being captured more than once (more specifically, 1 female was caught 4 times, 6 females were caught 3 times, and 19 females were caught twice). Weather and sea ice conditions often differ largely among areas in Svalbard, restricting choices of sampling areas. Females were thus opportunistically sampled throughout the Svalbard archipelago with the search effort largely depending on external factors. Females were immobilized by remote injection of a dart containing the drug Zoletil ® 100 (Virbac, France), fired from a helicopter (Eurocopter AS350 Écureuil). Following

immobilization, a vestigial premolar tooth was extracted and subsequently used to estimate the age of females (Calvert and Ramsay, 1998; Christensen-Dalsgaard et al., 2010). Blood was collected from the femoral vein using heparinised collecting tubes (kept on ice and in the dark) and centrifuged within 10 h (3500 rpm, 10 minutes). Plasma was frozen and stored at -20ºC and subsequently used to assess thyroid hormone, urea and creatinine concentrations as well as OHC levels (see below).

Body mass (BM) of females was obtained, to the nearest kg, by suspending them on a stretcher from two spring hanging scales (see Table 1). As one female could not be weighed, we estimated its body mass using morphometric measurements (i.e., axillary girth and dorsal straight-line body length) following Derocher and Wiig (2002). For all females, dorsal straight-line body length (SL) measures the straight line above the bear (lying in sternal recumbency) from the tip of the nose to the tip of the last tail vertebra. Body condition index (BCI) was thereafter calculated using the following formula described for polar bears by Cattet et al. (2002): BCI=(lnBM-3.07 x lnSL+10.76) / (0.17+0.009 x lnSL). BCI was expressed in arbitrary units with lower values indicating poorer body condition (see Table 1). Immobilization and handling procedures followed standard protocols (Stirling et al., 1989; Derocher and Wiig, 2002) and were approved by the National Animal Research Authority (NARA), Norway.

Mature females (4 to 28 years) were classified in three groups according to their breeding status: solitary (i.e., alone or together with a male in spring), with 1 or 2 cubs of the year (COY; cubs younger than 1 year old) or with 1 or 2 yearlings (YRL; cubs aged between 1 and 2 years). Among recaptures, only two females lost their cubs between spring and autumn of the same year, one female lost two cubs from one spring to the next and two females lost one cub from one autumn to the next.

Based on observed displacements recorded by marked individuals of the Barents Sea subpopulation (Lone et al., 2013) as well as sea ice characteristics, we categorized three sampling zones (Figure 1). For instance, sea ice is less extended and has a lower density along the West coast of Svalbard compared to the East coast (Vinje and Kvambekk, 1991; Hop et al., 2000). In contrast, the South-East area of Svalbard (i.e. Barentsøya and Edgeøya) experiences the largest amplitude of sea ice retreat during summer (Vinje and Kvambekk, 1991; Hop et al., 2000). Bears caught in the remainder of the archipelago, i.e. Nordaustlandet, along the North-East and southern coasts of Spitsbergen (the largest island of the Svalbard archipelago), frequently move among all regions (J. Aars, unpublished data), and we therefore pooled them into a third group. Consequently, we divided our sampling area into 3 main sampling zones: North-West (NW), South-East (SE) and North-East/South-West (NESW) (Figure 1).

2.2 Plasma thyroid hormones (THs) and urea to creatinine ratio (UCR)

Concentrations of THs in plasma (total tri-iodothyronine, TT3; free tri-iodothyronine, FT3; total thyroxine, TT4; free thyroxine, FT4) were simultaneously measured at the Department of Biology, Norwegian University of Science and Technology (NTNU, Trondheim, Norway). Concentrations were determined by radioimmunoassay (RIA) using commercially available ^{125}I RIA kits with antibody-coated tubes developed for humans (Coat-A-Count, Diagnostic Product Corporation, Los Angeles, CA, USA) and validated on polar bear plasma using parallelism tests (Braathen et al., 2004; Bytingsvik, 2012; Gabrielsen et al., 2015). The radioactivity in the samples was counted on a gamma counter (Cobra Auto- Gamma; Packard Instrument Company, Dowers Grove, IL, USA).

TT3 and FT3 assays were run in duplicate (using 100 µl of plasma per replicate) while TT4 and FT4 were run in triplicate (using 25 and 50 µl per replicate, respectively). For standard

reference material and samples run multiple times, the intra-assay variation was 5.33 % for TT3 (N=9), 6.19 % for FT3 (N=6), 4.26% for TT4 (N=9), and 2.40 % for FT4 (N=8) and the inter-assay variation was 7.14 % for TT3 (N=21), 10.66 % for FT3 (N=11), 10.06 % for TT4 (N=30), and 8.08 % for FT4 (N=26). TT4 and TT3 concentrations are expressed in nmol/L, and FT4 and FT3 concentrations in pmol/L (see Table 1). The analytical sensitivity was 0.11 nmol/L for TT3, 0.31 pmol/L for FT3, 3.22 nmol/L for TT4 and 0.13 pmol/L for FT4. Eleven samples had FT3 concentrations below the limit of detection (LOD) and were randomly assigned the arbitrary value of 0.005 pg/ml (or 0.00768 pmol/L).

Analysis of plasma urea (mmol/L) and creatinine (µmol/L) concentrations was performed using a dry clinical-chemical analyser, Reflotron® (Model IV, Boehringer-Mannheim GmhB, Mannheim, Germany) (Tartu et al., 2017; in revision). Plasma was thawed in the dark prior to analysis and samples were analysed in duplicates or triplicates when a high variance was observed between duplicates. The mean of the duplicates or triplicates was used for the statistical analysis. LOD was 3.33 mmol/l for urea and 44.50 µmol/l for creatinine. The urea to creatinine ratio (UCR) was thereafter calculated.

2.3 Contaminant levels

Plasma OHC analyses (ng/g wet weight concentrations) were performed at the Laboratory of Environmental Toxicology at The Norwegian University of Life Sciences in Oslo (NMBU), Norway, see Tartu et al. (2017) and references therein for details on the analyses of chlorinated and brominated compounds. Thirty eight organochlorine compounds were measured among which 18 congeners of PCBs (CB-99, -105, -118, -128, -137, -138, -153, -156, -157, -170, -180, -183, -187, -189, -194, -196, -206 and -209), 6 congeners of OCPs (oxychlordane, trans-nonachlor, alpha-, beta-hexachlorocyclohexanes (α-, *β*-HCH), hexachlorobenzene (HCB), *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE)), 4 congeners

of PBDEs (BDE-47, -99, -100, -153) and 10 phenolic compounds (4-OH-CB107, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187, 6-OH-BDE-47 and pentachlorophenol). In addition, 8 congeners of PFAS were analysed among which 2 perfluoroalkyl sulfononates (PFSAs) including perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) and 6 perfluoroalkylcarboxylates (PFCAs) including perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), fluorododecanoate (PFDoDA) and perfluorotridecanoate (PFTrDA). See Grønnestad et al. (2017) and Tartu et al. (2017; in revision) for details on the analyses.

2.4 Statistical analyses

Statistical analyses were conducted using R version 3.2.3 (R Core Team, 2016). Generalized linear mixed models (GLMMs; using the R-package `nlme` version 3.1.128; Pinheiro et al., 2015) were first used to test for the effects of sampling location (North West; South East or the North East South West diagonal), year (2012 or 2013), season (spring or autumn) and breeding status (solitary, with COYs or with YRLs) on body mass, BCI and plasma UCR and female ID was used as a random factor to account for the repeated measurements (among seasons and/or years). We performed an automated model selection (dredge function in MuMIn-package version 1.15.6; Barton, 2016) on a global model including 10 biologically relevant response variables applied as fixed factors (sampling location + season + year + breeding status + sampling location:season + sampling location:year + sampling location:status + season:year + season:status + year:status) and female ID as a random factor (Table 2). Thereafter, we used GLMMs with physiologically relevant fixed factors such as season, status, BCI and UCR (and their 2-way interactions) as predictors for variations in plasma thyroid hormone concentrations (TT3, FT3, TT4 and FT4). Model selection was based

on 10 biologically relevant models (Table 3). For all GLMMs, we used an information-theoretic approach (Burnham and Anderson, 2004) based on Akaike's information criterion corrected for small sample size (AICc, R-package AICcmodavg version 2.0.3, Mazerolle, 2015) to select the best GLMMs. The best model was taken to be the one with the smallest AICc, and/or the most parsimonious, i.e., other models with ΔAICc < 2 and lower k.

We used redundancy analysis (RDA, R-package vegan version 2.4.0; Oksanen et al., 2016) to explore the relationships between THs (response variables) and contaminant levels (explanatory variables) with season and status as catagorical factors. RDA is an extraction method that summarizes linear relationships between components of response variables that are "redundant" with a set of explanatory variables (Legendre and Anderson, 1999). Finally, GLMMs were used to examine the relationships between the THs and OHCs selected by the RDA analysis using female ID as a random factor. All OHC concentrations were log transformed for the GLMMs.

Only OHCs that were detected in more than 70% of the females were included in the statistical analyses. Compounds whose values were below LOD were assigned half of the LOD value. Due to inter-correlations among the organic contaminants, we used the sum ($\sum$) of 16 PCBs ($\sum_{16}$PCBs: CB-99, -105, -118, -137, -138, -153, -156, -157, -170, -180, -183, -187, -189, -194, -206 and -209), $\sum_4$OCPs (oxychlordane, trans-nonachlor, β-HCH, HCB), $\sum_8$OH-PCBs (4-OH-CB107, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187), $\sum_2$PBDEs (BDE-47, -153) and $\sum_8$PFAS (PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA) in the analyses.

Finally, we used diagnostic plots of residuals to check that the model assumptions were met (i.e., constant variance between residuals). When an interaction term was significant, we disregarded the effects of the main factors on the response variable and we used the least

squares means (LSM) method (*lsmeans* function in *Lsmeans* package; Lenth, 2015) to identify significantly different terms in a biologically relevant frame.

3. RESULTS

3.1 Influence of physiological and ecological factors on body mass, body condition and plasma urea to creatinine ratio

For each response variable, the five most competitive models are presented in Table 2. Body mass was influenced by the status of females ($F_{2,31}$=9.36, p=0.0007) with females with COYs being 26 kg (95% CI, [-38; -14]) lighter than solitary females during spring while females with YRLs were only 14 kg (95% CI, [-28; 1]) lighter compared to the latter group (see Table 1). However, because of large inter-individual variations in females' body mass, season only marginally influenced body mass ($F_{1,31}$=3.66, p=0.06) with females being 9 kg heavier in autumn compared to spring (regardless of their breeding status) (95% CI, [-1; 19]). Nevertheless, when restricting our analyses to females caught both during spring and autumn of the same year (N=32 occurrences including one female caught 4 times), we observed a highly significant effect of season (i.e., time) with females being on average 29 kg (95% CI, [17;49]) heavier in September compared to April (GLMM: Status: $F_{2,12}$=2.19, p=0.15; Season: $F_{1,12}$=24.98, p=0.0003; Status × Season: $F_{2,12}$=0.61, p=0.56).

BCI, which is a more accurate indicator of condition than body mass, was influenced by sampling location (GLMM: $F_{2,28}$=9.97, p=0.0005) and year ($F_{1,28}$=5.39, p=0.03) in addition to season ($F_{1,28}$=11.37, p=0.002) and status ($F_{2,28}$, p=0.002) (Figure 2). Females sampled in the North West of Svalbard showed poorer body condition (as expressed by lower BCI values) than females sampled in other areas of the archipelago (LSM: NW-SE: p=0.02; NW-NWSE: p=0.0001; NESW-SE: p=0.21). Moreover, BCI was lower in females with COYs

compared to solitary females (95% CI, [-0.81; -0.28]) while it did not differ between the two other groups (LSM: COYs-YRLs: p=0.22; solitary-YRLs: p=0.22). Finally, BCI was greater in autumn compared to spring (95% CI, [0.19; 0.72]) but lower in 2013 compared to 2012 (95% CI, [-0.48;-0.03]) regardless of the breeding status (see Table 1).

Plasma UCR was significantly influenced by season ($F_{1,33}$=21.54, p<0.0001) with UCR values lower in autumn compared to spring (95% CI, [-1.03; -0.40], see Table 1) reflecting a fasting state in autumn.

3.2 Effects of season and breeding status on thyroid hormone levels

Plasma concentrations of THs measured in the current study (see Table 1) were in accordance with those reported in previous studies using the same methodology (Skaare et al., 2001; Braathen et al., 2004; Knott et al., 2011; Villanger et al., 2011a; Gabrielsen et al., 2015).

The five highest ranked models explaining plasma TH concentrations are given in Table 3. TT3 and FT3 plasma concentrations were affected by season (TT3: $F_{1,29}$=58.38, p<0.0001; FT3: $F_{1,29}$=46.84, p<0.0001), breeding status (TT3: $F_{2,29}$=7.82, p=0.002; FT3: $F_{2,29}$=6.85, p=0.004) and their interaction (TT3: $F_{2,29}$=8.11, p=0.002; FT3: $F_{2,29}$=6.26, p=0.005) (Table 3). While plasma TT3 and FT3 concentrations in females measured during autumn were comparable in all females, regardless of their status (LSM: solitary-COYs: TT3: p=0.97, FT3: p=0.99; solitary-YRLs: TT3: p=0.99, FT3: p=0.85; COYs-YRLs: TT3: p=0.96, FT3: p=0.86), levels observed during spring were higher in solitary females compared to females with offspring (TT3: 95% CI, [-0.51; -0.21] in females with COYs and [-0.55; -0.18] in females with YRLs; FT3: 95% CI, [-0.99; -0.39] in females with COYs and [-0.93; -0.22] in females with YRLs) (Table 1). Indeed, plasma TT3 and FT3 in solitary females were also higher during spring than autumn (95% CI, [0.39; 0.66], and 95% CI, [0.69; 1.26], respectively; Table 1). However, in females with offspring (regardless of the age of the cub), plasma TT3

or FT3 levels did not differ between spring and autumn (LSM: TT3: Spring-autumn in females with COYs: p=0.09, Spring-autumn in females with YRLs: p=0.11; FT3: Spring-autumn in females with COYs: p=0.10, Spring-autumn in females with YRLs: p=0.16). TT4 and FT4 plasma concentrations were significantly affected by the season and decreased from spring to autumn for all females (95% CI, [-9.80; -5.69], and 95% CI, [-3.57; -1.96], respectively) (Table 1).

3.3 Relationships between thyroid hormones and contaminant levels

The plasma concentrations of contaminants (ng/g wet weight concentration) were as follows: $\sum_{16}$PCBs: 39.98 ± 3.84, $\sum_{4}$OCPs: 7.39 ± 0.50, $\sum_{8}$OH-PCBs: 65.14 ± 3.45, $\sum_{2}$PBDEs: 0.18 ± 0.01, $\sum_{8}$PFAS: 352.64 ± 15.99 ($\sum_{2}$PFSAs: 264.35 ± 12.45; $\sum_{6}$PFCAs: 88.28 ± 3.86).

The RDA model was highly significant (Monte-Carlo permutation test, 999 replicates, p=0.001). The RDA correlation triplot indicated that only TT3 and FT3 could be negatively related to plasma $\sum_{16}$PCBs, $\sum_{4}$OCPs and $\sum_{8}$PFAS (Figure A in supplementary information). We therefore selected the latter OHCs for further mixed model analyses to check whether these contaminant groups were significant predictors for TT3 and FT3 plasma concentrations. However, since the sample scores were separated by season (Figure A), we examined the above-described TH-OHC relationships separately in spring and autumn. While both TT3 and FT3 were negatively related to $\sum_{16}$PCBs (TT3: $F_{1,54}$=14.92, p=0.008; FT3: $F_{1,54}$=26.54, p=0.002; Figures 3A and 4A, respectively) and $\sum_{4}$OCPs (TT3: $F_{1,54}$=12.43, p=0.01; FT3: $F_{1,54}$=15.65, p=0.007; Figures 3B and 4B, respectively) in spring, none of these relationships were significant during autumn (data not shown, GLMM, 0.13<p<0.95; Figures 3C-D and 4D-E). In addition, FT3 was negatively related to $\sum_{8}$PFAS in spring ($F_{1,54}$=7.74, p=0.03; Figure 4C) but not in autumn ($F_{1,40}$=2.81, p=0.13; Figure 4F).

4. DISCUSSION

While examining the levels and patterns of OHCs is beyond the scope of this study, Tartu et al. (2017; in revision) investigated the main sources of variation in plasma lipophilic pollutants, phenolic compounds and PFAS for the females included in the present study. They reported that while body condition followed by diet were the most important drivers for concentrations of the highly lipophilic OHCs, breeding status was a significant predictor of concentrations of the less lipophilic OHCs (Tartu et al., 2017). In addition, they showed that diet was the most important predictor of PFAS concentrations with females feeding on high trophic level sea ice-associated prey being the most exposed to PFAS (Tartu et al., in revision).

This study documents seasonal and spatial variations in body condition and TH concentrations in three reproductive groups of free-ranging female polar bears in relation to pollution exposure. As expected, solitary females were overall in better condition than females caring for offspring and significantly so compared to females with cubs of the year, especially in spring. We also reported lower TH levels in autumn compared to spring, although this seasonal effect was mainly observed in solitary females. Finally, we highlighted season dependent possible alterations of the thyroid homeostasis (especially FT3) by PCBs, OCPs and PFAS.

4.1 Effects of sampling location on body condition

While body mass did not differ significantly between sampling locations, BCI revealed that females caught in the North-West were in poorer condition than females caught in other areas of the Svalbard archipelago (Figure 2). The spatial differences in body condition could be explained by the variations in sea ice conditions that accordingly appeared more clearly in the

South compared to the North West, since sea ice did not appear before late winter in the fjords of North Spitsbergen these years (Prop et al., 2015). Nevertheless, it is not only challenging to compute a parameter describing local sea ice extent but also very difficult to interpret its biological relevance for polar bears, such as the optimal sea ice cover needed for hunting. For example, the reduction of sea ice extent and duration have somewhat unknown consequences for the foraging behaviour of polar bears on ringed seals (*Phoca hispida*), and their primary prey (Stirling et al., 2007). Moreover, because of different hunting skills or experience of individuals (Stirling, 1974), identical sea ice condition can result in large inter-individual differences in fat store accumulation (i.e., body condition) of bears coming ashore once the sea ice melts (see Dyck and Kebreab, 2009). Yet, in our study based on the same females we reported diet variations among sampling areas based on carbon, nitrogen and lipid sources further highlighting diet specialization over a small geographic scale such as Svalbard (Tartu et al., 2016). The results of that study indicate that both NW and SE females ingest a larger proportion of terrestrial prey. Inter-individual differences were even larger in SE females who experienced the largest amplitude of sea ice retreat during summer (Tartu et al., 2016). Alternatively, another not mutually exclusive hypothesis to account for the spatial variation in BCI, at least in spring, could be the result of our population being composed of individuals with contrasted spatial behaviour: a pelagic and a near-shore ecotype (Mauritzen et al., 2001; 2002). Although a previous study based on telemetry movements of collared individuals suggested that pelagic females (with large home ranges) were located farther south than near-shore females (with smaller home ranges), it showed no differences in body mass between both groups (Olsen et al., 2003). Further studies should investigate differences in body condition between females of each ecotype.

4.2 Effects of breeding status and season on body condition

Overall and as predicted, both body mass and BCI tended to be poorer in females raising offspring (cubs or yearlings) compared to solitary females. These differences were expectedly significant during spring between solitary females and females with COYs that were caught shortly after den emergence (i.e., after sustaining a long fast).

We also highlighted seasonal variation in body mass and BCI indicating that females were leaner in spring compared to autumn. Previous estimations of body condition of polar bears sampled at different months of the year showed that while body condition was following an ascending phase in spring (feeding state) and a descending phase in autumn (fasting state), individuals exhibited yet lower BCI values in spring compared to autumn (Cattet, 2000). Accordingly, the lower UCR levels observed during autumn indicated that more females were in a fasting state in autumn compared to spring.

4.3 Effects of breeding status and season on thyroid hormone levels

The current study highlighted an effect of breeding status of females on their TH levels with solitary females exhibiting on average higher levels than females with offspring. We also reported seasonal variations in plasma concentrations of THs, which were overall higher in spring compared to autumn, although not observed for all groups. The reason for the observed seasonal differences is likely a combination of different factors. First, these variations could be interpreted as the result of females being in a fasting state in autumn, which is associated to decreased TH levels in black bears (*Ursus americanus*; Azizi et al., 1979; Tomasi et al., 1998). In addition, these differences could also be attributed to seasonal variations in environmental cues such as photoperiod and temperature, which can trigger physiological and endocrine changes. For example, because of the involvement of THs in thermoregulation (McNabb, 1992), colder spring temperatures could result in higher TH levels. Nevertheless, as

indicated by the significant interaction between season and breeding status, the season effect was mainly driven by the solitary females while the breeding status effect was only observed in spring (at least for TT3 and FT3). Indeed, solitary females showed significantly 1) higher TH levels than females with offspring and 2) higher TH levels in spring compared to autumn. On the other hand, plasma TH concentrations of females with COYs or YRLs were less affected by season or status. Based on these results, seasonal variation in abiotic factors alone cannot explain the lack of fluctuations between seasons in TH levels in these latter two groups. Females with COYs during spring could also show lower levels of THs than solitary females as a result of having recently sustained a longer and more energetically demanding fast compared to the other two groups. However, this is not fully supported by our data. Indeed, we showed no significant differences in TH levels between females with COYs and females with YRLs despite differences in BCI observed between both groups.

The influence of breeding status on TH could also be the result of TH and reproductive endocrine systems being intimately intricated (Nakao et al., 2008). For example, decreases in T3 serum levels were reported in lactating Crioula Lanada Serrana ewes compared to non-lactating females (Colodel et al., 2010). Milk production in nursing female polar bears could therefore explain the lower TT3 and FT3 plasma concentrations observed in this group compared to solitary females. Nevertheless, this scenario is no longer valid in autumn when plasma TT3 and FT3 were similar in all females. Alternatively, our results could be interpreted as solitary females showing the highest TH levels during spring as a consequence of estrous (i.e., receptive state), inducing a different hormonal state compared to anestrous females caring for young (Haave et al., 2003). Accordingly, plasma T3 was shown to decrease during the luteal phase of estrous of ewes (Peeters et al., 1989).

4.4 Anthropogenic drivers of thyroid hormone variations

The reported relationships between circulating concentrations of THs and contaminants suggest possible alterations of the THs homeostasis by organochlorines (PCBs and OCPs) and PFAS. Our results showed that in spring both TT3 and FT3 were negatively associated with Σ_{16}PCBs and Σ_4OCPs while only FT3 was negatively related to Σ_8PFAS. T3 (TT3 and FT3) therefore appeared to be the main hormone being influenced by OHCs further suggesting that T3 could be more sensitive than the other examined THs (T4) (Braathen et al., 2004; Debier et al., 2005). As T3 is the active hormone and FT3 represents the biologically available fraction, this may be of concern for the polar bear health. Accordingly, previous studies on mammals reported negative relationships between plasma concentrations of FT3 and PCBs in Svalbard female polar bears with COYs (Braathen et al., 2004), grey seal pups (*Halichoerus grypus*; Sørmo et al., 2005) and beluga whales (*Delphinapterus leucas*; Villanger et al., 2011b). Moreover, a study on pregnant women reported low levels of FT3 and TT3 in women with high blood concentrations of PFUnDA and PFDA, respectively, compared to women with low blood concentrations of these compounds (Berg et al., 2015). On the other hand, while Routti et al. (2010) found positive relationships between FT3 and OH-PCBs in ringed seals, Bytingsvik (2012) reported no significant relationships between FT3 nor TT3 and any of the examined OHCs, including PCBs and PFAS, in polar bear cubs.

While few studies consider physiological and environmental factors when reporting the endocrine disruptive potential of OHCs on THs, these factors can contribute to explain the discrepancies found between studies. In the current study, we report negative TH-OHC relationships in spring, but none of these relationships was significant in autumn. This emphasizes that environmental factors such as season can act as confounding factors. A non-mutually exclusive alternative explanation to contrasting TH-OHC relationships among and

within studies could be due to TH concentrations responding non-monotonously to OHC concentrations (Calabrese and Baldwin, 2003). Langer et al. (2007) examined TH and PCB concentrations in human serum and found inverse dose-dependent relationships between PCBs and FT4 and PCBs and TT3 at low doses, but positive dose-dependent relationships at higher doses (U-shaped dose-response). In the present study, contrasting seasonal variations in pollutant levels were highlighted with plasma concentrations of PCBs, OCPs and PBDEs being higher in spring compared to autumn, at least in 2013 (Tartu et al., 2017). Moreover, plasma PFNA and PFDA concentrations were higher in fasting females, a nutritional state that is more commonly observed during autumn (Tartu et al., in revision). Based on our observation that negative relationships between THs and OHCs were only observed in spring, our results therefore support a possible non-monotonous dose-response relationship linking PCBs, pesticides and PFAS to THs in polar bears. In addition, previous studies highlight that the reproductive status of female polar bears affected the TH-OHC relationships with negative relationships between TT4:TT3 and ΣPCBs in females with offspring but not in solitary females (Braathen et al., 2004). Similarly, correlations between *p,p'*-DDE and TT3 were negative in nursing polar bears but positive in solitary female bears (Villanger et al., 2011a).

4.5 Conclusions and implications

We reported variations in body condition and THs of female polar bears in relation to ecological factors. The partial mismatch between fluctuations in body condition and THs between groups does not suggest any direct relationship between both traits. It is nonetheless important to be aware of these spatial and temporal endogenous physiological changes since THs have been widely used as biomarkers of pollutant exposure in marine mammals (Jenssen, 2006; Routti et al., 2008) and polar bears in particular (Braathen et al., 2004; Knott et al,. 2011; Villanger et al., 2011a). The current study highlights possible alterations of THs by

OHCs and pinpoints contrasting relationships between THs and OHCs in relation to environmental factors such as season. We therefore emphasize the need to control for ecological factors when inferring about possible causative relationships between THs and contaminant exposure to avoid, or at least limit, the confounding effects of seasonal physiological processes. The combined effects of natural and anthropogenic stressors (i.e., climate change and endocrine disruptors, respectively) (Jenssen, 2006) on the homeostasis of THs remain however to be documented as it might impair the ability of individuals to adapt to ongoing climate changes (Jenssen et al., 2015).

5. ACKNOWLEDGMENTS

This study was supported by the Research Council of Norway (grant 216568/E10 to H.R) and by the Norwegian Polar Institute. Fieldwork in Svalbard was carried out under permit from the Governor of Svalbard. We thank the helicopter and Lance crews and Rupert Krapp for logistic help. We want to thank Magnus Andersen for both help in the field and fruitful discussions. Finally, we want to thank Vidar Torget for analyses of plasma urea creatinine.

6. REFERENCES

Andersen, M., Lie, E., Derocher, A.E., Belikov, S.E., Bernhoft, A., Boltunov, A.N., Garner, G.W., Skaare, J.U., Wiig, O. 2001. Geographic variation of PCB congeners in polar bears (*Ursus maritimus*) from Svalbard east to the Chukchi Sea. Polar Biol. 24, 231-238.

Andersen, M.S., Fuglei, E., König, M., Lipasti, I., Pedersen, Å.Ø., Polder, A., Yoccoz, N.G., Routti, H. 2015. Levels and temporal trends of persistent organic pollutants (POPs) in arctic foxes (*Vulpes lagopus*) from Svalbard in relation to dietary habits and food availability. Sci. Total Environ. 511, 112-122.

Azizi, F., Mannix, J.E., Howard, D., Nelson, R.A. 1979. Effect of winter sleep on pituitary-thyroid axis in the American black bear. Am. J. Physiol. 237, 227-230.

Bartoń, K. 2016. MuMIn: Multi-Model Inference. R package version 1.15.6. https://CRAN.R-project.org/package=MuMIn.

Berg, V., Nøst, T.H., Hansen, S., Elverland, A., Veyhe, A-S., Jorde, R., Odland, J.Ø., Sandanger, T.M. 2015. Assessing the Relationship between Perfluoroalkyl Substances, Thyroid Hormones and Binding Proteins in Pregnant Women; a Longitudinal Mixed Effects Approach. Environ. Int. 77, 63-69.

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

Burnham, K.P., Anderson, D.R. 2004. Model Selection and Multimodel Inference New York, NY: Springer New York.

Brouwer, A., Morse, D.C., Lans, M.C., Schuur, A.G., Murk, A.J., Klasson-Wehler. E., Bergman, Å, Visser, T.J. 1998. Interactions of persistent environmental organohalogens with

the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicol. Ind. Health 14, 59-84.

Bytingsvik, J. 2012. Organohalogenated Contaminants (OHCs) in Polar Bear Mother-Cub Pairs from Svalbard, Norway: Maternal Transfer, Exposure Assessment and Thyroid Hormone Disruptive Effects in Polar Bear Cubs. NTNU. Doctoral thesis.

Calabrese, E.J., Baldwin, L.A. 2003. Toxicology Rethinks Its Central Belief. Nature 421, 691.

Calvert, W., Ramsay, MA. 1998. Evaluation of age determination of polar bears bycounts of cementum growth layer groups. Ursus 10, 449-453.

Calvert, W., Ramsay, MA. 1998. Evaluation of age determination of polar bears bycounts of cementum growth layer groups. Ursus 10, 449-453.

Cattet, M.R.L. 2000. Biochemical and physiological aspects of obesity, high fat diet, and prolonged fasting in free-ranging polar bears. Ph.D. dissertation, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

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

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

Colodel, M.M., Martins, E., Martins, V.M.V., Marques Júnior, A.P. 2010. Serum concentration of thyroid hormones in *crioula lanada serrana* ewes in gestation and lactation. Arch. Zootec. 59 (228), 509-517.

Debier, C., Ylitalo, G.M., Weise, M., Gulland, F., Costa, D.P., Le Boeuf, B.J., De Tillesse, T., Larondelle, Y. 2005. PCBs and DDT in the Serum of Juvenile California Sea Lions: Associations with Vitamins A and E and Thyroid Hormones. Environ. Pollut. 134, 323-332.

Derocher, A.E., Lunn, N.J., Stirling, I. 2004. Polar bears in a warming climate. Integr. Comp. Biol. 44, 163-176.

Derocher, A.E., Nelson, R.A., Stirling, I., Ramsay, M.A. 1990. Effects of fasting and feeding on serum urea and serum creatinine levels in polar bears. Mar. Mammal Sci. 6, 196-203.

Derocher, A.E., Stirling, I., Andriashek, D. 1992. Pregnancy rates and serum progesterone levels of polar bears in western Hudson Bay. Can. J. Zool. 70, 561-566.

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

Dietz, R., Riget, F.F., Sonne, C., Born, E.W., Bechshoft, T., McKinney, M.A., Letcher, R.J. 2013a. Three decades (1983-2010) of contaminant trends in East Greenland polar bears (*Ursus maritimus*). Part 1: Legacy Organochlorine contaminants. Environ. Int. 59, 485-493.

Dietz, R., Riget, F.F., Sonne, C., Born, E.W., Bechshoft, T., McKinney, M.A., Drimmie, R.J., Muir, D.C.G., Letcher, R.J., 2013b. Three decades (1983-2010) of contaminant trends in East Greenland polar bears (Ursus maritimus). Part 2: Brominated flame retardants. Environ. Int. 59, 494-500.

Durner, G.M., Douglas, D.C., Nielson, R.M., Amstrup, S.C., McDonald, T.L., Stirling, I., Mauritzen, M., Born, E.W., Wiig, O., DeWeaver, E., Serreze, M.C., Belikov, S.E., Holland, M.M., Maslanik, J., Aars, J., Bailey, D.A., Derocher, A.E. 2009. Predicting 21st-century polar bear habitat distribution from global climate models. Ecol. Monogr. 79, 25-58.

Dyck, M.G., Kebreab, E. 2009. Estimating the Energetic Contribution of Polar Bear (*Ursus maritimus*) Summer Diets to the Total Energy Budget. J. Mammal. 90 (3), 585-593.

Gabrielsen, K.M., Krokstad, J.S., Villanger, G.D., Blair, D.A., Obregon, M-J., Sonne, C., Dietz, R., Letcher, R.J., Jenssen, B.M. 2015. Thyroid Hormones and Deiodinase Activity in Plasma and Tissues in Relation to High Levels of Organohalogen Contaminants in East Greenland Polar Bears (*Ursus maritimus*). Environ. Res. 136, 413.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T. 2015. EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, E1-E150.

Grønnestad, R., Villanger, G.D., Polder, A., Kovacs, K.M., Lydersen, C., Jenssen, B.M., Borgå, K. 2017. Maternal transfer of perfluoroalkyl substances in hooded seals. Environ. Toxicol. Chem. 36 (3), 763-770.

Haave, M., Ropstad, E., Derocher, A.E., Lie, E., Dahl, E., Wiig, Ø., Skaare, J.U., Jensen, B.M. 2003. Polychlorinated Biphenyls and reproductive hormones in female polar bears at Svalbard. Environ. Health Persp. 4 (111), 431-436.

Hop, H., Poltermann, M., Lønne, O.J., Falk-Petersen, S., Korsnes, R., Budgell. W.P. 2000. Ice amphipod distribution relative to ice density and under-ice topography in the northern Barents Sea. Polar Biol. 23, 357-367.

Hulbert, A.J. 2000. Thyroid hormones and their effects: a new perspective. Biol. Rev. 75, 519-631.

Jenssen, B.M. 2006. Endocrine-disrupting chemicals and climate change: a worst-case combination for arctic marine mammals and seabirds? Environ. Health Pers. 114, 76-80.

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

Kinnard, C., Zdanowicz, C.M., Fisher, D.A., Isaksson, E., de Vernal, A., Thompson, L.G. 2011. Reconstructed changes in Arctic sea ice over the past 1,450 years. Nature 479, 509-513.

Knott, K.K., Schenk, P., Beyerlein, S., Boyd, D., Ylitalo, G.M., O'Hara, T.M. 2011. Blood-based biomarkers of selenium and thyroid status indicate possible adverse biological effects of mercury and polychlorinated biphenyls in Southern Beaufort Sea polar bears. Environ. Res. 111, 1124-1136.

Kovacs, K., Lydersen, C., Overland, J., Moore, S. 2011. Impacts of changing sea-ice conditions on Arctic marine mammals. Marine Biodiversity 41, 181-194.

Laidre, K.L., Stern, H., Kovacs, K.M., Lowry, L., Moore, S.E., Regehr, E.V., Ferguson, S.H., Wiig, Ø., Boveng, P., Angliss, A.P., Born, E.W., Litovka, D., Quakenbush, L., Lydersen, C., Vongraven, D., Ugarte, F. 2015. A circumpolar assessment of Arctic marine mammals and sea ice loss, with conservation recommendations for the 21st century. Conserv. Biol. *29,* 724-737.

Laidre, K.L., Stirling, I., Lowry, L.F., Wiig, O., Heide-Jorgensen, M.P., Ferguson, S.H. 2008. Quantifying the sensitivity of arctic marine mammals to climate-induced habitat change. Ecol. Appl. 18, S97-S125.

Langer, P., Kocan, A., Tajtáková, M., Rádiková, Z., Petrík, J., Koska, J., Ksinantová, L., Imrich, R., Hucková, M., Chovancová, J., Drobná, B., Jursa, S., Bergman, A., Athanasiadou, M., Hovander, L., Gasperíková, D., Trnovec, T., Seböková, E., Klimes, I. 2007. Possible Effects of Persistent Organochlorinated Pollutants Cocktail on Thyroid Hormone Levels and Pituitary–Thyroid Interrelations. Chemosphere 70, 110.

Leatherland, J.F., Ronald, K. 1981. Plasma concentrations of thyroid hormones in a captive and feral polar bear (*Ursus maritimus*). Comp. Biochem. Physiol. 70A, 575-577.

Legendre, P., Anderson, M.J. 1999. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. Ecol. Monogr. 69, 1-24.

Lenth, R. 2015. Least-Square Means. R Package. The R Development Core Team.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995-3043.

Lone, K., Aars, J., Ims, R.A. 2013. Site fidelity of Svalbard polar bears revealed by mark-recapture positions. Polar Biol. 36, 27-39.

Lønø, O. 1970. The polar bear (*Ursus maritimus*) in the Svalbard area. Nor. Polarinst. Skr. 149, 1-117.

Mauritzen, M., Derocher, A.E., Wiig, O. 2001. Space-use strategies of female polar bears in a dynamic sea ice habitat. Can. J. Zool. 79, 1704-1713.

Mauritzen, M., Derocher, A.E., Wiig, Ø., Belikov, S.E., Boltunov, A.N., Hansen, E., Garner, G.W. 2002. Using satellite telemetry to define spatial population structure in polar bears in the Norwegian and western Russian Arctic. J. Appl. Ecol. 39, 79-90.

Mazerolle, M.J. 2015. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c).

McKinney, M.A., Letcher, R., Aars, J., Born, E., Branigan, M., Dietz, R., Evans, T., Gabrielsen, G.W., Peacock, E., Sonne, C. 2011. Flame retardants and legacy contaminants in polar bears from Alaska, Canada, East Greenland and Svalbard, 2005-2008. Environ. Int. 37, 365-374.

McNabb, F.M.A. 1992. Thyroid Hormones. Prentice Hall, Englewood Cliffs, New Jersey.

McNabb, F.M.A. 1995. Thyroid-hormones, their activation, degradation and effects on metabolism. J. Nutr. 125, S1773-S1776.

Muir, D.C.G., Backus, S., Derocher, A.E., Dietz, R., Evans, T.J., Gabrielsen, G.W., Nagy, J., Norstrom, R.J., Sonne, C., Stirling, I., Taylor, M.K., Letcher, R.J. 2006. Brominated flame retardants in polar bears (*Ursus maritimus*) from Alaska, the Canadian Arctic, East Greenland, and Svalbard. Environ. Sci. Technol. 40 (2), 449-455.

Muir, D.C.G., Kurt-Karakus, P., Stow, J. 2013. Chapter 4 - Occurrence and Trends in the Biological Environment, Canadian Arctic Contaminants Assessment Report On Persistent Organic Pollutants. Aboriginal Affairs and Northern Development Canada, Ottawa ON, pp. 273-422.

Nakao, N., Ono, H., Yoshimura, T. 2008. Thyroid hormones and seasonal reproductive neuroendocrine interactions. Reproduction 136 (1), 1-8.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H. 2016. Package Vegan. https://cran.r-project.org, https://github.com/vegandevs/vegan

Olsen, G.H., Mauritzen, M., Derocher, A.E., Sørmo, E.G., Skaare, J.U., Wiig, Ø., Jenssen, B.M. 2003. Space-use strategy is an important determinant of PCB concentrations in female polar bears in the Barents Sea. Environ. Sci. Technol. 37 (21), 4919-4924.

Peeters, R., Buys, N., Pauwels, I., Kuhn, E.R., Decuypere, E., Siau, O., Vanisterdael, J. 1989. Relationship between the thyroidal and gonadal axes during the estrous-cycle of ewes of different breeds and ages. Reprod. Nutr. Dev. 29, 237-245.

Pinheiro, J., Bates, D., Debroy, S., Sarkar, D., R core team, 2015. nlme: Linear and Nonlinear Mixed Effects Models.

Polischuk, S.C., Hobson, K.A., Ramsay, M.A. 2001. Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Can. J. Zool. 79, 499-511.

Porterfield. S.P. 1994. Vulnerability of the developing brain to thyroid abnormalities-environmental insults to the thyroid system. Environ. Health Perspect. 102, 125-130.

Prop, J., Aars, J., Bårdsen, B.-J., Hanssen, S.A., Bech, C., Bourgeon, S., de Fouw, J., Gabrielsen, G.W., Noreen, E., Oudman, T., Sittler, B., Stempniewicz, L., Tombre, I., Wolters, E., Moe, B. 2015. Climate change and the increasing impact of polar bears on bird populations. Front. Ecol. Evol. 3, 33.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsay, M.A., Stirling, I. 1988. Reproductive biology and ecology of female polar bears (*Ursus maritimus*). J. Zool. 214, 601-634.

Rigét, F., Bossi, R., Sonne, C., Vorkamp, K., Dietz, R. 2013. Trends of perfluorochemicals in Greenland ringed seals and polar bears: Indications of shifts to decreasing trends. Chemosphere 93, 1607-1614.

Rosa, C., Ohara, M.T., Hoekstra, F.P., Refsal, R.K., Blake, E.J. 2007. Serum thyroid hormone concentrations and thyroid histomorphology as biomarkers in bowhead whales (*Balaena mysticetus*). Can. J. Zool. 85 (5), 609-618.

Routti, H., Arukwe, A., Jenssen, B.M., Letcher, R.J., Nyman, M., Bäckman, C., Gabrielsen, G.W. 2010. Comparative endocrine disruptive effects of contaminants in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Comp. biochem. Phys. C 152, 306-312.

Routti, H., Nyman, M., Jenssen, B.M., Bäckman, C., Koistinen, J., Gabrielsen, G.W. 2008. Bone-related effects of contaminants in seals may be associated with vitamin D and thyroid hormones. Environ. Toxicol. Chem. 27, 873-880.

Skaare, J.U., Bernhoft, A., Wiig, Ø., Norum, K.R., Haug, E., Eide, D.M., Derocher, A.E. 2001. Relationships between plasma levels of organochlorines, retinol and thyroid hormones from polar bears (*Ursus maritimus*) at Svalbard. J. Toxicol. Env. Heal. A 62, 227-241.

Stern, H., Laidre, K.L. 2016. Sea-Ice indicators of polar bear habitat. The Cryosphere 10, 2027-2041.

Stirling, I. 1974. Midsummer observations on the behaviour of wild polar bears (*Ursus maritimus*). Can. J. Zool. 52, 1191-1198.

Stirling, I., Richardson, E., Thiemann, G.W., Derocher, A.E. 2007. Unusual Predation Attempts of Polar Bears on Ringed Seals in the Southern Beaufort Sea: Possible Significance of Changing Spring Ice Conditions. Arctic 61 (1), 14-22.

Stirling, I., Spencer, C., Andriashek, D. 1989. Immobilization of Polar Bears (*Ursus maritimus*) with Telazol in the Canadian Arctic. J. Wildl. Dis. 25, 159-168.

Stroeve, J., Notz, D. 2015. Insights on past and future sea-ice evolution from combining observations and models. Global Planet. Change 135**,** 119-132.

Sørmo, E.G., Jussi, I., Jussi, M., Braathen, M., Skaare, J.U., Jenssen, B.M. 2005. Thyroid Hormone Status in Gray Seal (*Halichoerus grypus*) Pups from the Baltic Sea and the Atlantic Ocean in Relation to Organochlorine Pollutants. Environ. Toxicol. Chem. 24, 610-616.

Tartu, S., Bourgeon, S., Aars, J., Andersen, M., Ehrich, D., Thiemann, G.W., Welker, J.M., Routti, H. 2016. Geographical area and life history traits influence diet in an Arctic marine predator. PLoS ONE 11 (5), e0155980.

Tartu, S., Bourgeon, S., Aars, J., Andersen, M., Lone, K., Jenssen, B.M., Polder, A., Thiemann, G.W., Torget, V., Welker, J.M., Routti, H. Diet and metabolic state are the main factors determining concentrations of perfluoroalkyl substances in female polar bears from Svalbard. In revision, Environmental Pollution.

Tartu, S., Bourgeon, S., Aars, J., Andersen, M., Polder, A., Thiemann, G.W., Welker, J.M., Routti, H. 2017. Sea ice-associated decline in body condition leads to increased concentrations of lipophilic pollutants in polar bears (Ursus maritimus) from Svalbard, Norway. Sci. Total Environ. 576, 409-419.

Tomasi, T.E., Hellgren, E.C., Tucker, T.J. 1998. Thyroid Hormone Concentrations in Black Bears (*Ursus americanus*): Hibernation and Pregnancy Effects. Gen. Comp. Endocr. 109, 192-199.

Verreault, J., Muir, D.C.G., Norstrom, R.J., Stirling, I., Fisk, A.T., Gabrielsen, G.W., Derocher, A.E., Evans, T., Dietz, R., Sonne, C., Sandala, G.M., Taylor, M.K., Nagy, J., Letcher, R.J. 2005. Chlorinated Hydrocarbon Contaminants and Metabolites in Polar Bears

(*Ursus maritimus*) From Svalbard, Greenland, Alaska and Canadian Arctic Populations: 1999-2002. Sci. Total Environ. 351-352, 369-390.

Villanger, G.D., Jenssen, B.M., Fjeldberg, R.R., Letcher, R.J., Muir, D.C.G., Kirkegaard, M., Sonne, C., Dietz, R. 2011a. Exposure to mixtures of organohalogen contaminants and associative interactions with thyroid hormones in East Greenland polar bears (*Ursus maritimus*). Environ. Int. 37, 694-708.

Villanger, G.D., Lydersen, C., Kovacs, K.M., Lie, E., Skaare, J.U., Jenssen, B.M. 2011b. Disruptive Effects of Persistent Organohalogen Contaminants on Thyroid Function in White Whales (*Delphinapterus leucas*) from Svalbard. Sci. Total Environ. 409, 2511-2524.

Vinje T., Kvambekk, Å.S. 1991. Barents Sea drift ice characteristics. Polar Res. 10, 59-68.

Zoeller, R.T., Dowling, A.L.S., Herzig, C.T.A., Iannacone, E.A., Gauger, K.J., Bansal, R. 2002. Thyroid hormone, brain development, and the environment. Environ. Health Perspect. 110, 355-61.

FIGURES

Figure 1. Division of our sampling area into three main zones: North-West, South-East and North-East/South-West (NESW) diagonal of Svalbard (Norway). Each dot represents the sampling of a female polar bear (112 samples representing 78 females).

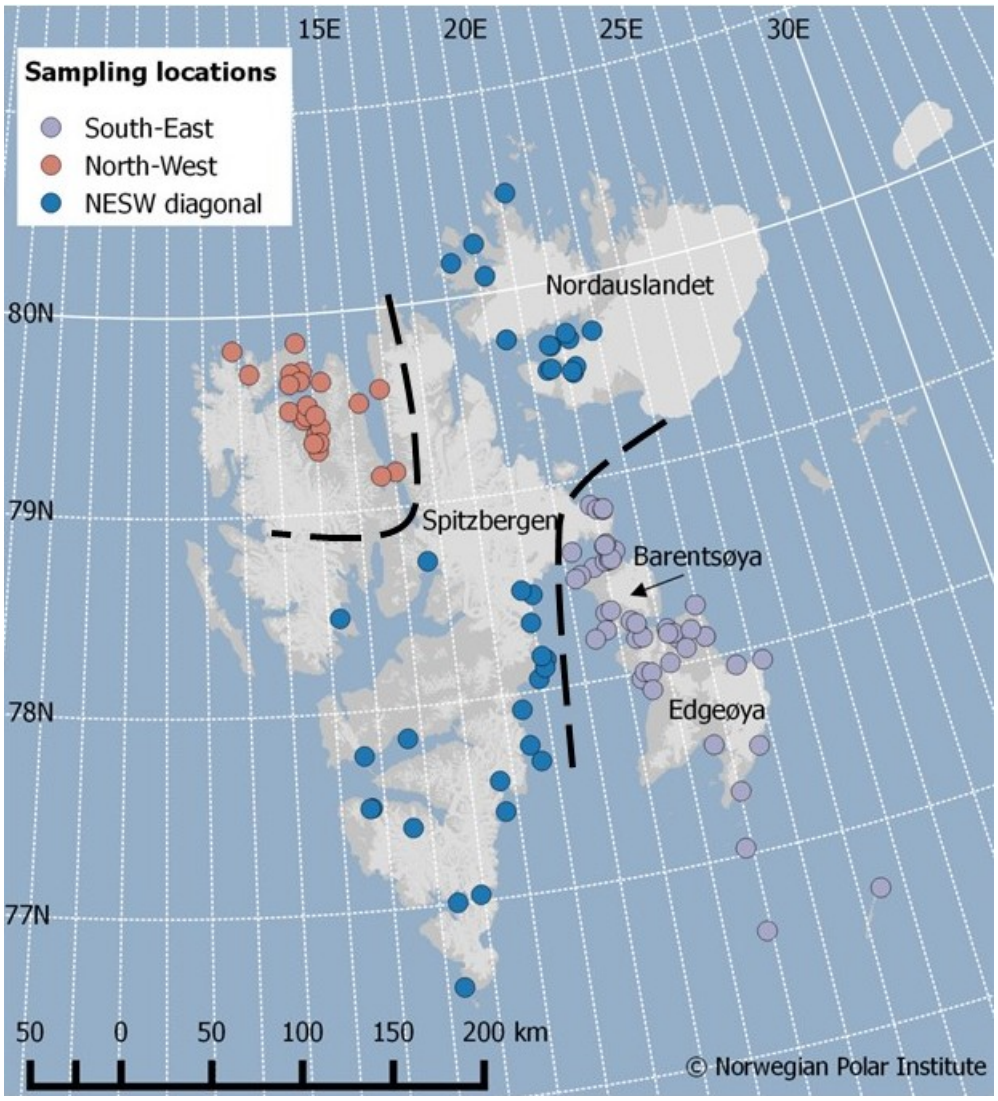


Figure 2. Boxplot illustrating body condition index (arbitrary units) according to sampling location (North-West, North-East/South-East diagonal or South-East of Svalbard) and season (spring in light grey and autumn in dark grey) in female polar bears (n=112) sampled in Svalbard in 2012 and 2013. On the plot, boxes are delimited by the 25th (lower bar) and 75th (upper bar) percentiles with the median represented by the thick horizontal line. Dots outside boxes illustrate potential outliers.

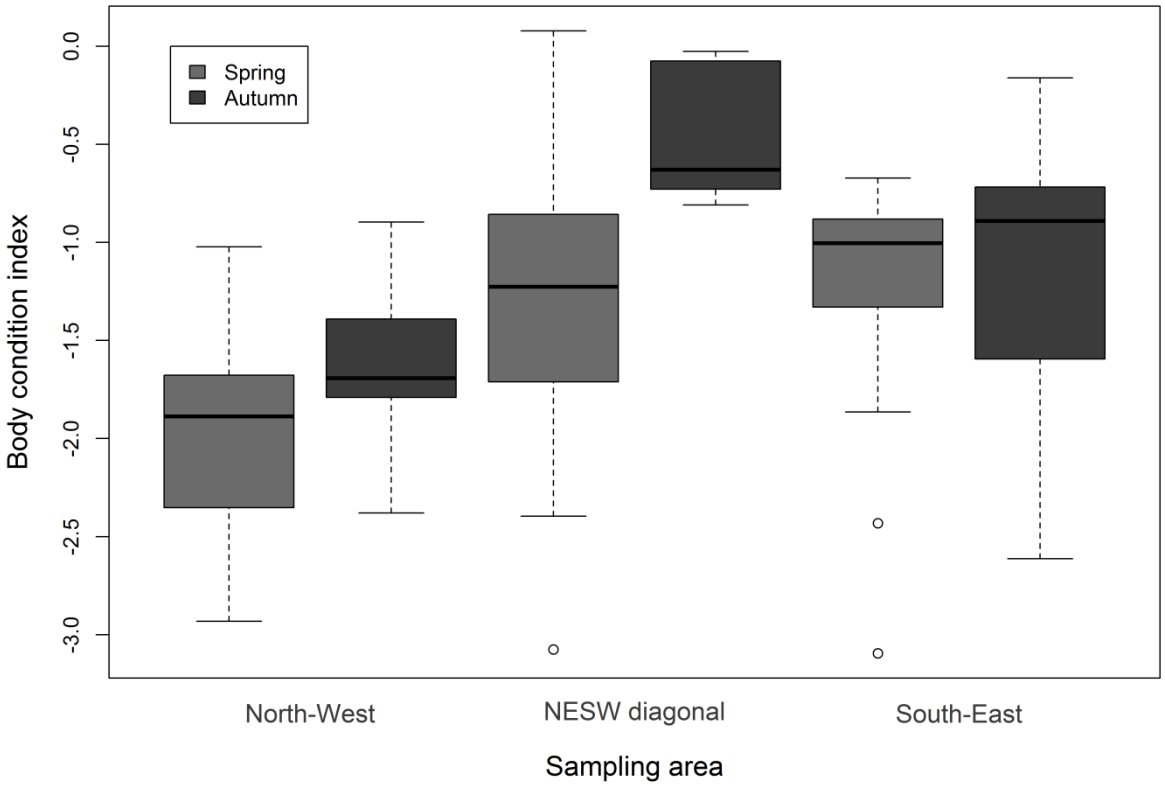


Figure 3. Relationships between plasma concentration of total triiodothyronine (TT3) and plasma sum of polychlorinated biphenyls ($\sum_{16}$PCBs) in A/ spring and C/ autumn and, between TT3 and plasma sum of organic chlorinated pesticides ($\sum_{4}$OCPs) in B/ spring and D/ autumn in female polar bears sampled in Svalbard in 2012-2013. The dots are the partial residuals, the solid line is the parameter estimate and the grey area represents its 95% confidence interval. Plasma concentrations of $\sum_{16}$PCBs and $\sum_{4}$OCPs are log transformed.

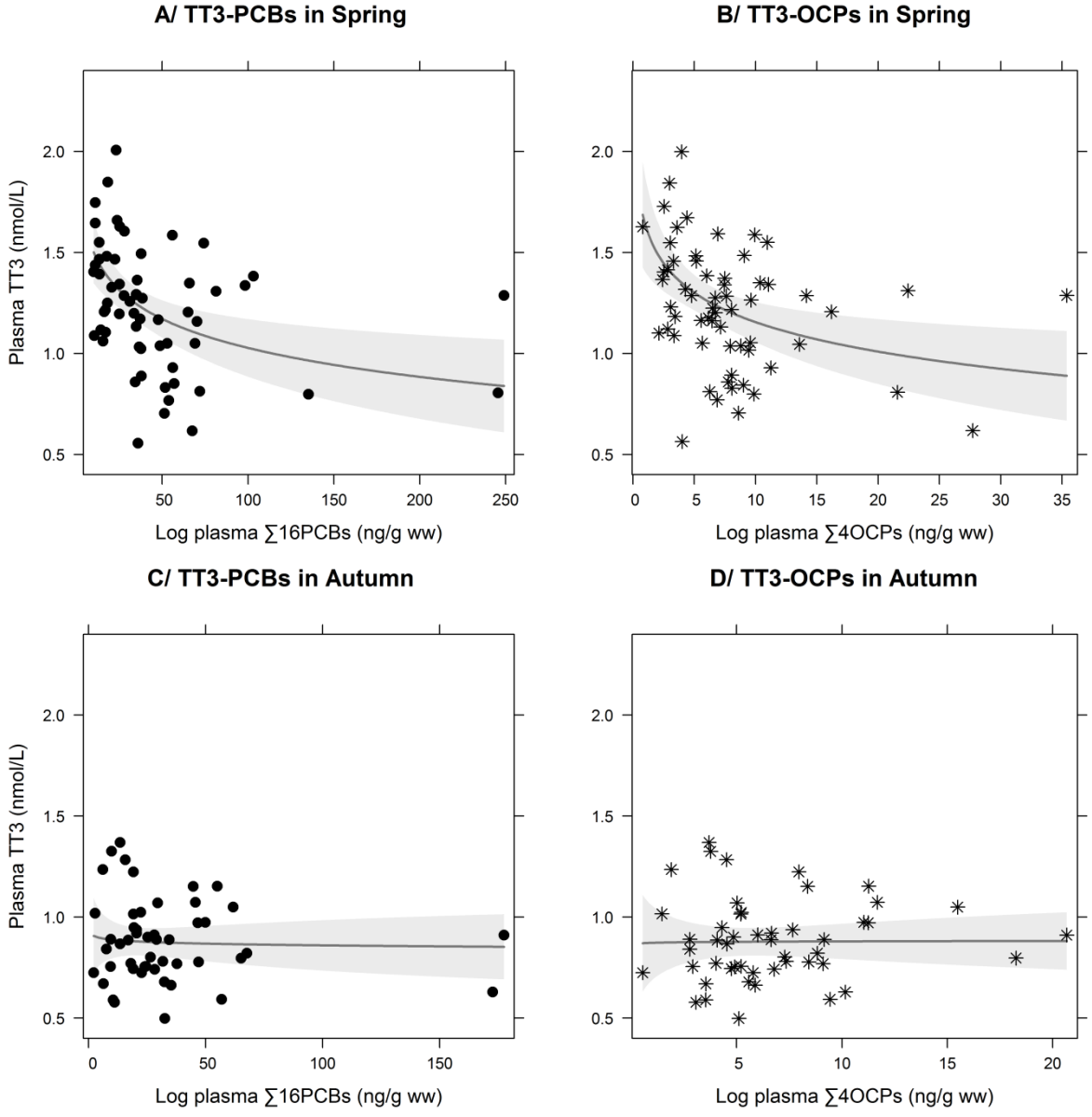
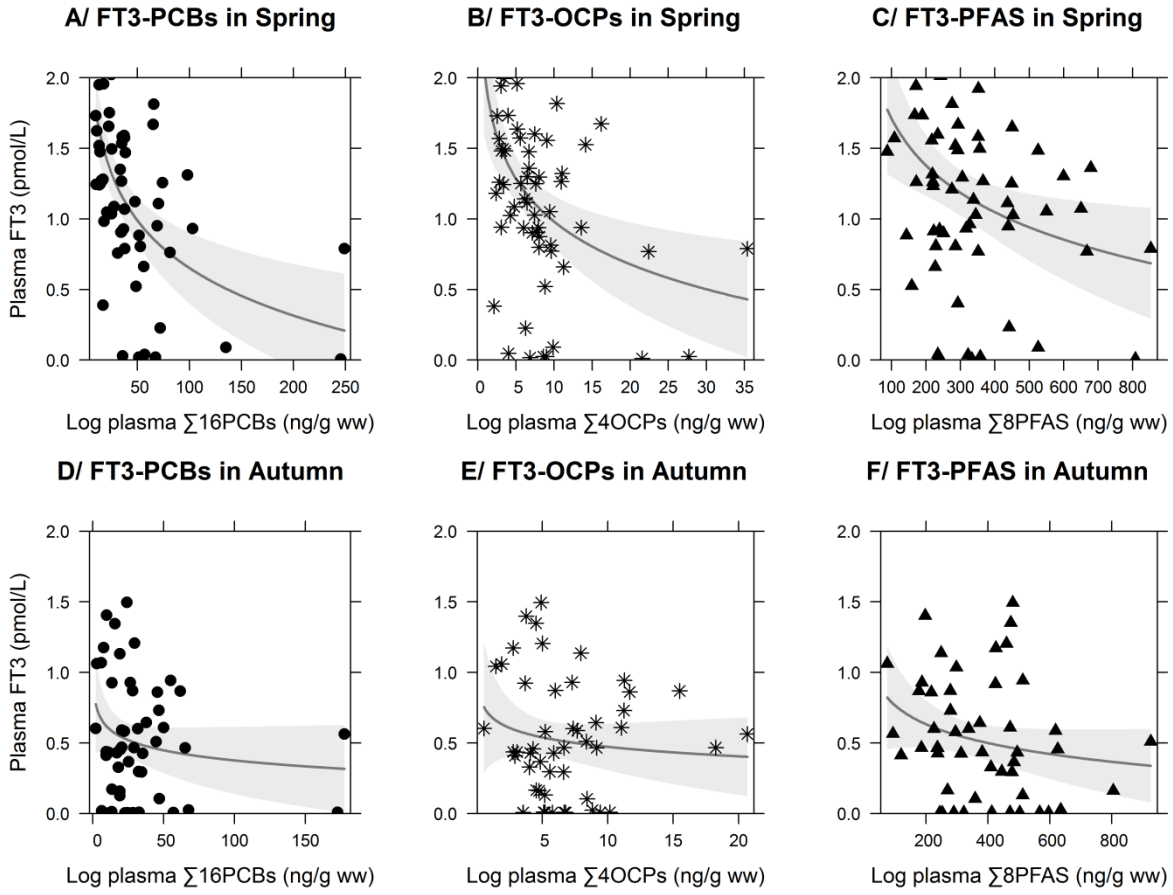
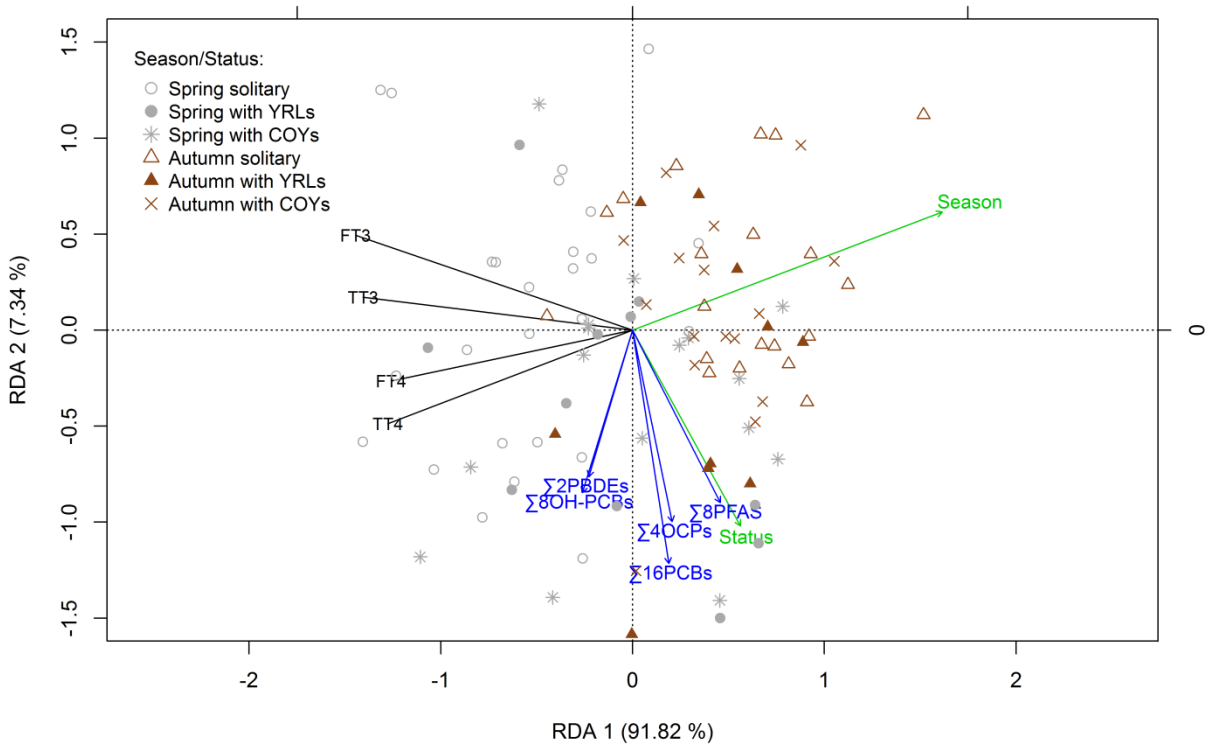


Figure 4. Relationships between plasma concentration of free triiodothyronine (FT3) and plasma sum of polychlorinated biphenyls ($\sum_{16}$PCBs) (A/ spring; D/ autumn), plasma sum of organic chlorinated pesticides ($\sum_{4}$OCPs) (B/ spring; E/ autumn) and, plasma sum of perfluoroalkyl substances ($\sum_{8}$PFAS) (C/ spring; F/ autumn) in female polar bears sampled in Svalbard in 2012-2013. The dots are the partial residuals, the solid line is the parameter estimate and the grey area represents its 95% confidence interval. Plasma concentrations of $\sum_{16}$PCBs, $\sum_{4}$OCPs and $\sum_{8}$PFAS are log transformed.



SUPPLEMENTARY INFORMATION

Figure A. Correlation triplot from redundancy analysis (RDA) illustrating the relationships between plasma concentrations of $\sum_{16}$PCB, $\sum_{4}$OCPs, $\sum_{2}$PBDEs, $\sum_{8}$OH-PCBs, $\sum_{8}$PFAS*, season, status, plasma concentrations of free and total triiodothyronine (FT3 and TT3, respectively) and thyroxine (FT4 and TT4, respectively). Female polar bears sampled in Svalbard in spring and autumn 2012-2013.



* sum (∑) of 16 PCBs ($\sum_{16}$PCBs: CB-99, -105, -118, -137, -138, -153, -156, -157, -170, -180, -183, -187, -189, -194, -206 and -209), $\sum_{4}$OCPs (oxychlordane, trans-nonachlor, *β*-HCH, HCB), $\sum_{2}$PBDEs (BDE-47, -153), $\sum_{8}$OH-PCBs (4-OH-CB107, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187), and $\sum_{8}$PFAS (PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA).

Table 1. Mean (± standard error; SE), range, median and 95% confidence interval (CI) of body mass, body condition and plasma concentrations of urea creatinine ratio (UCR), total and free triiodothyronine (TT3 and FT3) and total and free thyroxine (TT4 and FT4). The sample size (N) is indicated for each variable.

		Body mass (kg)		Body condition index (BCI; arbitrary units)		Urea Creatinine ratio (UCR; arbitrary units)		TT3 (nmol/L)		FT3 (pmol/L)		TT4 (nmol/L)		FT4 (pmol/L)	
		Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Solitary Females	N	33	22	33	22	32	22	33	22	33	22	33	22	32	22
	Mean ± SE	183 ± 4	185 ± 8	-1.22 ± 0.10	-1.02 ± 0.14	69.20 ± 9.68	44.24 ± 8.20	1.42 ± 0.05	0.85 ± 0.06	1.49 ± 0.09	0.51 ± 0.10	20.51 ± 1.04	10.07 ± 0.88	8.67 ± 0.42	4.86 ± 0.35
	Range	143; 232	121; 242	-2.37; -0.13	-2.38; -0.03	12.51; 186.43	8.35; 139.50	0.89; 2.53	0.292; 1.325	0.40; 2.61	0.01; 0.40	10.04; 38.15	1.31; 19.75	4.82; 17.71	4.71; 8.10
	Median	180	195	-1.13	-0.87	56.56	27.75	1.34	0.79	1.48	0.49	18.77	9.79	8.44	4.71
	95% CI	147; 224	127; 230	-2.12; -0.28	-2.31; -0.17	15.58; 180.73	8.97; 129.56	1.05; 1.90	0.50; 1.28	0.79; 2.33	0.01; 1.34	12.18; 29.95	4.15; 14.64	5.58; 11.17	2.84; 7.15
Females with COYs	N	18	16	18	16	18	16	18	16	18	16	18	16	18	16
	Mean ± SE	153 ± 6	161 ± 7	-1.92 ± 0.19	-1.34 ± 0.16	99.20 ± 13.52	42.83 ± 9.37	1.05 ± 0.06	0.89 ± 0.03	0.80 ± 0.13	0.50 ± 0.08	18.25 ± 1.57	11.99 ± 0.80	7.58 ± 0.57	5.45 ± 0.36
	Range	116; 212	123; 209	-3.09; -0.50	-2.47; -0.22	11.36; 241.33	9.73; 145.65	0.62; 1.45	0.59; 1.07	0.01; 1.73	0.01; 1.20	8.31; 34.56	5.27; 17.21	4.27; 13.30	3.06; 9.15
	Median	147	157	-1.77	-1.51	105.00	29.75	1.03	0.93	1.83	0.46	17.54	12.11	7.14	5.19
	95% CI	118; 204	130; 207	-3.08; -0.66	-2.29; -0.54	21.09; 209.61	12.11; 111.80	0.77; 1.42	0.65; 1.05	0.02; 1.60	0.02; 1.08	10.36; 28.22	5.87; 17.12	4.97; 12.17	3.58; 7.61
Females with YRLs	N	11	12	11	12	11	12	11	12	11	12	11	12	11	12
	Mean ± SE	173 ± 9	176 ± 7	-1.40 ± 0.20	-1.20 ± 0.19	99.04 ± 16.37	42.92 ± 12.07	1.05 ± 0.09	0.88 ± 0.06	0.91 ± 0.21	0.61 ± 0.14	18.25 ± 1.27	14.31 ± 1.51	7.55 ± 0.43	6.26 ± 0.63
	Range	138; 239	127; 210	-2.33; 0.08	-2.61; -0.07	37.81; 219.77	9.38; 139.55	0.56; 1.54	0.58; 1.37	0.01; 2.01	0.01; 1.49	12.59; 25.72	8.27; 26.02	5.64; 10.11	3.58; 10.80
	Median	170	179	-1.63	-1.05	86.15	22.15	1.13	0.86	0.94	0.45	17.31	12.05	7.33	5.55
	95% CI	144; 228	136; 204	-2.22; -0.39	-2.17; -0.43	39.43; 181.51	10.33; 118.35	0.63; 1.46	0.63; 1.25	0.01; 1.97	0.01; 1.32	12.92; 25.56	9.24; 22.58	5.93; 9.71	4.97; 12.17

Table 2. List of the five most competitive models that explain body mass, body condition index (BCI) and plasma urea creatinine ratio (UCR) in relation to season, breeding status, year and sampling location. All models (linear mixed models) include female identity as a random factor. The best model (in bold) was selected based on the lowest number of parameters (K) combined with a difference in AICc values between the "best" model and the model at hand (ΔAICc) below 2.

Response	Model	K	Log likelihood	AICc	ΔAICc
Body mass (kg)	Sampling area + Season + Status + Sampling area:Season	10	-524.00	1070.17	0
	Sampling location + Season + Status	8	-526.63	1070.66	0.48
	Sampling location + Season + Status + Year + Sampling location:Season	11	-523.30	1071.25	1.07
	Season + Status	6	-529.38	1071.57	1.40
	Sampling location + Season + Status + Sampling location:Season	9	-526.07	1071.9	1.72
BCI	Sampling location + Season + Status + Year + Sampling location:Year	11	-92.80	210.24	0
	Sampling location + Season + Status + Year	9	-95.30	210.36	0.11
	Sampling location + Season + Status + Year + Sampling location:Season + Sampling location:	13	-90.48	210.68	0.43
	Sampling location + Season + Status + Year + Season:Year	10	-94.40	210.98	0.73
	Sampling location + Season + Status + Year + Sampling location:Year + Season:Year	12	-91.96	211.06	0.82
UCR	**Season**	4	-100.96	210.46	0
	Season + Year + Season:Year	6	-98.92	210.98	0.52
	Season + Year	5	-100.50	211.80	1.34
	Season + Status	6	-99.60	212.33	1.88
	Season + Status + Year + Season:Year	8	-97.34	212.68	2.22

Table 3. List of candidate models to explain plasma concentrations of thyroid hormones (TT3, FT3, TT4 and FT4) in relation to season, breeding status, plasma urea creatinine ratio (UCR) and body condition index (BCI). All models (linear mixed models) include female identity as a random factor. The five most competitive models are presented for each response variable. The selected model (in bold) is the one with a null ΔAICc. ΔAICc is the difference in AICc between each candidate model and the model with the lowest AICc.

Candidate models

1- Season
2- Breeding status
3- Season + Breeding status + Season:Breeding status
4- Season + Breeding status
5- BCI
6- UCR
7- BCI + UCR + BCI:UCR
8- BCI + UCR
9- Breeding status + UCR
10- Breeding status + UCR + Breeding status:UCR
11- Null model

Response	Model	K	Log likelihood	AICc	ΔAICc
TT3	**Season + Status + (Season:Status)**	8	-3.25	23.89	0.00
	Season + Status	6	-11.22	35.23	11.34
	Season	4	-17.97	44.31	20.42
	Status + UCR	6	-23.18	59.17	35.28
	Status + UCR + (Status:UCR)	8	-21.35	36.22	36.22
FT3	**Season + Status + (Season:Status)**	8	-78.84	175.08	0.00
	Season + Status	6	-85.04	182.89	7.81
	Season	4	-91.18	190.73	15.65
	Status + UCR	6	-93.99	200.78	25.70
	Status + UCR + (Status:UCR)	8	-93.97	30.28	30.28
TT4	**Season**	4	-345.07	698.51	0.00
	Season + Status + (Season:Status)	8	-340.96	699.32	0.80
	Season + Status	6	-344.66	702.12	3.61
	UCR	4	-356.57	721.52	23.01
	BCI + UCR	5	-356.56	723.70	25.19
FT4	**Season**	4	-238.11	484.6	0
	Season + Status + (Season:Status)	8	-234.00	485.42	0.82
	Season + Status	6	-237.68	488.17	3.57
	UCR	4	-248.40	505.18	20.58
	BCI + UCR + (BCI:UCR)	6	-246.70	506.22	21.62

HIGHLIGHTS

- We assessed circulating thyroid hormones (TH) in 112 female polar bear samples.
- We reported seasonal variations in THs in relation to breeding status of females.
- TH levels were lower in autumn compared to spring, especially in solitary females.
- THs were negatively related to some contaminants in spring but not in autumn.