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Seasonal Variations in Thyroid Disrupting Effects of Persistent Organic Pollutants in Polar Bears (*Ursus maritimus*) from Svalbard

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Cover foto: Polar bear (*Ursus maritimus*), by Johanna Bergmann

ABSTRACT

Several persistent organic pollutants (POPs) have the ability to interfere with the thyroid hormone (TH) system in arctic mammals. This endocrine system is crucial for adapting to a changing environment, and it is an important regulator of growth, thermoregulation and metabolism. Seasonal variations in prey availability forces the polar bear (*Ursus maritimus*) to fast in longer periods, and consequently this causes seasonal variations in their body condition. Fasting has been related to depressed TH concentrations and redistribution of POPs from the adipose tissue to target organs. However, fasting (i.e. season) is rarely considered in studies examining TH-POP relationships. Therefore, it was aimed to examine concentrations and relationships between THs and POPs in free-ranging female polar bears in different seasons (spring or autumn) and reproductive status groups (solitary, with cubs of the year, or with yearlings). A total of 112 blood samples were collected in April and September 2012 and 2013 in Svalbard, Norway. Plasma concentrations of total triiodotyronine (TT3), free triiodotyronine (FT3), total thyroxine (TT4), and free thyroxine (FT4) were analysed. Concentrations of 52 individual POPs, including polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), per- and polyfluorinated alkyl substances (PFASs), hydroxylated PCBs (OH-PCBs), and hydroxylated PBDEs (OH-PBDEs), were also analysed in the plasma. The results showed seasonal and status differences in TH concentrations, and to a lesser degree in POP concentrations. THs were generally higher in spring compared to autumn, and higher in solitary females compared to females caring for offspring, which was explained by fasting status. Variations in POP concentrations could be related to body condition, which did not necessarily reflect the fasting status. FT3 was negatively related to ∑PCB, the sum of OCPs and PBDEs ("∑PESTBDE") and ∑PFAS. The ratio TT3:FT3 was positively correlated to ∑PCB and ∑PFAS, and TT4:FT4 was positively correlated to ∑PFAS. Seasonal differences in the TH-POP relationships were found for TT4:FT4-∑PFAS, TT4:TT3-∑PESTBDE, in addition to TT4:TT3-∑PFAS. In summary, the results of this study indicated that POPs may interfere with TH concentrations in polar bears, and they may be more sensitive to thyroid disruption in certain seasons. Altogether, the results emphasise the importance of accounting for environmental and biological variables, such as season and reproductive status, when examining variations of TH and POP concentrations and their relationships in arctic mammals.

SAMMENDRAG

Flere persistente organiske miljøgifter (POPer) er kjent for sine hormonforstyrrende egenskaper, og kan blant annet føre til endringer i konsentrasjoner av thyroidhormoner (TH) hos arktiske pattedyr. Thyroidhormonsystemet er viktig for at dyr skal kunne tilpasse seg et skiftende miljø, og det regulerer blant annet vekst, varmeregulering og stoffskifte. Isbjørnen (*Ursus maritimus*) faster i perioder på grunn av sesongvariasjoner i tilgjengeligheten av byttedyr, noe som fører til svingninger i deres kroppskondisjon. Faste har tidligere blitt relatert til lavere konsentrasjoner av THer og omfordeling av POPer fra fettvev til forskjellige organer. På tross av dette vurderes sesong eller faste sjeldent i studier som undersøker sammenhengen mellom konsentrasjoner av THer og POPer. I dette studiet ble det derfor undersøkt hvordan konsentrasjoner og relasjoner mellom THer og POPer i isbjørnbinner fra Svalbard varierte med hensyn på sesong (vår eller høst) og reproduksjonsstatus (enslige, unger fra 0-1 år og unger fra 1-2 år). 112 blodprøver ble innsamlet i april og september 2012 og 2013 på Svalbard. Plasmakonsentrasjoner av total trijodtyronin (TT3), fritt trijodtyronin (FT3), total tyroksin (TT4) og fritt tyroksin (FT4) ble analysert. I tillegg ble plasmaet analysert for 52 ulike POPer, deriblant polyklorinerte bifenyler (PCBer), organoklorine pestisider (OCPer), polybrominerte difenyletere (PBDEer), per- og polyfluorinerte alkylsubstanser (PFASer), hydroksylerte PCBer (OH-PCBer) og hydroksylerte PBDEer (OH-PBDEer). Resultatene viste sesong- og statusvariasjoner i TH-konsentrasjonene, og i mindre grad i POP-konsentrasjonene. TH-konsentrasjonene var generelt høyere på våren enn på høsten, og høyere hos enslige isbjørner sammenlignet med binner med unger, hvilket kunne forklares ut ifra fastestatus. Variasjonene i POP-konsentrasjonene kunne forklares ut ifra kroppskondisjon, som ikke nødvendigvis avspeilte fastestatus. FT3 var negativt korrelert med ∑PCB, summen av OCPer og PBDEer (”∑PESTBDE”) og ∑PFAS. Ratioen TT3:FT3 var positivt korrelert med ∑PCB og ∑PFAS, og TT4:FT4 var positivt korrelert med ∑PFAS. Sesongforskjeller ble funnet i relasjonen mellom TT4:FT4-∑PFAS, TT4:TT3-∑PESTBDE, i tillegg til TT4:TT3-∑PFAS. Kort oppsummert indikerte resultatene at POPer kan påvirke sirkulerende konsentrasjoner av THer i isbjørn, og at dyrene kan være mer sensitive for thyroidforstyrrende stoffer i ulike sesonger. I tillegg viser dette studiet hvor viktig det er å betrakte sesong og reproduksjonsstatus når man undersøker variasjoner i TH- og POP-konsentrasjoner og deres relasjoner i arktiske pattedyr.

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ABBREVIATIONS

ACE	Acetone
AIC	Akaikes Information Criterion
BCI	Body condition index
BM	Body mass
CHX	Cyclohexane
CI	Confidence interval
COIA	Co-inertia analysis
COY	Cubs younger than 1 year old
CV	Coefficient of variation
D1	Deiodinase enzyme type I
D2	Deiodinase enzyme type II
D3	Deiodinase enzyme type III
EDC	Endocrine-disrupting compounds
FT3	Free triiodotyronine
FT4	Free thyroxin
GC	Gas chromatography
HPT	Hypothalamic-pituitary-thyroid
ICC	Intra-class correlation coefficient
LME	Linear mixed-effect models
LSM	Least squares mean
LOD	Limit of detection
LOQ	Limit of quantification
NMBU	Norwegian University of Life Sciences
NP	Norwegian Polar Institute
NTNU	Norwegian University of Science and Technology
OCP	Organochlorine pesticide
OH-PBDE	Hydroxylated polybrominated diphenylether
OH-PCB	Hydroxylated polychlorinated biphenyl
PAPS	3′-phosphoadenosine-5′-phosphosulfate
PBDE	Polybrominated diphenylether
PCB	Polychlorinated biphenyl

PFAS	Per- and polyfluorinated alkyl substances
POP	Persistent organic pollutant
RDA	Redundancy analysis
rpm	Rounds per minute
rT3	Reverse triiodotyronine
sd	Standard deviation
SULT	Sulfotransferase
T3	Triiodotyronine
T4	Thyroxine
TDC	Thyroid-disrupting compounds
TH	Thyroid hormone
THR	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
TR	Thyroid receptor
TT3	Total triiodotyronine
TT4	Total thyroxine
TTR	Transthyretin
UDPGT	Uridine diphosphate glucuronosyltransferase
ww	Wet weight
YRL	Cubs aged between 1 and 2 years
ΣOH-PCB	Sum of hydroxylated polychlorinated biphenyls
ΣPCB	Sum of polychlorinated biphenyls
ΣPESTBDE	Sum of pesticides and polybrominated diphenylethers
ΣPFAS	Sum of per- and polyfluorinated alkyl substances

CONTENTS

1.0 INTRODUCTION

1.1 Contaminants in the Arctic

The Arctic is characterised by a low human population density and little industry, and only a small percentage of the pollution found there originates from local sources. Most contaminants reach the Arctic by long-range transport through air, ocean, rivers and ice from heavily industrialised areas (Barrie et al., 1992, Macdonald et al., 2002, Halsall, 2004, de Wit et al., 2006). Persistent organic pollutants (POPs) are organic chemical substances that, due to their physical and chemical properties, remain intact in the environment for long periods. With the aim of protecting human health and the environment from the negative effects of POPs, the Stockholm Convention went into force in 2004 (www.pops.int). The convention initially banned or restricted the production, use, release and unsafe disposal of twelve chemicals called "the dirty dozen"[1], and new chemicals were added to the list in 2009[2], 2011[3] and 2013[4]. Despite the regulations, high levels of these compounds are still found in the environment due to their persistent character, and most of these POPs have been detected in both abiotic and biotic components of the arctic environment (Hunter et al., 2010, Letcher et al., 2010). As many of the POPs are lipophilic and persistent, they tend to accumulate in animals and are biomagnified through food webs (Fisk et al., 2001).

Apex predators, such as the polar bear (*Ursus maritimus*), are exposed to high concentrations of POPs through their diet and are therefore highly susceptible to toxic effects (Norstrom et al., 1998, Letcher et al., 2010). There has been a decreasing trend in concentrations of legacy POPs in arctic biota, including the polar bear, during the last decades (Braune et al., 2005, Rigét et al., 2010, McKinney et al., 2011, Bytingsvik et al., 2012a, Dietz et al., 2013a). Contrastingly, some per- and polyfluorinated alkyl substances (PFASs) and brominated flame-retardants may have increased during the same period (Dietz et al., 2008, Butt et al., 2010, de Wit et al., 2010, Bytingsvik et al., 2012b, Dietz et al., 2013b).

[1] aldrin, chlordane, dichlorodipheyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, polychlorinated biphenyls (PCBs) polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs).

[2] chlordecone, gamma-hexachlorocyclohexane (γ-HCH, lindane), alpha-hexachlorocyclohexane (α-HCH), beta-hexachlorocyclohexane (β-HCH), hexa-, hepta-, tetra- and pentabromodiphenyl ether (PBDEs), hexabromobiphenyl, pentachlorobenzene (PeCB), perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOS-F).

[3] alpha- and beta-endosulfan

[4] hexabromocyclododecane (HBCD)

However, some of these novel POPs have recently started to decrease (McKinney et al., 2011, Rigét et al., 2013). The decreasing trends of legacy POPs generally reflect reduced emissions (Rigét et al., 2010, Dietz et al., 2013b); however, climate change may also directly or indirectly affect the distribution of POPs in biota (McKinney et al., 2016).

1.2 POPs in a warming climate

The interaction between climate change and POP exposure has attracted scientific attention for its potential to act as an additional threat to ecosystems (Jenssen, 2006, Noyes et al., 2009, Letcher et al., 2010, Jenssen et al., 2015). The negative effects of multiple stressors could be enhanced in environments where species are living at the edge of their physiological tolerance, such as in the Arctic (Noyes et al., 2009). Amplification of positive feedbacks, associated with melting of ice and snow, makes polar regions particularly susceptible to climate change (Moritz et al., 2002). The release, distribution and degradation of POPs are highly dependent on environmental conditions, thus climate change and increased climate variability will affect the life cycle of POPs (Macdonald et al., 2003). It is difficult to predict to which extent the different pollutants will be affected, but there is a concern that increasing temperatures will generally increase POP levels in the Arctic due to increased revolatilization from melting snow and ice (Ma et al., 2011). Furthermore, reduced summer sea ice could lead to increased shipping, exploitation of resources, industrial activities and tourism in the Arctic Ocean, and thus change the emission sources of novel emerging POPs, with unknown toxic effects (AMAP, 2012). Several studies have additionally shown that climate change may cause a diet change in wildlife populations due to earlier sea ice breakup, thus leading to an altered contaminant exposure pattern (McKinney et al., 2009, Gaden et al., 2012, McKinney et al., 2013). Climate change may challenge the endocrine system in wildlife, while it regulates timing and duration of life history stages (e.g. reproduction). When environmental conditions are altered and become less predictable, as a consequence of climate change, this may cause inappropriate timing of life history events with a possible fatal outcome (Wingfield, 2008).

1.3 Thyroid hormones

Endocrine-disrupting chemicals (EDCs) are exogenous compounds, capable of disrupting the normal function of endogenous hormones by interfering with the synthesis, secretion, metabolism, binding, function or elimination of endogenous hormones (Colborn et al., 1993).

The endocrine system plays a key role in adaptation, and exposure to EDCs may therefore affect an animal's capacity to respond to a changing environment (Wingfield, 2008). Thyroid hormones (THs) are critical for normal growth and cell differentiation, brain development, thermoregulation and the control of basal metabolism (Crisp et al., 1998, Diamanti-Kandarakis et al., 2009). Disruptions in the thyroid system may thus disturb the response to seasonal temperature changes (Oki and Atkinson, 2004) and the negative energy balance, which polar bears may experience during the summer (Jenssen et al., 2015). Natural variations in TH concentrations have been associated with sex, age, stress, fasting, moulting, and physiological and reproductive status (McNabb, 1992, Ortiz et al., 2003, Oki and Atkinson, 2004, Routti et al., 2010).

The thyroid system is regulated by the hypothalamic-pituitary-thyroid (HPT) axis, which is activated by the secretion of thyrotropin-releasing hormone (TRH) from the hypothalamus (Hiller-Sturmhöfel and Bartke, 1997). When reaching the pituitary gland, TRH stimulates the release of thyroid-stimulating hormone (TSH), which further stimulates the production and secretion of the THs; triiodotyronine (T3) and thyroxine (T4). T4 is the most abundant hormone and only 10% of the hormones produced in the thyroid gland are T3s (Hiller-Sturmhöfel and Bartke, 1997). T3 has a much higher affinity for the thyroid receptors (TR) than T4 and is therefore considered the active hormone (Visser et al., 1990, McNabb, 1995). Consequently, most T4 hormones are converted into T3 in the liver and kidneys by deiodinase enzymes (McNabb, 1995). Deiodinase type I (D1) and type II (D2) remove an iodine atom from the outer ring of T4 converting it into T3, and deiodinase type III (D3) converts T4 into reverse T3 (rT3) by removing an iodine atom from its inner ring. Deiodinases are likely to play key roles in the control of tissue/cellular levels of T3. When circulating throughout the body, T4 is strongly bound to one of the three transport proteins; thyroxine-binding-globulin, transthyretin (TTR) and albumin (Crisp et al., 1998). These proteins facilitate transport to target tissues, where the THs bind to TRs and mediate gene expression. THs are cleared from the blood in the liver as a result of sulfation by sulfotransferases (SULTs), or glucuronidation by uridine diphosphate glucuronosyltransferase (UDPGT). The modified thyroid hormones are then eliminated through the bile (Visser and Peeters, 2000). To end the circle, T3 and T4 ultimately provide a negative feedback effect on the hypothalamus and pituitary, consequently self-regulating the concentration of THs in the body.

A variety of POPs have been assessed as thyroid-disrupting compounds (TDCs) while being able to act directly on the thyroid gland (Wade et al., 2002), bind to TH-transport proteins in blood (Lans et al., 1994, Gutleb et al., 2010, Simon et al., 2011, Bytingsvik et al., 2013) and thus displacing THs and influencing TH homeostasis in the blood, interfere with the TRs (Cheek et al., 1999, Kitamura et al., 2005, Miyazaki et al., 2008) resulting in altered TH-mediated gene expression, disrupt enzyme systems (deiodinases, UDPGTs, and SULTs) resulting in altered enzyme levels or activities (Beetstra et al., 1991, Van Raaij et al., 1993, Schuur et al., 1998a, Schuur et al., 1998b, Schuur et al., 1999, Wade et al., 2002, Kato et al., 2004, Yu et al., 2009, Routti et al., 2010, Gabrielsen et al., 2015b) and thus affecting TH production, conversion of T4 to T3, biotransformation and excretion of THs. Several correlative field studies as well as mechanistic *in vitro* studies suggest thyroid disrupting effects by POPs in polar bears (Skaare et al., 2001, Braathen et al., 2004, Villanger et al., 2011a, Bytingsvik et al., 2013, Gabrielsen et al., 2015b).

1.3 Polar bears

Polar bears are apex predators of the arctic marine ecosystem (Amstrup, 2003). They have a circumpolar distribution and the global population is estimated to approximately 26000 individuals (www.iucnredlist.org). Their principal habitat is the sea ice and they are therefore classified as marine mammals (Amstrup, 2003). Polar bears mainly feed on seals, especially ringed seal (*Phoca hispida*) (Derocher et al., 2002) and harp seals (*Pagophilus groenlandicus*) (Kleivane et al., 2000), but they are also opportunistic scavengers feeding on for instance whale carcasses and seabird eggs (Prop et al., 2015). Today, the primary conservation concern for polar bears is habitat loss and reduced access to their primary prey due to climate change. The sea ice is declining as a response to global warming and this has been associated with declines in body condition, birth rates, survival and abundance of polar bears (Derocher et al., 2004, Regehr et al., 2007, Laidre et al., 2008, Robbins et al., 2012, Stirling and Derocher, 2012). Climate change may also cause alterations in trophic structures, food sources and migratory patterns, which will influence bioaccumulation and biomagnification of some POPs (McKinney et al., 2016). The effect of this will be most pronounced for species positioned at the highest levels of the trophic chain (McKinney et al., 2009, Noyes et al., 2009). Consequently, polar bears are in a stressful situation when trying to adapt to the new conditions. The Barents Sea subpopulation of polar bears is expected to be especially susceptible to habitat loss as a consequence of climate change (Durner et al., 2009), and is

also found to be one of the most contaminated subpopulations in the Arctic (Verreault et al., 2005, Letcher et al., 2010). A modelling study by Amstrup et al. (2008) predicted that two-thirds of the global polar bear population, including the Barents Sea subpopulation, will be extinct by mid-century mainly due to declines in sea ice habitat.

Most POPs are stored in the adipose tissue due to their lipophilicity. Polar bears and arctic mammals in general are highly dependent on their fat reserves due to their adaption to short periods of high productivity and long seasons with less available food sources (Barrie et al., 1992). During summer, most polar bears are forced to fast because melting sea ice makes hunting difficult. In order to prepare themselves for this fasting period they build up fat reserves during spring when food sources are abundant. In late summer their nutritional status can be rather poor, until hunting becomes possible again in autumn or early winter (Atkinson et al., 1996). Pregnant polar bears enter maternity dens in early winter where they may fast up to 8 months and lose more than 40% of their body mass. Reproduction is very energy consuming, and the fact that this coincides with fasting in polar bears makes body fat critically important for reproductive success (Atkinson and Ramsay, 1995).

During fasting, when the fat storage declines, contaminants may be redistributed to target organs such as the liver, and this may lead to complications of the bear's health (Polischuk et al., 1995, Polischuk et al., 2002). It has been shown that contaminants are mobilized from the adipose tissues to the blood stream and thereby redistributed to target organs during fasting or starvation in arctic and non-arctic mammals (Lydersen et al., 2002, Debier et al., 2006, Helgason et al., 2013). This redistribution can increase the concentrations of contaminants in target tissues, and thereby increase the toxic effect (Helgason et al., 2013). Thus, animals experiencing long periods of emaciation, fat mobilization and redistribution of POPs seem to be particularly vulnerable (Fuglei et al., 2007, Letcher et al., 2010).

1.4 Aims

Previous studies have found varying trends in the relationships between THs and POPs in wildlife, most likely due to confounding factors. It is known that THs have natural seasonal variations and that POP concentrations also vary as an effect of for instance fasting. However, the effect of season is usually not considered when studying TH-POP relationships in arctic mammals. Annual monitoring of polar bears in Svalbard is performed during spring (March-May) (www.mosj.no), and little data is available for polar bears sampled in autumn. Thus,

using adult female polar bears as a model species, this project aimed to investigate 1) the seasonal variations in the concentrations of THs and POPs in accordance to reproductive status, 2) relationships between THs and POPs, and 3) if these relationships can be related to fasting status, i.e. season.

1.5 Expectations

TH levels were expected to vary between seasons as a result of changes in metabolism due to differences in prey availability and energetic demands. A lower metabolism associated with fasting has previously been related to decreased TH concentrations in American black bears (*Ursus americanus*) (Schussler and Orlando, 1978, Azizi et al., 1979, Tomasi et al., 1998). The study by Torget (2015), which used the same polar bear samples as the present study, found that the polar bears generally appeared to be feeding in spring and fasting in autumn. It was therefore hypothesised that TH concentrations would be lower and POP concentrations higher in fasting animals.

Lactation has been related to low TH concentrations in humans (Strbak et al., 1978) and rats (Kahl et al., 1987). Consequently, it was hypothesised that females caring for offspring would have lower TH concentrations than solitary bears. Most POPs are transferred from mother to offspring through the milk (Polischuk et al., 2002, Gabrielsen et al., 2011, Bytingsvik et al., 2012a, Bytingsvik et al., 2012b, Frouin et al., 2012), and lactation is therefore considered an important excretion route. Based on this, it was hypothesised that the concentrations of POPs would be lower in females with offspring compared to solitary females.

Both positive and negative associations between THs and POPs in polar bears have been reported previously (Skaare et al., 2001, Braathen et al., 2004, Bertinussen, 2009, Villanger et al., 2011a, Gabrielsen et al., 2015b). Thus, it was expected to detect TH-POP relationships in the present study as well, however, it was not possible to predict the direction of these. As contrasting patterns were expected in the concentrations of THs (lowest in fasting animals) and POPs (highest in fasting animals), it was hypothesised that the TH-POP relationships would be different in non-fasting and fasting animals, manifested as seasonal differences. This would provide an indication of whether polar bears are more sensitive to the effect of TDCs in certain periods of the year.

2.0 MATERIAL AND METHODS

2.1 Field sampling

Blood samples from 112 sexually mature free-ranging female polar bears from the Barents Sea population were collected in April and September 2012 and 2013. In 2012 a number of 57 samples was obtained, 33 in April and 24 in September, and in 2013 additionally 55 samples were taken, 29 in April and 26 in September. Altogether this represented 78 individuals, and 26 of them were captured more than once (hereafter referred to as recaptured). The search effort of the sampling was mainly dependent on external factors, e.g. weather and sea ice conditions, resulting in an opportunistic sampling regime.

The polar bears were immobilised from a helicopter (Eurocopter AS350 Ecureuil) by remote injection of a dart containing the anaesthetic drug Zoletil ® 100 (Virbac, Carros, France). Each bear was individually marked with an ear tag and a tattoo to enable identification when recaptured. For the age determination of the bears, a vestigial premolar tooth was extracted and later analysed following the methods of Calvert and Ramsay (1998) and Christensen-Dalsgaard et al. (2010). Using heparinised collecting tubes, the blood samples were collected from the femoral vein, stored on ice and centrifuged within 10 hours (3500 rounds per minute, rpm, 10 min). The plasma was frozen immediately after centrifugation and stored at -20°C until further use. Body mass (BM) was determined (to the nearest kg) by suspending the bear on a stretcher from two spring hanging scales. One of the bears could not be weighed and its BM was therefore estimated using morphometric measurements (i.e. auxiliary girth and dorsal straight-line body length) as described by Derocher and Wiig (2002).

Immobilisation and all the described handling procedures followed standard protocols (Stirling et al., 1989, Derocher and Wiig, 2002) and were approved by the National Animal Research Authority of Norway and the Governor of Svalbard.

According to reproductive status, the polar bears were divided into three groups: 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 the polar bears that were recaptured, some of the cubs were lost. Two females lost their single cubs between spring and autumn of the same year, one female lost two cubs from the first spring to the next and two females lost one cub from the first autumn to the next.

2.2 Hormone analyses

The hormone analyses were conducted at the Department of Biology, NTNU, Trondheim, Norway. The concentration of T3 and T4 in plasma was determined using a solid phase radioimmunoassay. This method is based on competitive binding of radioactive-labelled THs (T3*, T4*) and unlabelled THs (T3, T4) to tubes covered with antibodies. The amount of bound T3* and T4* was quantified using a gamma counter, and the concentration of unlabelled THs was then estimated based on a calibration curve.

A total of four TH variables, total and free T3 (TT3, FT3) and total and free T4 (TT4, FT4), were analysed using a Coat-A-Count® kit (Coat-A-Count TT4, Coat-A-Count FT4, Coat-A-Count TT3, Coat-A-Count FT3, Siemens Healthcare Diagnostics, Los Angeles, CA, USA), following the procedure provided by the manufacturer. The kits are commercially available ^{125}I kits originally developed for humans, and have been validated and used successfully on polar bear plasma previously (Braathen et al., 2004, Bytingsvik, 2012, Gabrielsen et al., 2015a).

The desired amount of each sample and calibrator was added to the pre-coated tubes in addition to 1 mL radioactive-labelled THs. The solution was mixed and incubated in a water bath from 1-3 hours at 37°C. Afterwards the tubes were decanted thoroughly and counted for 1 minute in a gamma counter (Cobra Auto-Gamma; Packard Instrument Company, Dowers Grove, IL, USA). The TH concentrations were calculated using calibration curves generated by the gamma counter software (SpectraWorks, Spectrum Analysis Software, Meriden, USA).

2.2.1 Quality control

TT3 and FT3 were run in duplicates (using 100 μL of plasma per replicate) while TT4 and FT4 were run in triplicates (using 25 and 50 μL per replicate, respectively). Instrument detection limits were 0.016 and 2.048 nmol/L for TT3 and TT4, respectively, and 0.012 and 0.425 pmol/L for FT3 and FT4, respectively. The coefficients of variation (CVs) were generally acceptable (CV<20%) for TT3, TT4, and FT4. 17% of the FT3 samples had high CVs (CV>20%), however, these all had low TH concentrations and were therefore accepted.

A series of calibrators with known TH levels were analysed to obtain a calibration curve for each analysed kit. When two kits were analysed simultaneously, only one calibration curve was created. Three levels of standard reference material (SRM, Lyphochek ® Immunoassay

Plus Control, Levels 1-3, BIO-RAD, CA, USA), bovine serum and one additional plasma sample were analysed in each run, in order to check intra- and inter-assay variation. The intra-assay variation was between 2.40-6.19%, determined as 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). 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) (Bourgeon et al., unpublished). TT3 and TT4 concentrations are expressed in nmol/L, whereas FT3 and FT4 concentrations are expressed in pmol/L. 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. Three TH ratios (TT3:FT3, TT4:FT4 and TT4:TT3) were also calculated.

2.3 Contaminant analysis

The plasma samples were analysed with gas chromatography (GC) at the Laboratory of Environmental Toxicology, NMBU, Oslo, Norway. The laboratory is accredited (Norwegian Accreditation, Kjeller, Norway) for determination of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenylethers (PBDEs) in biological matrices of animal origin according to the requirements of NS-EN ISO/IEC 17025:2005 (Test 137). The method for detecting PFASs, hydroxylated polychlorinated biphenyls (OH-PCBs) and hydroxylated polybrominated diphenylethers (OH-PBDEs) is not accredited, but it was performed and validated following the same principles as the accredited method for PCBs, OCPs and PBDEs.

The following 52 compounds were analysed: PCB-99, -105, -118, -128, -137, -138, -153, -156, -157, -170, -180, -183, -187, -189, -194, -196, -206, -209, α-HCH, β-HCH, *p,p'*-DDE, HCB, Oxychlordane, *trans*-Nonachlor, BDE-47, -99, -100, -153, PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, 4'-OH-CB106, 4-OH-CB107, 4'-OH-CB108, 3-OH-CB118, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187, 4-OH-BDE42, 3-OH-BDE47, 6-OH-BDE47, 4'-OH-BDE49 and 2'-OH-BDE68 (see Appendix A for full names). The analytical standards were provided by Cambridge Isotope Laboratories, Inc., Andover, MA, USA (PCBs, OCPs, BDEs); Ultra Scientific, Rhode Island, USA (PCBs); Supelco, Bellefonte, PA, USA (OCPs); LGC Promochem, Wesel, Germany (HCB); Wellington Laboratories Inc., Ontario, Canada (OH-PCBs). All the compound concentrations are given in nmol/L wet weight (ww).

The clean-up method is based on a liquid-liquid extraction that was first described by Brevik (1978) and later modified by Polder et al. (2008). The analysis of the OH-metabolites was performed according to the modifications done by Gabrielsen et al. (2011).

Approximately 2 g (±0.100 g) of the plasma were weighed in 80 mL centrifuge glasses. The samples were spiked with internal standards; PCB-29, -112 and -207 (Ultra Scientific, Rhode Island, USA), BDE-77, -119, -181 and -[$^{13}C_{12}$]-209 (Cambridge Isotope Laboratories, Inc., Andover, MA, USA), 4'-OH-[$^{13}C_{12}$]-CB-159 and 4-OH-[$^{13}C_{12}$]-CB-187. Knowledge from previous analyses was used to add internal standards in amounts corresponding to the levels expected in the samples.

Solvents were added to the samples in the following order: 2 mL 6% NaCl, 10 mL 1M H_2SO_4, 15 mL acetone (ACE) and 20 mL cyclohexane (CHX). The samples were then homogenized for 1 minute using an ultra sound sonicator (Cole Parmer CPX 750, Vernon Hills IL, USA) followed by centrifugation (3000 rpm, 10 min, Allegra X-12R, Beckman Coulter, Fullerton CA, USA). If an emulsion occurred after the centrifugation, the sample was frozen for at least one hour before centrifuged again. If the supernatant was clear and there was no sign of emulsion, it was transferred to Zymark® glasses. The samples were then evaporated to 1 mL at 40°C with a gentle stream of N_2 (purity: 99.6 %; AGA AS, Oslo, Norway, pressure 0.6 bar) using a Zymark® evaporator (TurboWap II, Zymark Corporation, Hopkinton, MA, USA). A new round of solvents were added to the centrifuge glasses in the following order: 5 mL ACE and 10 mL CHX. The samples were then sonicated for a second time (30 sec), followed by another centrifugation (3000 rpm, 10 min). The supernatant was added to the same Zymark® glass, and the evaporation was repeated to reach approximately 1 mL. The concentrated sample was then ready for the lipid determination.

Due to low amounts of lipids and relatively small sample volumes of the plasma, the complete extract was used for the lipid determination. The concentrated extract from the last evaporation was transferred quantitatively from the Zymark® glass to a pre-weighed 10 mL conical glass. The samples were then evaporated to dryness on a sand bath (40°) with a gentle stream of N_2, tempered to room temperature followed by weighing. The evaporation and weighing was continued until a constant weight was achieved (variation less than ±0.0020 g).

Afterwards the lipid percentage was determined using the following formula:

$$\text{lipid\%} = \frac{(\text{weight of glass with fat} - \text{weight of empty glass}) \cdot 100}{\text{weighed quantity}}$$

1 mL of CHX was then added and the sample was mixed on a Whirlimixer (MS2 Minishaker, IKA® Works, INC.) and left for 10 min (until homogeneous) before further preparation.

In order to remove fat and protein residues, concentrated sulphuric acid (6 mL, 97.5 % H_2SO_4, Fluka Analytical, Sigma-Aldrich, St. Louis MO, USA) was added to the samples, which were then mixed and reversed carefully. The samples were left in darkness in room temperature for one hour, followed by centrifugation (3000 rpm, 10 min). The supernatant was transferred to a test tube, and the acid layer was washed with CHX, turned upside-down and centrifuged (3000 rpm, 5 min). The "washed" supernatant was then added to the test tube.

The alkaline extraction was performed in order to extract the OH-metabolites in their own phase, to avoid interference with other similar substances. Before the alkaline extraction, the samples were evaporated on a sand bath (40° with a gentle stream of N_2) to approximately 1 mL. Potassium hydroxide (1M KOH in 50% ethanol, 5 mL) was added to each sample, mixed and centrifuged (3000 rpm, 5 min). The heaviest phase was then transferred to large test tubes, and the alkaline extraction was repeated once.

Approximately 2 mL of purified Grade 1 water was added to the organic phase left in the test tubes and then centrifuged (3000 rpm, 5 min). The supernatant was transferred to a calibrated (400 μL) conical glass. Approximately 2 mL of CHX was used to wash the tube, and the centrifugation was repeated (3000 rpm, 5 min), before transferring the supernatant to the same conical glass. When calibrating the glass, the appropriate amount of keeper (300 μL 2% decane in CHX) was added to a glass, and the meniscus was marked on the glass. The keeper contained less volatile solvents, which helps to prevent evaporation of the analytes. The samples were then evaporated on a sand bath (40° with a gentle stream of N_2) to the calibration mark, washed once with approximately 0.5 mL CHX followed by another evaporation to the calibration mark. Afterwards, the remaining sample was transferred to amber vials with inlets for analysis.

Concentrated H_2SO_4 was added carefully to the alkaline phase, until a pH of 1-2 was obtained. The samples were mixed properly and 5 mL CHX was added before a second

mixing. The samples were then left until the phases had separated, and the supernatant was transferred to Zymark® glasses. The extraction was repeated once. Before transferring the samples to new test tubes, they were evaporated to 1 mL on the Zymark® evaporator. After transferring the samples, each Zymark® glass was washed three times with CHX. Once more, the samples were evaporated on a sand bath (40° with a gentle stream of N_2) to 1 mL followed by derivatisation.

In order to avoid peak tailing on the GC, the hydroxyl groups on the OH-metabolites were exchanged with acetyl groups through a derivatisation process. 60 μL mix of acetic anhydride and pyridine (1:1) was added to each of the samples, mixed and placed in a heating cabinet (60°C, 30 min). When the samples had achieved room temperature again, they were extracted by adding 2 mL of Grade 1 water, mixed and centrifuged (3000 rpm, 5 min). The organic phase was then transferred to a calibrated conical glass (400 μL) and evaporated on a sand bath (40° with a gentle stream of N_2) to the calibration mark. They were then washed once with 0.5 mL CHX followed by evaporation to the calibration mark. Afterwards, the remaining sample was transferred to GC-glass for analysis.

The POP concentrations were quantified using a high resolution GC (Agilent 6890 Series GC system, Agilent Technologies, Santa Clara, CA, USA) equipped with an auto sampler (Agilent 7683 Series, Agilent Technologies). When analysing PCB and OCP concentrations, the system was coupled to two ^{63}Ni micro electron-capture detectors (Agilent 6890 μ-ECD, Agilent Technologies). A mass spectrometer (Agilent 5973 Network Mass Selective Detector, Agilent Technologies) was configured with the GC for quantification of PBDEs and OH-PCBs. Details of the methods can be found in Polder et al. (2008) and Gabrielsen et al. (2011), with the exception of the following modifications for PCBs and OCPs made for the present study: constant flow of the hydrogen carrier gas was increased to 1.2 mL/min and final holding time at 275°C was increased to 21 min, making the total run time 76.6 min.

2.3.1 Quality control

For each series of 16 samples, 3 blank samples, 1 blind and 2 recovery samples were included, as well as the in-house reference materials of seal blubber and seal blood. The blank samples consisted only of internal standards and solvents. The matrix used for the blind and recovery samples was a mixture provided by NMBU, mainly consisting of plasma from domestic animals, which were expected to have low concentrations of environmental

pollutants. Standard procedures were used to ensure adequate quality assurance and control, and the accuracy, linearity, and sensitivity of the analyses were within the laboratory's accreditation requirements.

The limit of detection (LOD) was defined as three times the average background noise in the chromatograms of the sample extracts and ranged from 0.010-0.370 ng/g ww for the PCBs, 0.10-0.140 ng/g ww for the OCPs, 0.010-0.018 ng/g ww for the PBDEs, 0.050-0.500 ng/g ww for the PFASs, 0.035-0.210 ng/g ww for the OH-PCBs, and 0.060-0.115 ng/g ww for the OH-PBDEs. Due to coeluting compounds, the limits of quantification (LOQ) for PCB-28, -52, -101 *p,p'*-DDT, and *cis*-chlordane were set to ten times the noise level.

The relative recovery for each sample series was calculated from two samples of low-contaminated material spiked with a standard containing all the analytes. For the PCBs it ranged from 92-115%, 93-145% for the OCPs, 92-105% for the PBDEs, 86-124% for the PFASs, 57-104% for the OH-PCBs, and 43-76% for the OH-PBDEs. α-HCH, β-HCH, HCB, and *trans*-Nonachlor were corrected for recovery in two of the sample series. PCB-153, -138 and -209 were corrected for procedural blank values. The analyses of the laboratory's internal reference material proved satisfactory reproducibility.

2.4 Statistical analyses

Only POPs that were detected in more than 60% of the samples were included in the following statistical analyses. For the compounds included in the statistical analyses, values below LOD were assigned half the LOD value. All samples had concentrations of TT3, TT4, and FT4 above LOD; however, the samples with FT3 concentrations below LOD were assigned the arbitrary value 0.005 pg/mL (0.00768 pmol/L).

All statistical analyses were conducted using the software R version 3.2.2 (R Core Team, 2015) and RStudio version 0.99.465 (RStudio, Inc.) with additional packages: "ade4" version 1.7-2 for redundancy analysis (RDA), "nlme" version 3.1-121 and "lme4" version 1.1-9 for linear mixed-effect models (LME), "AICcmodavg" version 2.0-3 for model selection, "lsmeans" version 2.20-23 for calculating least squares means (LSM), "effects" version 3.0-4 for generating effect plots, and "sjmisc" version 1.2 and "sjplot" version 1.8.4 for generating tables. The total sample number was 112 in all analyses, except from the RDA and all models

involving FT4, where the sample number was 111 due to one missing sample in the FT4 analysis. Results were considered statistically significant when $p<0.05$.

Multivariate analyses were performed to illustrate the relationships between season, reproductive status, POPs and THs. An RDA was performed with THs as response variables and POPs as explanatory variables (hereafter referred to as predictors). Prior to the analysis all the data was centred and scaled. The relationship between the response variables and the predictors were further examined with a co-inertia analysis (COIA) (Dray et al., 2003, Josse et al., 2008), which provided us with an RV coefficient of 0.075. The COIA was then tested using a Monte-Carlo permutation test. Sample scores were plotted with respect to season and status to illustrate their influence on the response variables. To reduce the number of variables in the further analyses the POPs were divided in four groups (∑PCB: PCB-99, -105, -118, -128, -137, -138, -153, -156, -157, -170, -180, -183, -187, -189, -194, -206 and -209; ∑PESTBDE: β-HCH, *p,p'*-DDE, HCB, Oxychlordane, *trans*-Nonachlor, BDE-47 and -153; ∑PFAS: PFHxS, PFOS, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTriA; ∑OHPCB: 4-OH-CB107, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180, 4-OH-CB187) based on the loadings from the RDA.

Maximum likelihood LMEs were used to test for the effects of season (spring and autumn), reproductive status (alone, COY and YRL) and POP groups (∑PCB, ∑PESTBDE, ∑PFAS and ∑OHPCB) on plasma TH concentrations. LMEs consist of two types of predictors, non-random (hereafter referred to as fixed factors) and random effects. Including random effects in the model is a way of accounting for repeated measurements of some of the individuals. The identification number of each polar bear was therefore included as a random effect in all the following models.

The influence of season and status on THs and POPs was tested by creating a model for each of the response variables (TT3, FT3, TT4, FT4, TT3:FT3, TT4:FT4 TT4:TT3, ∑PCB, ∑PESTBDE, ∑PFAS and ∑OHPCB) with season, status and their interaction (season:status) as predictors. The differences between categories in the predictors were investigated by analysing the LSMs (Lenth, 2015) and reported as *p*-values.

In order to reduce the number of models for further investigation, model selection was performed using Akaikes Information Criterion (AIC) (Johnson and Omland, 2004). For each response variable (TT3, FT3, TT4, FT4, TT3:FT3, TT4:FT4 and TT4:TT3) a set of models

were created representing 25 combinations of the five fixed factors (POP, season, status, season:POP and season:status). Four sets of 25 models were made for each response variable, one per POP group (∑PCB, ∑PESTBDE, ∑PFAS and ∑OHPCB). Within each set the most parsimonious model was determined as the one with the lowest AICc, which resulted in 17 final models. The influence of the predictors on the response variables was reported as parameter estimates (β) with 95% confidence intervals (CI) from the LMEs, *p*-values from a Wald chi-squared test and the intra-class correlation coefficient (ICC) for each model. It should be emphasised that there is a disagreement among statisticians on how to calculate *p*-values for mixed models, and they should therefore be considered a supplement to the confidence intervals for assigning significance level.

Diagnostic plots of residuals against fitted values were created for each model to check if the model assumptions were met, i.e. constant variance between residuals. The response variable TT3:FT3 did not meet the assumptions and was therefore log-transformed in all the relevant models. ∑PCB and ∑PESTBDE were also log-transformed in the models explaining FT3 and TT3:FT3 levels, as they were better predictors than the non-log-transformed variables.

3.0 RESULTS

3.1 THs in relation to season and status

Figure 1 depicts the mean concentrations ± standard deviation (sd) of THs for females in different breeding status groups during spring and autumn (Appendix B and C). LMEs with THs as response variables and season, status and their interaction as predictors showed that, independent of status, the concentrations of TT3, FT3, TT4 and FT4 were higher in spring compared to autumn (LSM, $p<0.0001$). This trend was mainly driven by the solitary females and to a lesser degree by females with offspring (Alone: $p<0.0001$; COY: $p<0.066$; YRL: $p<0.251$). For polar bears captured in spring, the solitary females had higher concentrations of TT3 and FT3 than females with offspring ($p<0.007$), whereas concentrations of TT4 and FT4 did not differ significantly between status groups ($p>0.183$). In autumn, females with YRL had higher concentrations of TT4 than solitary females ($p=0.048$), whereas females with COY did not differ from the other groups ($p>0.291$). Concentrations of TT3, FT3 and FT4 did not differ significantly between status groups in autumn ($p>0.108$). Independent of status, TT4:TT3 was the only one of the ratios showing significant seasonal variation by being higher in spring compared to autumn ($p=0.002$). However, solitary females had lower levels of TT3:FT3 and higher levels of TT4:FT4 in spring compared to autumn ($p<0.018$). Solitary females sampled in autumn had significantly lower levels of TT4:TT3 than females with YRL ($p=0.008$). There were no status differences for TT3:FT3 and TT4:FT4 in any of the seasons ($p>0.124$).

3.2 POPs in relation to season and status

40 compounds, out of the 52 that were examined, were detected in more than 60% of the samples (Figure 2, Appendix D). When expressed as nmol/L, ∑PFAS was the most abundant POP group constituting around 70% of the total POP load. PFOS had the highest mean concentration of all POPs (466.64±241.60 nmol/L) and represented 46% of the total POP load and 65% of ∑PFAS. ∑OHPCB constituted approximately 17% of the total POP load, and the two congeners 4-OH-CB146 and 4-OH-CB187 represented 84% of ∑OHPCB. ∑PCB constituted about 11% of the total POP load with PCB153 and PCB180 representing 60% of ∑PCB. The least abundant group was ∑PESTBDE constituting only 2% of the total POP load with Oxychlordane representing 63% of ∑PESTBDE.

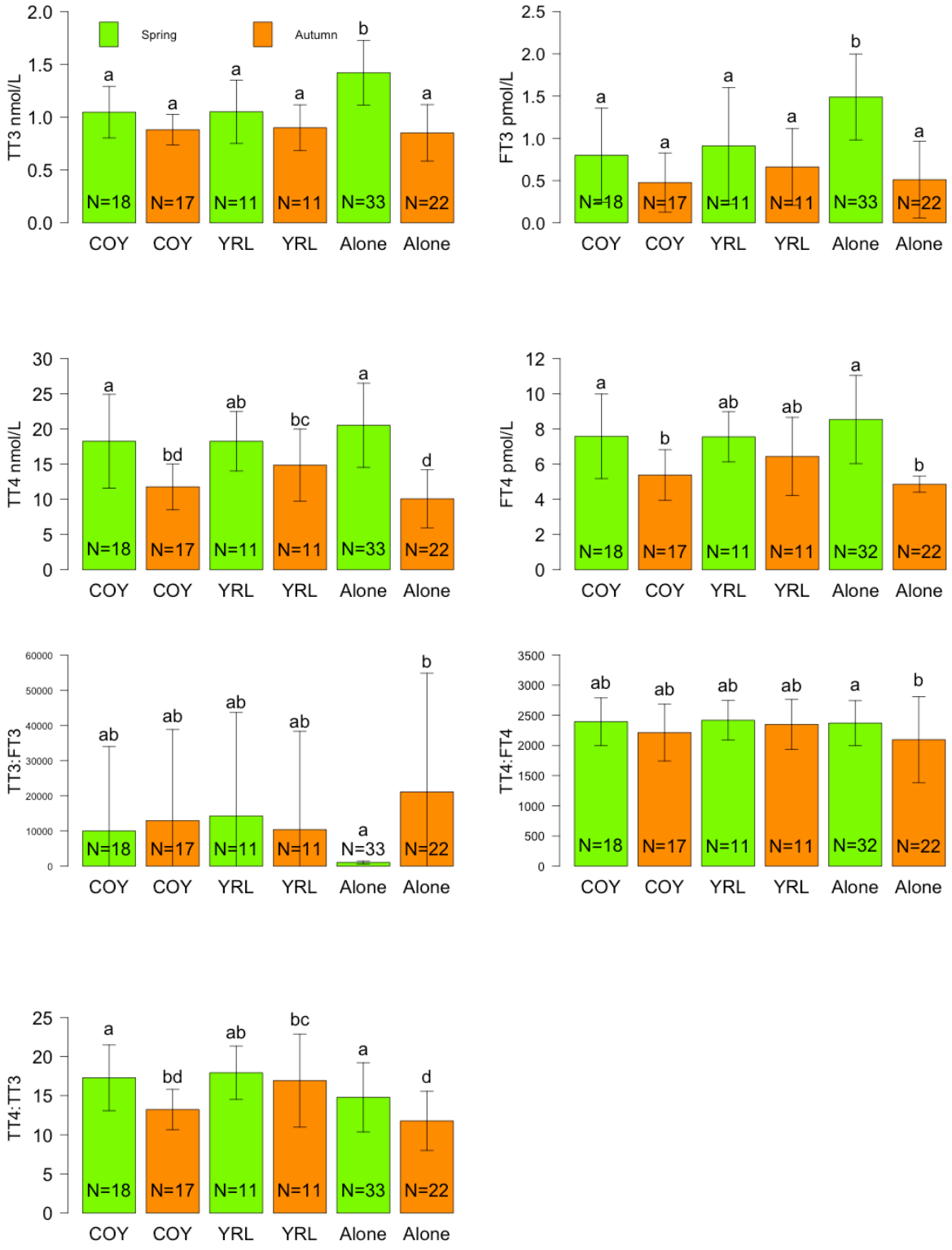

Figure 1 Mean concentrations ± standard deviation (nmol/L wet weight) of thyroid hormones and ratios measured in plasma from free-ranging female polar bears sampled in April and September 2012-13 in Svalbard. The polar bears were grouped by reproductive status (Alone, COY or YRL) and season (spring or autumn). Groups without a common letter (a, b, c, d) are significantly different from each other ($p \leq 0.05$) based on least squares means comparisons. Abbreviations: total triiodothyronine (TT3), free triiodothyronine (FT3), total thyroxine (TT4), free thyroxine (FT4), females with cubs of the year (COY), and with yearlings (YRL).

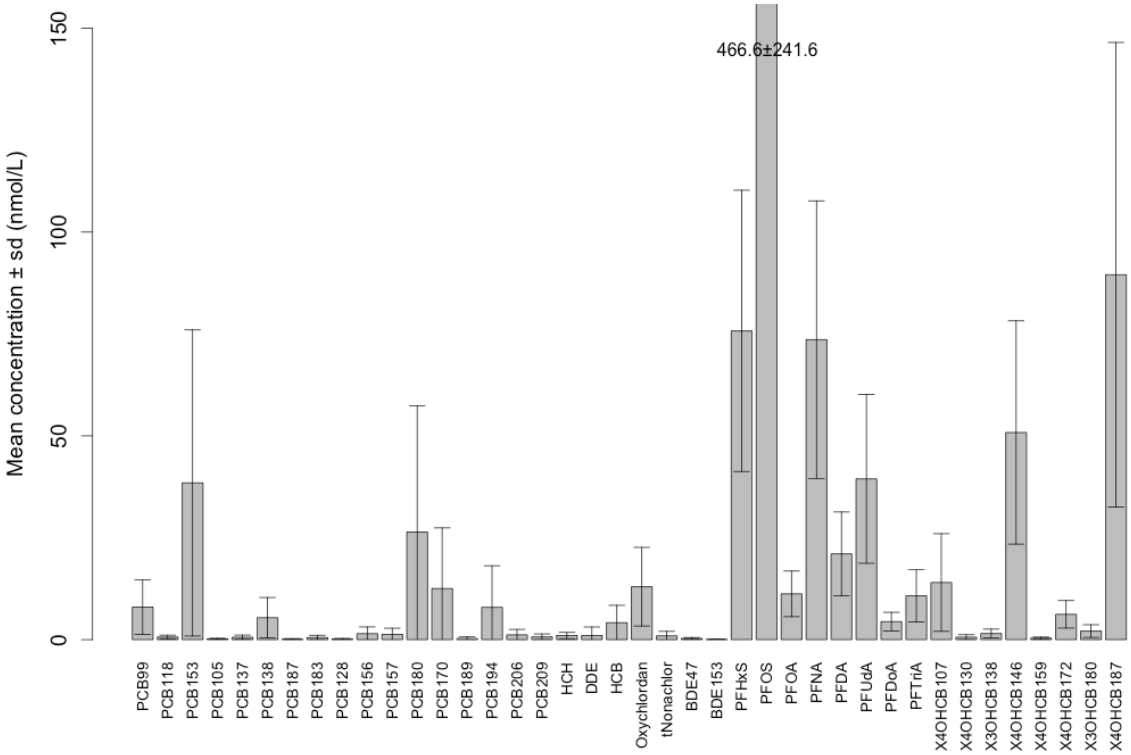

Figure 2 Mean concentrations ± standard deviation (nmol/L wet weight) of 40 POPs measured in plasma from free-ranging female polar bears (N=112) sampled in spring and autumn 2012-13 in Svalbard. Abbreviations: persistent organic pollutants (POPs), polychlorinated biphenyl (PCB), beta-hexachlorocyclohexane (HCH), dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), brominated diphenyl ether (BDE), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluoroctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUdA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTriA) and hydroxylated polychlorinated biphenyl (OHPCB).

Figure 3 depicts the mean concentrations ± sd of POPs in the polar bears when grouped by season and status. The following results are derived from LMEs with POPs as response variables and season, status and their interaction as predictors. ∑PCB concentrations were higher in spring compared to in autumn (LMS, p=0.030), which was primarily due to seasonal differences in females with COY (p=0.011). The interaction of season and status showed that females with COY had higher ∑PCB concentrations than solitary females sampled in spring (p=0.028), but none of the other groups differed between seasons (p>0.131). ∑PESTBDE, ∑PFAS and ∑OHPCB concentrations in polar bears was not significantly correlated to season (p>0.162), status (p>0.131) or their interaction term (p>0.057).

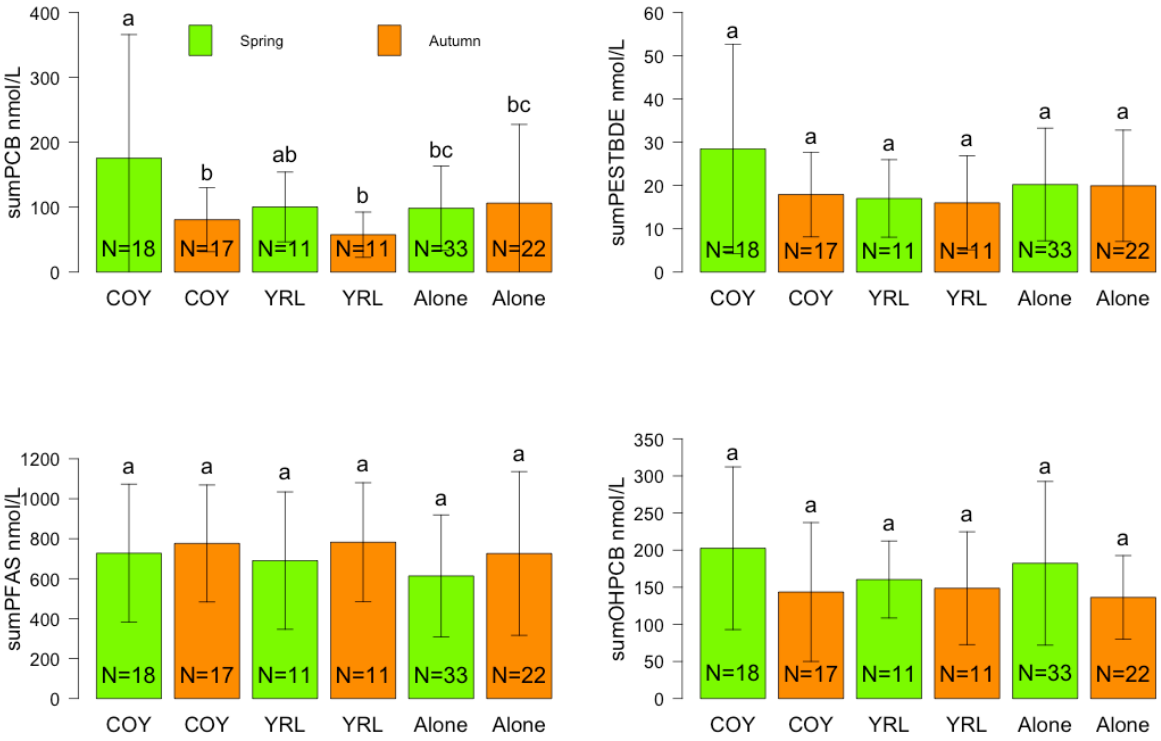

Figure 3 Mean concentrations ± standard deviation (nmol/L wet weight) of POP groups measured in plasma from free-ranging female polar bears sampled in spring and autumn 2012-13 in Svalbard. The polar bears were grouped by reproductive status (Alone, COY or YRL) and season (spring or autumn). Groups without a common letter (a, b, c) are significantly different from each other ($p \leq 0.05$) based on least squares means comparisons. Abbreviations: persistent organic pollutants (POPs), sum of polychlorinated biphenyls (∑PCB), sum of pesticides and polybrominated diphenyl ethers (∑PESTBDE), sum of per- and polyflourinated alkyl substances (∑PFAS), sum of hydroxylated polychlorinated biphenyls (∑OHPCB), females with cubs of the year (COY), and with yearlings (YRL).

3.3 Effects of POPs on THs

Figure 4A illustrates the relationship between THs and POPs in an RDA loading plot. Based on this plot the POPs were divided into four groups in order to reduce the number of variables in the LMEs. Although these groups were not all clear in the biplot, most compounds within a group were situated in the same area, and considering their chemical structure, it was reasonable to group them together as ∑PCB (PCB-99, -105, -118, -128, -137, -138, -153, -156, -157, -170, -180, -183, -187, -189, -194, -206 and -209), ∑PESTBDE (β-HCH, *p,p'*-DDE, HCB, Oxychlordane, *trans*-Nonachlor, BDE-47 and -153), ∑PFAS (PFHxS, PFOS, PFOA, PFNA, PFDA, PFUdA, PFDoA, and PFTriA) and ∑OHPCB (4-OH-CB107, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4'-OH-CB172, 3'-OH-CB180 and 4-OH-CB187). A new RDA with the THs as response variables and the POP groups as

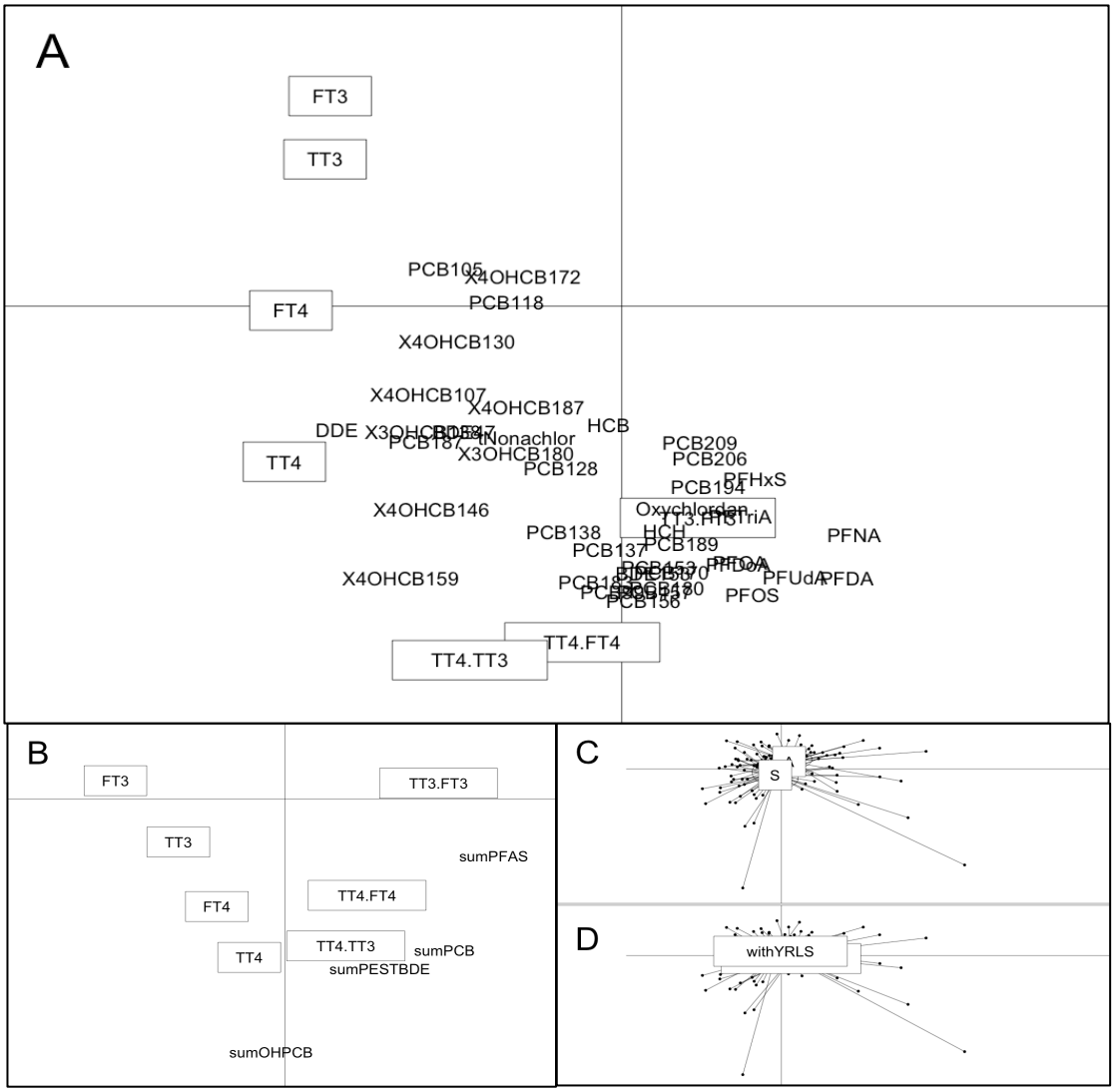

Figure 4A-D Results from RDA illustrating the relationship between THs and POPs in plasma from free-ranging female polar bears sampled in spring and autumn 2012-13 in Svalbard. A) Loading plot from RDA with THs as response variables and single POPs as predictors. The 1st axis explains 45.7% of the variation and the 2nd axis 29.3%. B) Loading plot from RDA with THs as response variables and POP groups as predictors. The 1st axis explains 70.7% of the variation and the 2nd axis 21.0%. C-D) Scores plot from the second RDA illustrating how season and breeding status may influence the relationship between THs and POPs. Abbreviations: redundancy analysis (RDA), thyroid hormones (THs), persistent organic pollutants (POPs), thyroid hormones (THs), total triiodothyronine (TT3), free triiodothyronine (FT3), total thyroxine (TT4), free thyroxine (FT4), polychlorinated biphenyl (PCB), beta-hexachlorocyclohexane (HCH), dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), brominated diphenyl ether (BDE), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluoroctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUdA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTriA), hydroxylated polychlorinated biphenyl (OHPCB), sum of polychlorinated biphenyls (sumPCB), sum of pesticides and polybrominated diphenyl ethers (sumPESTBDE), sum of per- and polyflourinated alkyl substances (sumPFAS), sum of hydroxylated polychlorinated biphenyls (sumOHPCB), autumn (A), spring (S), females with cubs of the year (COY), and with yearlings (YRL).

predictors was created and illustrated in a loading plot (Figure 4B). From this plot ∑PFAS was expected to be negatively related to FT3 and TT3 and positively related to the ratio of TT3:FT3. ∑PESTBDE and ∑PCB were expected to be positively related to TT4:TT3 and negatively related to FT3. Figures 4C-D are scores plots illustrating that season could have an influence THs-POP relationships, whereas status is less likely to influence these. Results from the Monte-Carlo permutation test of the COIA verified the RDA, as the RV coefficient differed significantly from zero (1000 replicates, $p = 0.008$), meaning that there was a correlation between the THs and the POPs, and it was greater than what could be expected by chance.

Model selection resulted in 28 models (Appendix E) that were further examined. Generally, season was important in all models, and most often status or its interaction with season was important as well. At least one POP group or its interaction with season was important in all set of models, except for TT4 and FT4. ∑PFAS was the most frequently occurring POP group in the parsimonious models.

The results from the LMEs (Table 1-2) supported parts of the expectations from the RDA. Increasing concentrations of ∑PCB, ∑PESTBDE and ∑PFAS were significantly related to decreasing levels of FT3 (log(∑PCB): β=-0.23, [-0.34, -0.13], $p<0.001$; log(∑PESTBDE): β=-0.22, [-0.37, -0.08], $p=0.002$; ∑PFAS: β=-0.0004, [-0.0007, -0.00009], $p=0.007$). Increasing concentrations of ∑PCB and ∑PFAS were significantly related to increasing levels of TT3:FT3 (log(∑PCB: β=0.50, [0.18, 0.82], $p=0.002$; ∑PFAS: (β=0.0001, [0.0001, 0.001], $p=0.016$). Increasing concentrations of ∑PFAS were significantly related to increasing levels of TT4:FT4 (β=0.73, [0.38, 1.08], $p<0.001$). Figure 5 depicts the above stated effects of POPs on THs.

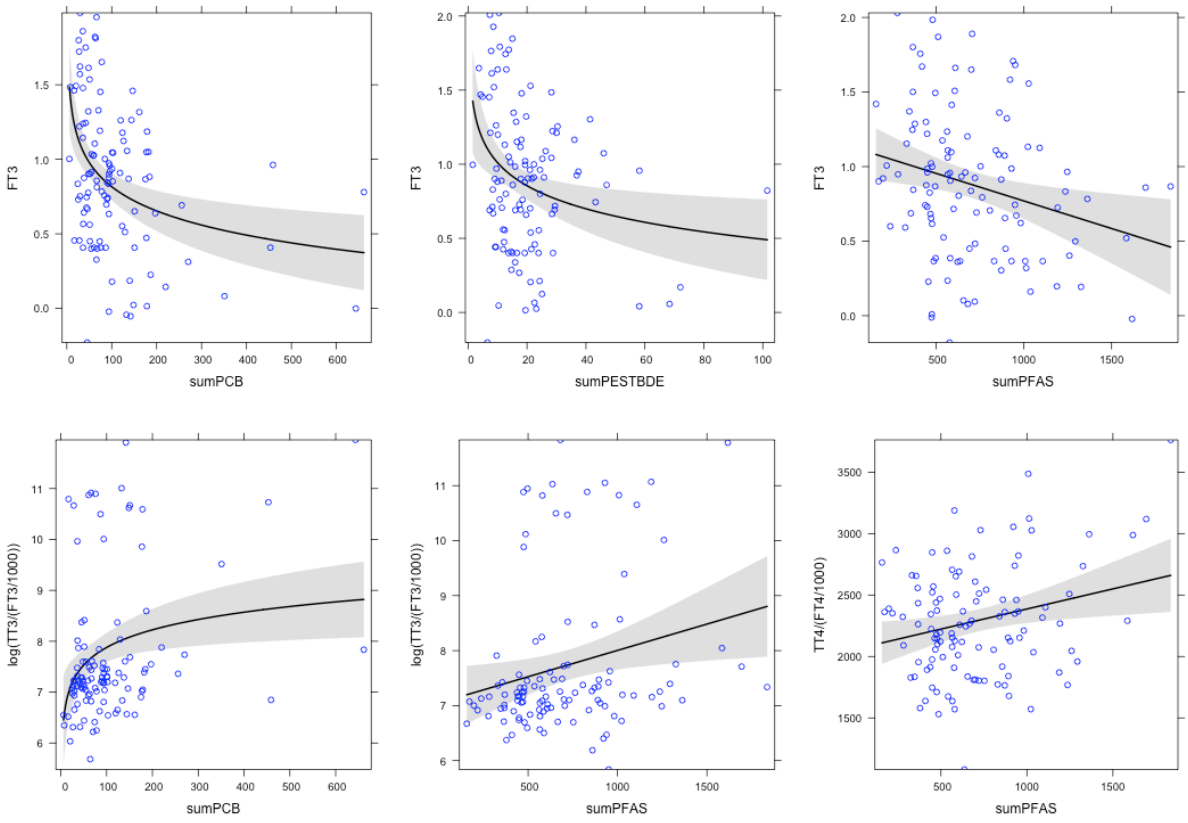

Figure 5 Illustrations of how POPs influence THs measured in free-ranging female polar bears sampled in spring and autumn 2012-13 in Svalbard. The plots are partial residual plots illustrating the effects of POPs on THs when the other factors in the linear mixed-effect models have been controlled for. The blue dots are the partial residuals, the black line is the parameter estimate and the grey area represents its 95% confidence interval. sumPCB and sumPESTBDE were log-transformed in the FT3 and TT3:FT3 models. Abbreviations: persistent organic pollutants (POPs), thyroid hormones (THs), total triiodothyronine (TT3), free triiodothyronine (FT3), total thyroxine (TT4), free thyroxine (FT4), sum of polychlorinated biphenyls (sumPCB), sum of pesticides and polybrominated diphenyl ethers (sumPESTBDE) and sum of per- and polyflourinated alkyl substances (sumPFAS).

Table 1 Results from LMEs explaining how THs and POPs are related in free-ranging female polar bears sampled in April (spring) and September (autumn) 2012-2013 in Svaøbard. FT3 and TT3:FT3 are the response variables and season, status and their interaction in addition to either log(sumPCB), log(sumPESTBDE) or sumPFAS are predictors. The models have been through a selection process, which is the reason why results from all predictors are not shown for every response variable. Results are presented as parameter estimates with 95% confidence intervals, *p*-values derived from a Wald chi-squared test, number of individuals (N_{ID}), the intra-class correlation coefficient (ICC_{ID}) and number of observations. Abbreviations: linear mixed-effect models (LMEs), thyroid hormones (THs), persistent organic pollutants (POPs), total triiodothyronine (TT3), free triiodothyronine (FT3), sum of polychlorinated biphenyls (sumPCB), sum of pesticides and polybrominated diphenyl ethers (sumPESTBDE), sum of perflourinated alkyl substances (sumPFAS), autumn (A), spring (S), females with cubs of the year (COY), and with yearlings (YRL).

Coefficients	*Response*								
	FT3			FT3			FT3		
	Estimate	*Conf. Int.*	*p-value*	*Estimate*	*Conf. Int.*	*p-value*	*Estimate*	*Conf. Int.*	*p-value*
Fixed Parts									
Intercept	1.50	1.00 – 1.99	**<.001**	1.13	0.70 – 1.57	**<.001**	0.78	0.50 – 1.06	**<.001**
log(sumPCB)	-0.23	-0.34 – -0.13	**<.001**						
SeasonS	1.02	0.78 – 1.27	**<.001**	0.99	0.74 – 1.24	**<.001**	0.93	0.68 – 1.19	**<.001**
StatuswithCOYS	-0.05	-0.33 – 0.24	.766				-0.02	-0.32 – 0.28	.539
StatuswithYRLS	0.08	-0.25 – 0.41	.766				0.17	-0.17 – 0.51	.539
SeasonS:StatuswithCOYS	-0.57	-0.96 – -0.18	**.003**	-0.63	-0.90 – -0.36	**<.001**	-0.63	-1.03 – -0.22	**.001**
SeasonS:StatuswithYRLS	-0.64	-1.10 – -0.19	**.003**	-0.61	-0.93 – -0.29	**<.001**	-0.72	-1.18 – -0.25	**.001**
log(sumPESTBDE)				-0.22	-0.37 – -0.08	**.002**			
SeasonA:StatuswithCOYS				-0.04	-0.34 – 0.26	**<.001**			
SeasonA:StatuswithYRLS				0.11	-0.23 – 0.45	**<.001**			
sumPFAS							-0.00	-0.00 – -0.00	**.007**
Random Parts									
N_{ID}		78			78			78	
ICC_{ID}		0.000			0.000			0.024	
Observations		112			112			112	

Coefficients	*Response*					
	TT3:FT3			TT3:FT3		
	Estimate	*Conf. Int.*	*p-value*	*Estimate*	*Conf. Int.*	*p-value*
Fixed Parts						
Intercept	6.12	4.76 – 7.47	**<.001**	7.43	6.72 – 8.14	**<.001**
log(sumPCB)	0.50	0.18 – 0.82	**.002**			
SeasonS	-0.98	-1.46 – -0.50	**<.001**	-0.70	-1.17 – -0.23	**.004**
sumPFAS				0.00	0.00 – 0.00	**.016**
Random Parts						
N_{ID}		78			78	
ICC_{ID}		0.318			0.320	
Observations		112			112	

3.3.1 Seasonal differences in TH-POP relationships

The interaction terms season:∑PFAS and season:∑PESTBDE showed seasonal differences in the TH-POP relationships for TT4:FT4 and TT4:TT3, which are depicted in figure 4. TT4:FT4 was positively affected by the season spring compared to autumn (β=726.99, [367.16, 1086.82], p<0.001), but the association between TT4:FT4 and ∑PFAS was greater in autumn compared to spring (Spring:∑PFAS: β=-0.73, [-1.21, -0.26], p=0.002). The opposite was the case for TT4:TT3, which was positively related to ∑PFAS in spring samples but not in autumn samples (Spring:∑PFAS: β=0.003, [0.001, 0.005], p=0.001). ∑PESTBDE was positively related to TT4:TT3 in spring samples but negatively related in autumn samples (Spring:∑ PESTBDE: β=0.12, [0.06, 0.18], p<0.001).

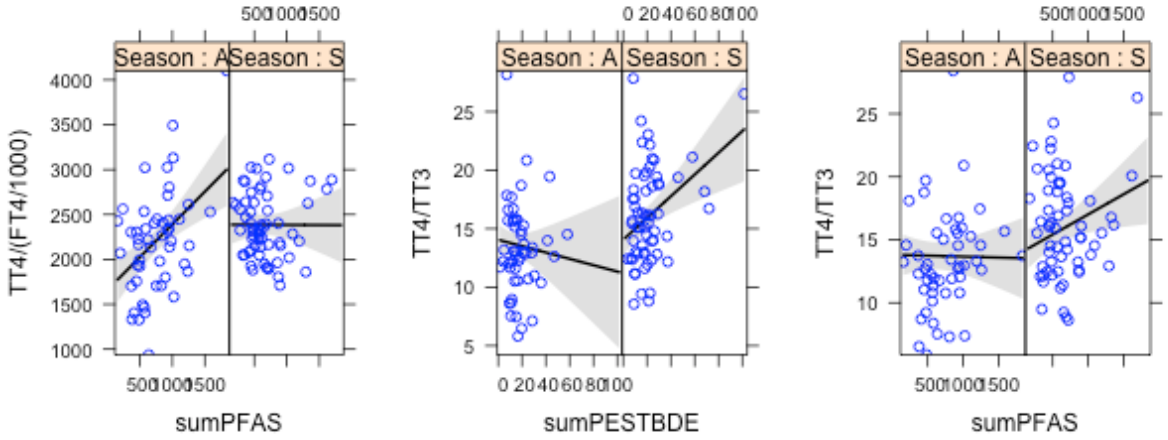

Figure 6 Illustrations of how season affects the relationship between POPs and THs measured in free-ranging female polar bears sampled in April (spring) and September (autumn) 2012-13 in Svalbard. The plots are partial residual plots illustrating the seasonal difference in effects of POPs on THs when the other factors in the linear mixed-effect models have been controlled for. The blue dots are the partial residuals, the black line is the parameter estimate and the grey area represents its 95% confidence interval. Abbreviations: persistent organic pollutants (POPs), thyroid hormones (THs), spring (S), autumn (A), total triiodothyronine (TT3), total thyroxine (TT4), free thyroxine (FT4), sum of pesticides and polybrominated diphenyl ethers (sumPESTBDE) and sum of perflourinated alkyl substances (sumPFAS).

Table 2 Results from LMEs explaining seasonal differences in the relationship between THs and POPs in free-ranging female polar bears sampled in April (spring) and September (autumn) 2012-2013. TT4:FT4 and TT4:TT3 are the response variables and season is the predictor together with some of the following variables, sumPFAS, sumPESTBDE, status, interaction of season and sumPFAS or interaction of season and sumPESTBDE. The models have been through a selection process, which is the reason why results from all predictors are not shown for every response variable. Results are presented as parameter estimates with 95% confidence intervals, *p*-values derived from a Wald chi-squared test, number of individuals (N_{ID}), the intra-class correlation coefficient (ICC_{ID}) and number of observations. Abbreviations: linear mixed-effect models (LMEs), thyroid hormones (THs), persistent organic pollutants (POPs), total triiodothyronine (TT3), free triiodothyronine (FT3), total thyroxine (TT4), free thyroxine (FT4), sum of polychlorinated biphenyls (sumPCB), sum of pesticides and polybrominated diphenyl ethers (sumPESTBDE), sum of per- and polyflourinated alkyl substances (sumPFAS), autumn (A), spring (S), females with cubs of the year (COY), and with yearlings (YRL).

Coefficients	*Response*								
	TT4:FT4			TT4:TT3			TT4:TT3		
	Estimate	*Conf. Int.*	*p-value*	*Estimate*	*Conf. Int.*	*p-value*	*Estimate*	*Conf. Int.*	*p-value*
Fixed Parts									
Intercept	1659.94	1366.75 – 1953.14	**<.001**	12.74	11.24 – 14.24	**<.001**	12.45	10.48 – 14.41	**<.001**
sumPFAS	0.73	0.38 – 1.08	**<.001**				-0.00	-0.00 – 0.00	.902
SeasonS	726.99	367.16 – 1086.82	**<.001**						
sumPFAS:SeasonS	-0.73	-1.21 – -0.26	**.002**				0.00	0.00 – 0.01	**.001**
sumPESTBDE				-0.03	-0.10 – 0.04	.457			
StatuswithCOYS				1.64	-0.05 – 3.33	**<.001**	1.94	0.19 – 3.70	**.001**
StatuswithYRLS				4.09	2.08 – 6.09	**<.001**	3.89	1.80 – 5.98	**.001**
sumPESTBDE:SeasonS				0.12	0.06 – 0.18	**<.001**			
Random Parts									
N_{ID}		77			78			78	
ICC_{ID}		0.321			0.194			0.198	
Observations		111			112			112	

4.0 DISCUSSION

The aims of the present study were to investigate seasonal variations in concentrations and relationships between THs and POPs in female polar bears representing different reproductive status groups. TH concentrations, and to some extent POP concentrations, were significantly related to season and reproductive status. Fasting seemed to be the underlying factor explaining these seasonal and status variations in THs, whereas POP variations appeared to follow variations in body condition index (BCI). FT3 was the only single hormone that was significantly related to ΣPCB, ΣPESTBDE and ΣPFAS. The ratio TT3:FT3 was significantly related to ΣPCB and ΣPFAS, and TT4:FT4 was significantly related to ΣPFAS. Seasonal differences in the TH-POP relationships were detected in the relationship between TT4:FT4 and ΣPFAS in addition to the relationships TT4:TT3-ΣPESTBDE and TT4:TT3-ΣPFAS.

4.1 THs in relation to season and status

The plasma concentrations of TT3 measured in the present study were within the range of those reported previously in female polar bears (Skaare et al., 2001, Braathen et al., 2004, Knott et al., 2011, Gabrielsen et al., 2015a). Although within the same range, there seemed to be some variation between studies. This could most likely be explained by differences in sampling time (ranging from January-October), geographical location (East Greenland, Svalbard or Southern Beaufort Sea) or method and matrix (plasma or serum) used for analysis.

All the TH concentrations (TT3, FT3, TT4, FT4) reported in the present study were lower in autumn samples compared to spring samples. This was in accordance with the expectations, as fasting has been related to decreased TH concentrations American black bear (Schussler and Orlando, 1978, Azizi et al., 1979, Tomasi et al., 1998). Seasonal fluctuations in THs with peaks in the winter months have also been observed in captive polar bears that were maintained in a constant environment with regard to temperature, photoperiod and diet (Leatherland and Ronald, 1981). Although the polar bears in the present study appeared to be feeding in spring and fasting in autumn (Torget, 2015), this was not reflected in their BM or BCI (Bourgeon et al., unpublished). Bourgeon et al. (unpublished) examined the relationships between TH concentrations, BM and BCI in the same polar bear samples as used for the present study. They found that the mean BM and BCI were higher in autumn relative to

spring, although only significantly different for BCI. Cattet (2000) reported a similar scenario with lacking reflections of fasting state in the BM and BCI of polar bears. However, Cattet (2000) showed that the BCI followed an ascending phase in spring (feeding state) and a descending phase in autumn (fasting state), although the mean BCI values were lower in spring compared to autumn. The bears in the present study were sampled in the very beginning of the hyperphagic period that polar bears undergo in spring, when the seals come ashore to give birth to their pups (Lydersen, 2014). The polar bears are still lean after the winter, but they build up fat reserves quickly. The hyperphagic period can be followed by a period of low activity in the summer (Whiteman et al., 2015), and the polar bears start to burn their fat reserves. However, there is a delay from when fasting begins until it shows in the BCI, and consequently the BCI cannot be interpreted as a direct indicator of fasting status. The autumn sampling in the present study took place in the period of low activity, but at a time when the bears still had fat reserves left.

TT3 and FT3 were significantly lower in females with offspring relative to solitary females. Contrastingly, females with YRL had significantly higher levels of TT4 compared to solitary females, but only when sampled in autumn. The results were in accordance with the study by Braathen et al. (2004), in which female polar bears with COY had significantly lower concentrations of TT3 relative to solitary females during spring. Females caring for offspring were expected to have lower TH concentrations than solitary bears due to the influence of lactation, which was supported by the results for TT3 and FT3. The TH levels may also reflect fasting in females with COY, as samples were taken just after they emerged from their maternity dens. Bourgeon et al. (unpublished) found that the BM and BCI were significantly lower in females with COY compared to solitary females, especially in spring, which again reflects that they were still affected by their recent fasting period when sampled in spring. The low concentrations of TT3 an FT3 may thus be a reflection of both lactation and fasting in females with COY, but only lactation in females with YRL. However, this does not explain why the mean concentration of TT4 was higher in females with YRL compared to solitary females in autumn. Another possible explanation for the status differences in THs could be that the thyroid and the reproductive hormone systems are closely related (Moenter et al., 1991, Webster et al., 1991, Anderson et al., 2002, Krassas et al., 2010), and that the reproductive system can alter circulating TH levels and vice versa.

Stress associated with handling and capturing wild animals may also lead to depressed concentrations of THs, as discovered in beluga whales (*Delphinapterus leucas*) (St. Aubin and Geraci, 1988). Experiments with rats have shown that stress-induced elevation of glucocorticosteroid concentrations affect TSH levels and consequently circulating TH concentrations (Wilber and Utiger, 1969, Bianco et al., 1987). Although the sampling procedure in the present study aimed to be as little stressful as possible, it cannot be ruled out that some individuals may have had increased glucocorticosteroid concentrations, which could have affected the TH concentrations. However, since baseline levels of THs in polar bears are not well established (Letcher et al., 2010), it is difficult to determine whether the detected concentrations where different and possibly a result of stress induction.

4.2 POPs in relation to season and status

The present study reported concentrations of 40 different POPs detected in polar bear plasma, and these concentrations were generally in the same range or lower than recently reported in polar bears from Svalbard and East Greenland sampled in February-April (Bytingsvik et al., 2012a, Bytingsvik et al., 2012b, Gabrielsen et al., 2015b).

PFASs was by far the most abundant POP group in the present study. The group of OH-PCBs constituted only one-fourth of the PFASs, followed by even lower abundances of PCBs, OCPs and PBDEs. PCB-153 was the most abundant PCB-congener followed by PCB-180 and -170. The same congeners were dominating in sub-adult polar bears (N=7) from East Greenland in 2011 (Gabrielsen et al., 2015b), but the mean concentration of PCB-153 was almost one-third higher than in the present study. Oxychlordane dominated the group of OCPs and PBDEs in the present study, and levels of the next-most abundant compounds, HCB and β-HCH, were much lower. Oxychlordane was also dominating among pesticides in the study by Gabrielsen et al. (2015b), although found in higher concentrations than in this study. The mean BDE-47 concentration from Gabrielsen et al. (2015b) was rather similar to what was detected in this study. PFOS was by far the most abundant single compound among all the POPs, and it was detected at more than three times the concentrations of PFHxS and PFNA. Bytingsvik et al. (2012b) also found PFOS and PFNA to be the dominant compounds in polar bear mothers (N=9) from Svalbard in 2008. With respect to the OH-PCBs, the most abundant compound was 4-OH-CB187, followed by 4-OH-CB146 and 4-OH-CB107. Also Bytingsvik

et al. (2012a) and (Gabrielsen et al., 2015b) found 4-OH-CB187 to be the most dominating OH-PCB.

There was a great variation in the POP concentrations, and there was no obvious seasonal pattern. The only significant seasonal difference was found in females with COY, showing a decrease in ΣPCB concentrations from spring to autumn. As the females with COY was the only group that had significantly higher BCI in autumn compared to spring (Bourgeon et al., unpublished), this explains why a seasonal pattern only was detected in this group. Knott et al. (2011) also found PCB concentrations in blood to be highest in polar bears with a poor condition. Although the two other groups seemed to be fasting in autumn, it was not reflected in their BCI and consequently not in their concentrations of POPs either. Plasma levels of organochlorines were generally not influenced by fasting in the study by Polischuk et al. (2002), except for chlordanes that were significantly higher in females with COY after the fast. However, seasonal variations in adipose tissue concentrations of POPs was detected in adult female polar bears (N=25) from East Greenland Dietz et al. (2004). They found that the concentrations of ΣPCB, ΣHCH, ΣHCL and Dieldrin were highest in spring and lowest in autumn/winter, ΣDDT was highest in late summer and ΣCBz showed little variation.

Only significant status differences for ΣPCB were detected in the present study, and concentrations were significantly higher in females with COY compared to solitary females sampled in spring. As mentioned, females with COY had significantly lower BCI compared to solitary females, especially in spring, which explains their higher concentrations of ΣPCB. POP concentrations were expected to be lower in females with offspring due to lactation being an important excretion route (Gabrielsen et al., 2011, Bytingsvik et al., 2012a, Bytingsvik et al., 2012b, Frouin et al., 2012). The results did not support this expectation, but most likely the effect of fasting masked the lactation effect. The findings by Polischuk et al. (1995) support this, as female polar bears with cubs had higher contaminant levels in adipose tissue than solitary females. They explained this by the fact that solitary females had 20- 50% more adipose tissue than females with cubs. Altogether, the results indicate that BCI is a better predictor of POP concentrations in plasma than fasting or reproductive status.

4.3 Effects of POPs on THs

In the following discussion, it was assumed that POP concentrations in blood reflect blubber concentrations (Bernhoft et al., 1997, Tartu et al., unpublished), and studies examining the relationship between circulating concentrations of THs and of POPs in both plasma and blubber were therefore included.

FT3 was the only single hormone that was significantly influenced by POPs, and it therefore appeared to be more sensitive than the other examined THs. This is supported by the results from Braathen et al. (2004) that also found FT3 to be the most affected hormone. Braathen et al. (2004) and Debier et al. (2005) both report that T4 was associated with contaminant exposure to a lesser degree than T3. As T3 is the active hormone and FT3 represents the biologically available fraction, this may be of concern for the polar bear health.

TH ratios were also included in the present study, as alternative indicators of thyroid disruption. Levels of the single hormones may vary substantially between individuals as a result of different plasma protein levels for instance (Zoeller et al., 2007). However, the ratios are more constant, which makes them more accurate indicators of the proportion of the circulating hormone available to target tissues (Skaare et al., 2001).

The results showed that FT3 was negatively associated to ΣPCB, ΣPESTBDE and ΣPFAS. Many studies on TH-POP relationships only include TT4 and TT3, and sometimes FT4, but seldom FT3. Nevertheless, the following three studies found FT3-POP relationships in arctic mammals that corresponded to the present results. Braathen et al. (2004) found a negative correlation between plasma concentrations of FT3 and ΣPCB_5 (PCB-99, -153, -156, -180 and -194) in female polar bears with COY (N=17). Negative correlations between FT3 and the blubber concentrations of three single PCBs (-118, -149 and -180) were also found in grey seal pups (*Halichoerus grypus*) (N=23) (Sørmo et al., 2005). Villanger et al. (2011b) found a negative correlation between FT3 and the concentration of PCB-105, PBDEs (PBDE-28, -47, -99, -100, and -154) and HCB in adipose tissue in beluga whales (N=12). On the other side, Routti et al. (2010) found positive relationships between FT3 and OH-PCBs in ringed seals (N=32). Only few studies have examined effects of PFASs on FT3. Bytingsvik (2012) did not find any significant relationships between FT3 (or TT3 and rT3) and any of the examined POPs, including PCBs and PFASs, in polar bear cubs (N=31). Similarly, Nøst et al. (2012) did not find any significant correlations between FT3 and PFASs in Black-legged kittiwake

(*Rissa tridactyla*) (N=15) and Northern fulmar chicks (*Fulmarus glacialis*) (N=15). Contrastingly, a study of pregnant women reported lower levels of FT3 and TT3 in women with higher blood concentrations of PFUnDA and PFDA, respectively, compared to women with lower blood concentrations of these compounds (Berg et al., 2015). In summary, the detection of negative relationships between FT3, ΣPCB, ΣPESTBDE and ΣPFAS was in accordance with most of the few studies that exist on FT3-POP relationships.

Concentrations of TT3, TT4 and FT4 were only significantly influenced by season and status. Contrastingly, other studies have found both positive and negative relationships between these three hormones and PCBs in wildlife (Brouwer et al., 1989, Braathen et al., 2004, Debier et al., 2005, Hall and Thomas, 2007, Villanger et al., 2011a, Villanger et al., 2011b, Bytingsvik, 2012, Gabrielsen et al., 2015b), OCPs (Verreault et al., 2004, Hall and Thomas, 2007, Villanger et al., 2011a, Villanger et al., 2011b), PBDEs (Hall and Thomas, 2007, Villanger et al., 2011a, Villanger et al., 2011b), PFASs (Braune et al., 2011, Bytingsvik, 2012, Nøst et al., 2012, Ask, 2015) and OH-PCBs (Bertinussen, 2009, Gabrielsen et al., 2015b).

TT3:FT3 was positively related to ΣPCB and ΣPFAS. For TT3:FT3 to increase either TT3 has to increase or FT3 has to decrease. As it was found that ΣPCB and ΣPFAS seemed to be related to declining levels of FT3 this may explain the corresponding increase in the ratio.

Independent of season, TT4:FT4 was positively related to ΣPFAS. However, the interaction term season:PFAS showed that this only occurred in autumn samples. ΣPFAS was the only POP group that had consistently higher mean concentrations in autumn compared to spring in all status groups, although the difference was not significant. On the other hand, the mean TT4:FT4 was lower in autumn compared to spring in all status groups, although only significant for the solitary females. This could indicate a dose-dependent response for the effects of ΣPFAS on TT4:FT4.

TT4:TT3 was positively related to ΣPESTBDE and ΣPFAS, when the relationships were examined independent of season. However, the interaction terms season:PESTBDE and season:PFAS showed that this only occurred in spring samples. ΣPESTBDE was negatively related to TT4:TT3 in autumn samples. TT4:TT3 was consistently higher in spring samples in all status groups, although not significant for females with YRL. ΣPESTBDE did not show a consistent pattern between seasons in any of the status groups, but ΣPFAS was slightly higher in autumn compared to spring samples as already mentioned. The reason why these

relationships differ between the two seasons is hard to determine from the present data, especially because of the missing seasonal pattern in the mean ΣPESTBDE concentrations. Perhaps this pattern would have been clearer if each compound had been analysed individually, since ΣPESTBDE consists of a wide variety of compounds.

Mostly negative relationships have been reported between TT3:FT3 (Gabrielsen et al., 2011, Villanger et al., 2013), TT4:FT4 (Skaare et al., 2001), TT4:TT3 (Verreault et al., 2007, Bertinussen, 2009, Gabrielsen et al., 2011, Bytingsvik, 2012), and POPs in arctic mammals. However, also some positive relationships were found between TT3:FT3 (Villanger et al., 2013) Routti2010b, TT4:FT4 (Skaare et al., 2001) and POPs. Positive relationships between TT3:FT3, TT4:FT4, ΣPCB and ΣPFAS, were found independent of season. However, both positive and negative relations were found between TT4:FT4, TT4:TT3, ΣPESTBDE and ΣPFAS, when the interaction with season was considered.

An explanation to why contrasting relationships are often detected could be that TH-POP relationships are dose-dependent. Langer et al. (2007) examined TH and PCB concentrations in human serum and found dose-dependent TH-PCB relationships. For instance, FT4 and TT3 were negatively related to PCBs at low doses (<530ng/g lipid) but positively related at high doses (530-2000 ng/g lipid). This could indicate that TH concentrations may have a hormetic (bell-shaped) response curve (Calabrese and Baldwin, 2003) to POP exposure, and thus cause contrasting results at low and high exposure levels. In relation to this, the HPT axis might be activated in response to subtle decreases in TH concentrations, and this activation could possibly compensate for the altered TH plasma concentrations (Boas et al., 2006, Knott et al., 2011, Bytingsvik, 2012). It has also been shown that TH effects of POP mixtures may depend on the relative concentration of each compound in the mixture (Langer et al., 2007, Villanger et al., 2011a). The polar bears in the present study have much lower POP concentrations than what is normally used in experimental exposure studies, and using these as explanations for TH-POP relationships in free-ranging polar bears may therefore be misleading. However, these theories do not explain why studies on polar bears with similar POP concentrations still find contrasting relationships.

The present study has shown that TH-POP relationships can be contrasting between season, which is an example of a confounding factor that only few of the studies have considered. In addition, the results from Braathen et al. (2004) and Villanger et al. (2011a) indicate that there might also be status differences in the TH-POP relationships. For instance, TT4:TT3 was

negatively related to ΣPCBs in females with offspring but not in solitary females (Braathen et al., 2004). Villanger et al. (2011a) found that *p,p'*-DDE was negatively correlated with TT3 in nursing females, but positively correlated with TT3 in solitary females. Thus, seasonal and status differences in thyroid sensitivity could therefore be part of the explanation of contradictory findings in TH-POP relationship studies. However, there is still a lot of uncertainty connected to these sensitivity differences, and they should therefore be examined more thoroughly in future studies.

4.3.1 Mechanisms behind thyroid toxicity

The mechanisms behind the thyroid disrupting properties of POPs have been investigated in both wildlife and laboratory animals, *in vivo* as well as *in vitro*. However, extrapolating results from laboratory animals to wildlife should be performed with caution. Wild animals are often chronically exposed to low doses of POP mixtures (Letcher et al., 2010), and the influence of confounding factors can be difficult to interpret. On the other hand, laboratory animals are often exposed to single compounds or perhaps simple mixtures, while kept in a controlled environment. Nevertheless, laboratory experiments offer the opportunity of testing causal relationships in opposition to relationships based only on correlations. Wade et al. (2002) demonstrated that an environmentally relevant mixture of organochlorines and heavy metals was thyroid disrupting, although the concentrations of the single compounds were below the lowest dose at which each substance has been shown to cause significant adverse effects *in vivo*. This is an example of how a mixture of TDCs may cause both dose-dependent additivity and synergism, which has also been suggested by Letcher et al. (2010) and Crofton et al. (2005). However, different modes of action by different POPs may also lead to opposite effects and in the end neutralize the overall disruption.

One of the well-documented modes of action by TDCs is competitive binding to the TTR. Due to their structural similarity to T4, several studies have shown that OH-PCBs have higher affinities to TTR than the natural ligand T4 (Lans et al., 1993, Lans et al., 1994, Cheek et al., 1999, Gutleb et al., 2010, Simon et al., 2011, Bytingsvik et al., 2013, Simon et al., 2013). Quite surprisingly, significant correlations between concentrations of THs and ΣOHPCBs were not detected in this study, despite the rather similar concentrations compared to previous studies. Other POPs like PCBs, OCPs, PBDEs, OH-PBDEs, and PFASs may also bind to TTR, although with a much lower affinity than T4 (Cheek et al., 1999, Weiss et al., 2009, Simon et al., 2011, Bytingsvik et al., 2013). Despite the low TTR-binding potential of some

POPs, they may still contribute when occurring in high enough concentrations. For instance, PFASs have a 12-50 times lower affinity for TTR than T4, but they are found in much higher concentrations than the other POPs (Weiss et al., 2009). The PFASs occurring in highest concentrations in the present study (PFOS and PFHxS) are also those with the highest binding affinity to TTR within this group of compounds (Weiss et al., 2009). Gutleb et al. (2010) found that TTR-binding sites in polar bear plasma from Svalbard in 1995-1996 were completely saturated by exogenous compounds, suggesting that competitive binding to TTR is an important mechanism for thyroid disruption. When T4 is displaced from TTR binding sites the excretion of FT4 is facilitated, which should decrease the proportion of circulating T4 relative to T3, thus leading to a decline in the TT4:TT3 ratio (Brouwer et al., 1998, Knott et al., 2011). Reduced T4 in the target tissues would also result in a decrease in the substrate for deiodinases and subsequently decrease the availability of T3 to TR (Ucán-Marín et al., 2009). In our study we found a positive relationship between TT4:TT3, ΣPESTBDE and ΣPFAS in spring samples, and negative relationships in autumn samples. The results from the spring samples do not support the theory of competitive binding to TTR as an important mechanism for thyroid disruption. Although the results from autumn samples could support the theory, the negative relationships were not very strong. If POPs bind to TTR, this may cause an additional effect, as it can facilitate their transport across the blood-brain and blood-placental barrier and lead to accumulation of POPs in the brain or foetus (Boas et al., 2012).

Another mechanism behind thyroid toxicity may be altered metabolism of the THs, by disrupting enzymes involved in deiodination, glucoronidation or sulfation. Deiodination enzymes (D1, D2, D3) are involved in both bioactivation and degradation of THs, depending on if they have outer-ring deiodinase activity, inner-ring deiodinase activity or both (Visser and Peeters, 2000). These enzymes are important for local modification of TH bioactivity independent of circulating TH levels. The expression of these enzymes is under direct control by THs, but several studies have found that also POPs may interfere with deiodinase levels or activities. The relationships between deiodinases and POPs have been shown to be both positive (Yu et al., 2009, Routti et al., 2010, Gabrielsen et al., 2015b) and negative (Wade et al., 2002, Kato et al., 2004, Yu et al., 2009, Gabrielsen et al., 2015b), depending on which tissues and POPs that where examined. Altered deiodinase activity should be possible to relate to TT4:TT3 ratios. If D1 activity is increased this would mean increased conversion of T4 to T3, resulting in lower T4 levels and higher T3 levels, and thus a decreasing TT4:TT3 ratio. Contrastingly, if D3 activity increased as a result of POPs exposure, resulting in

increased conversion of T3 into rT3, the TT4:TT3 ratio would increase. A negative correlation between TT4:TT3 and ΣPESTBDE in autumn samples and a positive correlation between TT4:TT3 and ΣPESTBDE and ΣPFAS in spring samples were found in this study. These results could indicate an induction of D1 activity by ΣPESTBDE in autumn and an induction of D3 activity by ΣPESTBDE and ΣPFAS in spring. However, this is a very simplified explanation of the mechanism, and altered deiodinase levels or activity may have different effects in different tissues and depending on which type of deiodinase is affected.

The second part of TH metabolism is mediated by sulfation and glucuronidation, which increase the water-solubility of the substrates, and thus facilitate their biliary or urinary excretion (Visser and Peeters, 2000). SULTs catalyse a transfer of a sulfate group from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to an acceptor group of the substrate (Negishi et al., 2001), whereas UDPGT catalyse the transfer of the glucoronic acid component of UDP-glucoronic acid to an acceptor group of the substrate. Iodothyronine glucuronides are found in much higher concentrations in plasma, bile and urine compared to iodothyronine sulfates (Visser et al., 1990). This is due to iodothyronine sulfates being rapidly degraded by D1, which means that sulfate conjugation is a primary step leading to the irreversible inactivation of THs (Visser, 1994). In this way sulfate conjugates represent a reservoir of inactive hormones, from which active T3 can be recovered readily (Visser, 1994, Wu et al., 2005). Several studies report induction of UDPGT activity or levels as a result of POP exposure (Beetstra et al., 1991, Van Raaij et al., 1993, Wade et al., 2002, Yu et al., 2009), and they generally found a corresponding decrease in T4 concentrations, but no effect on T3 concentrations. It has been suggested that induction of the UDPGT activity on T4, and the following increased clearance of circulating T4, is the most significant mechanism by which PCBs, dioxins, and other xenobiotics induce thyroid toxicity (Wade et al., 2002). Schuur et al. (1998a, 1998b, 1999) found that OH-PCBs were significant inhibitors of SULT activity towards THs *in vitro*, and that an important structural requirement is a hydroxyl group on the *para* or *meta* position. As no significant relationships were detected between TT4, FT4 and any of the POP groups, the results do not support the theory of induced conjugation enzyme activity by POPs. Negative relationships between FT3, ΣPCB, ΣPESTBDE and ΣPFAS were detected in the present study, in opposition to previous studies, which found that T3 concentrations were not affected by induced conjugation enzyme activity. However, these studies primarily looked at TT3 and rT3, not FT3. The only part of the results that supports

the metabolism-mechanism is the weak negative relationship between TT4:TT3 and ΣPESTBDE detected in autumn samples.

4.4 Implications of findings and future perspectives

The amount of variables available for this study was very high, and it was necessary to narrow the focus in order to remain within the scope of this project. Inevitably this lead to a simplification of the relationships in the data set, which makes it difficult to interpret the biological significance of the results. Although two important confounding factors were included, reproductive status and season, other possible confounding factors, such as age, sampling year and location, were not considered. This was done in spite of previous studies showing that for instance age may influence both TH and POP concentrations in polar bears (Bernhoft et al., 1997, Braathen et al., 2004, Knott et al., 2011, Villanger et al., 2011a). With respect to the reproductive status it would have been useful to have more information about the solitary females. They might have lost a cub recently or be pregnant, which could influence their TH concentrations (Berg et al., 2015). BM or BCI were not included as separate predictors, as these were nested in the variable season. However, as the study by Bourgeon et al. (unpublished) showed, the seasonal pattern in BM and BCI was not as clear as expected. Therefore, it could be argued that for instance BCI would have been a more appropriate predictor than season. Nevertheless, it was decided to use season instead of BCI based on the fasting indications that Torget (2015) found.

In contrast to many other studies, POP concentrations were examined on a molar basis instead of a weight basis. This decision was based on the theory that single molecules exert the toxic effect of POPs, and this effect is mostly independent of the weight of the molecules. It was also decided not to correct for lipid content in the plasma, although the concentration of the lipophilic POPs usually depends on this. Again, the argument was related to the mechanisms, which is most likely independent on plasma lipids. Also concentrations of POPs in plasma tend to be more stable than lipid concentrations (Polischuk et al., 2002). Some of these decisions were supported by Bytingsvik (2012), showing that plasma lipids and body mass were not important for explaining the relationship between concentrations of TT4, FT4, TT4:TT3 and POPs in polar bear cubs.

Another drawback of the present study is how the POPs were grouped. The ideal would have been to analyse each compound individually, but with 40 different compounds this was not

possible within the scope of this project. The groups that were used were not obvious in the RDA loading plot, which suggests that there could have been some contrasting effects. Especially PCB-105 and -118 differed from the rest of the PCBs, and it would have been reasonable to divide the PCB group further, in for instance *ortho*- and non-*ortho*-PCBs. PCB-105 and -118 have previously been demonstrated to be oppositely related to THs compared to other congeners (Braathen et al., 2004, Villanger et al., 2011a, Bytingsvik, 2012), further supporting an alternative grouping of especially the PCBs.

Plasma concentrations of POPs generally reflect adipose tissue concentrations, but they may differ from target tissues, such as the liver. In the end, the effects of TH disruption takes place in the target tissues, and it would therefore be more relevant to assess each tissue individually. Additionally, THs are regulated locally in response to tissue-specific needs, and this does not necessarily alter plasma concentrations (Gereben et al., 2008, Gabrielsen et al., 2015a). This suggests that plasma concentrations are not as good indicators of thyroid disruption as previously assumed (Knott et al., 2011). However, blood sampling is a non-destructive procedure and is therefore one of the few sampling procedures that are relevant when studying protected animals like the polar bears from Svalbard.

Although apparent changes in circulating hormone concentrations are not detected, this does not necessarily mean that, the hormonal homeostasis is unaffected (Boas et al., 2006). Consequently, effect assessments of TDCs should preferably be based on evaluations of a broader group of biomarkers in different tissues, not solely on circulating TH concentrations and correlative associations with sums of contaminant groups. Lastly, it should be emphasised that correlations between biomarker endpoints and tissue concentrations of POPs not necessarily reflect a causal relationship, and they should therefore be interpreted with caution.

5.0 CONCLUSION

Season and reproductive status were found to be important variables for predicting TH concentrations in free-ranging female polar bears from Svalbard. Out of all POP groups examined, ΣPCB was the only one significantly influenced by season, and status differences were only found in the spring samples. The results indicated that fasting was the underlying factor for explaining seasonal and status variations in TH concentrations, whereas POP concentrations were better explained by BCI variations.

FT3 was significantly related to ΣPCB, ΣPESTBDE and ΣPFAS, however, no significant relationships were found between TT3, TT4 or FT4 and any of the POPs. The ratio TT3:FT3 was positively related to ΣPCB and ΣPFAS, and TT4:FT4 was positively related to ΣPFAS. Seasonal differences in the TH-POP relationships were detected in the relationship between TT4:FT4 and ΣPFAS in addition to the relationships TT4:TT3-ΣPESTBDE and TT4:TT3-ΣPFAS. TT4:FT4-ΣPFAS and TT4:TT3-ΣPFAS showed that TH concentrations only were related to ΣPFAS concentrations in one of the seasons, either autumn or spring, respectively. On the other hand, TT4:TT3-ΣPESTBDE showed that TH concentrations were related to ΣPESTBDE in opposite directions, depending on season.

Although statistical correlations not necessarily reflect biological cause-effect relationships, the results of the present study indicated that POPs may interfere with TH concentrations in polar bears. Furthermore, they showed that there are seasonal variations in TH-POP relationships. However, there was no obvious season where polar bears seemed to be more sensitive to thyroid disruption, as this depended on which TH and POP group that were examined. Therefore, seasonal variations in TH-POP relationships appear to be complex, which support the need for further investigations of this in future studies. Altogether, the results emphasise the importance of considering confounding factors, such as season and reproductive status, when examining variations of TH and POP concentrations and their relationships in arctic mammals.

6.0 REFERENCES

AMAP 2012. Arctic Climate Issues 2011: Changes in Arctic Snow, Water, Ice and Permafrost. SWIPA 2011 Overview Report. *Arctic Monitoring and Assessment Programme (AMAP).*

AMSTRUP, SC 2003. Polar Bear, *Ursus maritimus*. Chapter 27 in Wild Mammals of North America: Biology, Management, and Conservation. *John Hopkins University Press, Baltimore*, 587.

AMSTRUP, SC, MARCOT, BG & DOUGLAS, DC 2008. A Bayesian Network Modeling Approach to Forecasting the 21st Century Worldwide Status of Polar Bears. *Arctic Sea Ice Decline: Observations, Projections, Mechanisms, and Implications*, 213.

ANDERSON, GM, CONNORS, JM, HARDY, SL, VALENT, M & GOODMAN, RL 2002. Thyroid Hormones Mediate Steroid-Independent Seasonal Changes in Luteinizing Hormone Pulsatility in the Ewe. *Biology of Reproduction,* 66, 701.

ASK, A 2015. Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) Affect the Thyroid Hormone System, Body Condition, and Body Mass in Two Arctic Seabird Species. *NTNU. Master thesis.*

ATKINSON, SN & RAMSAY, MA 1995. The Effects of Prolonged Fasting of the Body Composition and Reproductive Success of Female Polar Bears (*Ursus maritimus*). *Functional Ecology,* 9, 559.

ATKINSON, SN, NELSON, RA & RAMSAY, MA 1996. Changes in the Body Composition of Fasting Polar Bears (*Ursus maritimus*): The Effect of Relative Fatness on Protein Conservation. *Physiological Zoology*, 304.

AZIZI, F, MANNIX, J, HOWARD, D & NELSON, R 1979. Effect of Winter Sleep on Pituitary-Thyroid Axis in American Black Bear. *American Journal of Physiology - Gastrointestinal and Liver Physiology,* 237, 227.

BARRIE, LA, GREGOR, D, HARGRAVE, B, LAKE, R, MUIR, D, SHEARER, R, TRACEY, B & BIDLEMAN, T 1992. Arctic Contaminants: Sources, Occurrence and Pathways. *Science of the Total Environment,* 122, 1.

BEETSTRA, J, VAN ENGELEN, J, KARELS, P, VAN DER HOEK, H, JONG, M, DOCTER, R, KRENNING, E, HENNEMANN, G, BROUWER, A & VISSER, T 1991. Thyroxine and 3,3′,5-Triiodothyronine Are Glucuronidated in Rat Liver by Different Uridine Diphosphate-Glucuronyltransferases. *Endocrinology,* 128, 741.

BERG, V, NØST, TH, HANSEN, S, ELVERLAND, A, VEYHE, A-S, JORDE, R, ODLAND, JØ & SANDANGER, TM 2015. Assessing the Relationship between Perfluoroalkyl Substances, Thyroid Hormones and Binding Proteins in Pregnant Women; a Longitudinal Mixed Effects Approach. *Environment International,* 77, 63.

BERNHOFT, A, WIIG, Ø & UTNE SKAARE, J 1997. Organochlorines in Polar Bears (*Ursus maritimus*) at Svalbard. *Environmental Pollution,* 95, 159.

BERTINUSSEN, HO 2009. Effects of Hydroxylated PCBs on Thyroid Hormone Levels in Mother-Cub Pairs of Polar Bears (*Ursus maritimus*) from Svalbard. *NTNU. Master thesis.*

BIANCO, AC, NUNES, MT, HELL, NS & MACIEL, RM 1987. The Role of Glucocorticoids in the Stress-Induced Reduction of Extrathyroidal 3,5,3′-Triiodothyronine Generation in Rats. *Endocrinology,* 120, 1033.

BOAS, M, FELDT-RASMUSSEN, U, SKAKKEBÆK, NE & MAIN, KM 2006. Environmental Chemicals and Thyroid Function. *European Journal of Endocrinology,* 154, 599.

BOAS, M, FELDT-RASMUSSEN, U & MAIN, KM 2012. Thyroid Effects of Endocrine Disrupting Chemicals. *Molecular and Cellular Endocrinology,* 355, 240.
BRAUNE, BM, OUTRIDGE, PM, FISK, AT, MUIR, DCG, HELM, PA, HOBBS, K, HOEKSTRA, PF, KUZYK, ZA, KWAN, M, LETCHER, RJ, LOCKHART, WL, NORSTROM, RJ, STERN, GA & STIRLING, I 2005. Persistent Organic Pollutants and Mercury in Marine Biota of the Canadian Arctic: An Overview of Spatial and Temporal Trends. *Science of the Total Environment,* 351–352, 4.
BRAUNE, BM, TRUDEAU, S, JEFFREY, DA & MALLORY, ML 2011. Biomarker Responses Associated with Halogenated Organic Contaminants in Northern Fulmars (*Fulmarus glacialis*) Breeding in the Canadian Arctic. *Environmental Pollution,* 159, 2891.
BREVIK, E 1978. Gas Chromatographic Method for the Determination of Organochlorine Pesticides in Human Milk. *Bulletin of Environmental Contamination and Toxicology,* 19, 281.
BROUWER, A, REIJNDERS, PJH & KOEMAN, JH 1989. Polychlorinated Biphenyl (PCB)-Contaminated Fish Induces Vitamin a and Thyroid Hormone Deficiency in the Common Seal (*Phoca vitulina*). *Aquatic Toxicology,* 15, 99.
BROUWER, A, MORSE, DC, LANS, MC, GERLIENKE SCHUUR, A, MURK, AJ, KLASSON-WEHLER, E, BERGMAN, Å & VISSER, TJ 1998. Interactions of Persistent Environmental Organohalogens with the Thyroid Hormone System: Mechanisms and Possible Consequences for Animal and Human Health. *Toxicology and Industrial Health,* 14, 59.
BRAATHEN, M, DEROCHER, AE, WIIG, Ø, SØRMO, EG, LIE, E, SKAARE, JU & JENSSEN, BM 2004. Relationships between PCBs and Thyroid Hormones and Retinol in Female and Male Polar Bears. *Environmental Health Perspectives,* 112, 826.
BUTT, CM, BERGER, U, BOSSI, R & TOMY, GT 2010. Levels and Trends of Poly-and Perfluorinated Compounds in the Arctic Environment. *Science of the Total Environment,* 408, 2936.
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*.
BYTINGSVIK, J, LIE, E, AARS, J, DEROCHER, AE, WIIG, Ø & JENSSEN, BM 2012a. PCBs and OH-PCBs in Polar Bear Mother–Cub Pairs: A Comparative Study Based on Plasma Levels in 1998 and 2008. *Science of the Total Environment,* 417–418, 117.
BYTINGSVIK, J, VAN LEEUWEN, SPJ, HAMERS, T, SWART, K, AARS, J, LIE, E, NILSEN, EME, WIIG, Ø, DEROCHER, AE & JENSSEN, BM 2012b. Perfluoroalkyl Substances in Polar Bear Mother–Cub Pairs: A Comparative Study Based on Plasma Levels from 1998 and 2008. *Environment International,* 49, 92.
BYTINGSVIK, J, SIMON, E, LEONARDS, PEG, LAMOREE, M, LIE, E, AARS, J, DEROCHER, AE, WIIG, Ø, JENSSEN, BM & HAMERS, T 2013. Transthyretin-Binding Activity of Contaminants in Blood from Polar Bear (*Ursus maritimus*) Cubs. *Environmental Science & Technology,* 47, 4778.
CALABRESE, EJ & BALDWIN, LA 2003. Toxicology Rethinks Its Central Belief. *Nature,* 421, 691.
CALVERT, W & RAMSAY, MA 1998. Evaluation of Age Determination of Polar Bears by Counts of Cementum Growth Layer Groups. *Ursus*, 449.
CATTET, M 2000. Biochemical and Physiological Aspects of Obesity, High Fat Diet, and Prolonged Fasting in Free-Ranging Polar Bears. *University of Saskatchewan. Doctoral thesis*.

CHEEK, AO, KOW, K, CHEN, J & MCLACHLAN, JA 1999. Potential Mechanisms of Thyroid Disruption in Humans: Interaction of Organochlorine Compounds with Thyroid Receptor, Transthyretin, and Thyroid-Binding Globulin. *Environmental Health Perspectives,* 107, 273.
CHRISTENSEN-DALSGAARD, SN, AARS, J, ANDERSEN, M, LOCKYER, C & YOCCOZ, NG 2010. Accuracy and Precision in Estimation of Age of Norwegian Arctic Polar Bears (*Ursus maritimus*) Using Dental Cementum Layers from Known-Age Individuals. *Polar Biology,* 33, 589.
COLBORN, T, VOM SAAL, FS & SOTO, AM 1993. Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans. *Environmental Health Perspectives,* 101, 378.
CRISP, TM, CLEGG, ED, COOPER, RL, WOOD, WP, ANDERSON, DG, BAETCKE, KP, HOFFMANN, JL, MORROW, MS, RODIER, DJ & SCHAEFFER, JE 1998. Environmental Endocrine Disruption: An Effects Assessment and Analysis. *Environmental Health Perspectives,* 106, 11.
CROFTON, KM, CRAFT, ES, HEDGE, JM, GENNINGS, C, SIMMONS, JE, CARCHMAN, RA, CARTER, WH & DEVITO, MJ 2005. Thyroid-Hormone-Disrupting Chemicals: Evidence for Dose-Dependent Additivity or Synergism. *Environmental Health Perspectives,* 113, 1549.
DE WIT, CA, ALAEE, M & MUIR, DCG 2006. Levels and Trends of Brominated Flame Retardants in the Arctic. *Chemosphere,* 64, 209.
DE WIT, CA, HERZKE, D & VORKAMP, K 2010. Brominated Flame Retardants in the Arctic Environment — Trends and New Candidates. *Science of the Total Environment,* 408, 2885.
DEBIER, C, YLITALO, GM, WEISE, M, GULLAND, F, COSTA, DP, LE BOEUF, BJ, 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. *Environmental Pollution,* 134, 323.
DEBIER, C, CHALON, C, LE BŒUF, BJ, DE TILLESSE, T, LARONDELLE, Y & THOMÉ, J-P 2006. Mobilization of Pcbs from Blubber to Blood in Northern Elephant Seals (*Mirounga angustirostris*) During the Post-Weaning Fast. *Aquatic Toxicology,* 80, 149.
DEROCHER, AE & WIIG, Ø 2002. Postnatal Growth in Body Length and Mass of Polar Bears (*Ursus maritimus*) at Svalbard. *Journal of Zoology,* 256, 343.
DEROCHER, AE, WIIG, Ø & ANDERSEN, M 2002. Diet Composition of Polar Bears in Svalbard and the Western Barents Sea. *Polar Biology,* 25, 448.
DEROCHER, AE, LUNN, NJ & STIRLING, I 2004. Polar Bears in a Warming Climate. *Integrative and Comparative Biology,* 44, 163.
DIAMANTI-KANDARAKIS, E, BOURGUIGNON, J-P, GIUDICE, LC, HAUSER, R, PRINS, GS, SOTO, AM, ZOELLER, RT & GORE, AC 2009. Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. *Endocrine Reviews,* 30, 293.
DIETZ, R, RIGET, F, SONNE, C, LETCHER, R, BORN, E & MUIR, D 2004. Seasonal and Temporal Trends in Polychlorinated Biphenyls and Organochlorine Pesticides in East Greenland Polar Bears (*Ursus maritimus*), 1990–2001. *Science of the Total Environment,* 331, 107.
DIETZ, R, BOSSI, R, RIGET, FF, SONNE, C & BORN, E 2008. Increasing Perfluoroalkyl Contaminants in East Greenland Polar Bears (*Ursus maritimus*): A New Toxic Threat to the Arctic Bears. *Environmental Science & Technology,* 42, 2701.
DIETZ, R, RIGÉT, FF, SONNE, C, BORN, EW, BECHSHØFT, T, MCKINNEY, MA, DRIMMIE, RJ, MUIR, DCG & LETCHER, RJ 2013a. Three Decades (1983–2010) of

Contaminant Trends in East Greenland Polar Bears (*Ursus maritimus*). Part 2: Brominated Flame Retardants. *Environment International,* 59, 494.
DIETZ, R, RIGÉT, FF, SONNE, C, BORN, EW, BECHSHØFT, T, MCKINNEY, MA & LETCHER, RJ 2013b. Three Decades (1983–2010) of Contaminant Trends in East Greenland Polar Bears (*Ursus maritimus*). Part 1: Legacy Organochlorine Contaminants. *Environment International,* 59, 485.
DRAY, S, CHESSEL, D & THIOULOUSE, J 2003. Co-Inertia Analysis and the Linking of Ecological Data Tables. *Ecology,* 84, 3078.
DURNER, GM, DOUGLAS, DC, NIELSON, RM, AMSTRUP, SC, MCDONALD, TL, STIRLING, I, MAURITZEN, M, BORN, EW, WIIG, Ø & DEWEAVER, E 2009. Predicting 21st-Century Polar Bear Habitat Distribution from Global Climate Models. *Ecological Monographs,* 79, 25.
FISK, AT, HOBSON, KA & NORSTROM, RJ 2001. Influence of Chemical and Biological Factors on Trophic Transfer of Persistent Organic Pollutants in the Northwater Polynya Marine Food Web. *Environmental Science & Technology,* 35, 732.
FROUIN, H, LEBEUF, M, HAMMILL, M & FOURNIER, M 2012. Transfer of PBDEs and Chlorinated POPs from Mother to Pup During Lactation in Harp Seals (*Phoca groenlandica*). *Science of the Total Environment,* 417, 98.
FUGLEI, E, BUSTNES, JO, HOP, H, MØRK, T, BJÖRNFOTH, H & VAN BAVEL, B 2007. Environmental Contaminants in Arctic Foxes (*Alopex lagopus*) in Svalbard: Relationships with Feeding Ecology and Body Condition. *Environmental Pollution,* 146, 128.
GABRIELSEN, KM, VILLANGER, GD, LIE, E, KARIMI, M, LYDERSEN, C, KOVACS, KM & JENSSEN, BM 2011. Levels and Patterns of Hydroxylated Polychlorinated Biphenyls (OH-PCBs) and Their Associations with Thyroid Hormones in Hooded Seal (*Cystophora cristata*) Mother–Pup Pairs. *Aquatic Toxicology,* 105, 482.
GABRIELSEN, KM, KROKSTAD, JS, OBREGON, M-J, VILLANGER, GD, SONNE, C, DIETZ, R & JENSSEN, BM 2015a. Thyroid Hormones and Deiodinase Activities in Plasma and Tissues from East Greenland Polar Bears (*Ursus maritimus*) During Winter Season. *Polar Biology,* 38, 1285.
GABRIELSEN, KM, KROKSTAD, JS, VILLANGER, GD, BLAIR, DA, OBREGON, M-J, SONNE, C, DIETZ, R, LETCHER, RJ & JENSSEN, BM 2015b. Thyroid Hormones and Deiodinase Activity in Plasma and Tissues in Relation to High Levels of Organohalogen Contaminants in East Greenland Polar Bears (*Ursus maritimus*). *Environmental Research,* 136, 413.
GADEN, A, FERGUSON, SH, HARWOOD, L, MELLING, H, ALIKAMIK, J & STERN, G 2012. Western Canadian Arctic Ringed Seal Organic Contaminant Trends in Relation to Sea Ice Break-Up. *Environmental Science & Technology,* 46, 4427.
GEREBEN, B, ZAVACKI, AM, RIBICH, S, KIM, BW, HUANG, SA, SIMONIDES, WS, ZEOLD, A & BIANCO, AC 2008. Cellular and Molecular Basis of Deiodinase-Regulated Thyroid Hormone Signaling. *Endocrine Reviews,* 29, 898.
GUTLEB, AC, CENIJN, P, VELZEN, MV, LIE, E, ROPSTAD, E, SKAARE, JU, MALMBERG, T, BERGMAN, Å, GABRIELSEN, GW & LEGLER, J 2010. In Vitro Assay Shows That PCB Metabolites Completely Saturate Thyroid Hormone Transport Capacity in Blood of Wild Polar Bears (*Ursus maritimus*). *Environmental Science & Technology,* 44, 3149.
HALL, AJ & THOMAS, GO 2007. Polychlorinated Biphenyls, DDT, Polybrominated Diphenyl Ethers, and Organic Pesticides in United Kingdom Harbor Seals (*Phoca vitulina*) – Mixed Exposures and Thyroid Homeostasis. *Environmental Toxicology and Chemistry,* 26, 851.

HALSALL, CJ 2004. Investigating the Occurrence of Persistent Organic Pollutants (POPs) in the Arctic: Their Atmospheric Behaviour and Interaction with the Seasonal Snow Pack. *Environmental Pollution,* 128, 163.
HELGASON, LB, WOLKERS, H, FUGLEI, E, AHLSTRØM, Ø, MUIR, D & JØRGENSEN, EH 2013. Seasonal Emaciation Causes Tissue Redistribution and an Increased Potential for Toxicity of Lipophilic Pollutants in Farmed Arctic Fox (*Vulpes lagopus*). *Environmental Toxicology and Chemistry*, 1784.
HILLER-STURMHÖFEL, S & BARTKE, A 1997. The Endocrine System: An Overview. *Alcohol Health and Research World,* 22, 153.
HUNTER, CM, CASWELL, H, RUNGE, MC, REGEHR, EV, AMSTRUP, SC & STIRLING, I 2010. Climate Change Threatens Polar Bear Populations: A Stochastic Demographic Analysis. *Ecology,* 91, 2883.
JENSSEN, BM 2006. Endocrine-Disrupting Chemicals and Climate Change: A Worst-Case Combination for Arctic Marine Mammals and Seabirds? *Environmental Health Perspectives,* 114, 76.
JENSSEN, BM, VILLANGER, GD, GABRIELSEN, KM, BYTINGSVIK, J, BECHSHØFT, TØ, CIESIELSKI, TM, SONNE, C & DIETZ, R 2015. Anthropogenic Flank Attack on Polar Bears: Interacting Consequences of Climate Warming and Pollutant Exposure. *Frontiers in Ecology and Evolution,* 3, 1.
JOHNSON, JB & OMLAND, KS 2004. Model Selection in Ecology and Evolution. *Trends in Ecology & Evolution,* 19, 101.
JOSSE, J, PAGÈS, J & HUSSON, F 2008. Testing the Significance of the RV Coefficient. *Computational Statistics & Data Analysis,* 53, 82.
KAHL, S, CAPUCO, A & BITMAN, J 1987. Serum Concentrations of Thyroid Hormones and Extrathyroidal Thyroxine-5′-Monodeiodinase Activity During Lactation in the Rat. *Experimental Biology and Medicine,* 184, 144.
KATO, Y, IKUSHIRO, S, HARAGUCHI, K, YAMAZAKI, T, ITO, Y, SUZUKI, H, KIMURA, R, YAMADA, S, INOUE, T & DEGAWA, M 2004. A Possible Mechanism for Decrease in Serum Thyroxine Level by Polychlorinated Biphenyls in Wistar and Gunn Rats. *Toxicological Sciences,* 81, 309.
KITAMURA, S, JINNO, N, SUZUKI, T, SUGIHARA, K, OHTA, S, KUROKI, H & FUJIMOTO, N 2005. Thyroid Hormone-Like and Estrogenic Activity of Hydroxylated PCBs in Cell Culture. *Toxicology,* 208, 377.
KLEIVANE, L, SEVERINSEN, T & SKAARE, JU 2000. Biological Transport and Mammal to Mammal Transfer of Organochlorines in Arctic Fauna. *Marine Environmental Research,* 49, 343.
KNOTT, KK, SCHENK, P, BEYERLEIN, S, BOYD, D, YLITALO, GM & O'HARA, TM 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. *Environmental Research,* 111, 1124.
KRASSAS, G, POPPE, K & GLINOER, D 2010. Thyroid Function and Human Reproductive Health. *Endocrine Reviews,* 31, 702.
LAIDRE, KL, STIRLING, I, LOWRY, LF, WIIG, Ø, HEIDE-JØRGENSEN, MP & FERGUSON, SH 2008. Quantifying the Sensitivity of Arctic Marine Mammals to Climate-Induced Habitat Change. *Ecological Applications,* 18, 97.
LANGER, P, KOČAN, A, TAJTÁKOVÁ, M, RÁDIKOVÁ, Ž, PETRÍK, J, KOŠKA, J, KŠINANTOVÁ, L, IMRICH, R, HUČKOVÁ, M, CHOVANCOVÁ, J, DROBNÁ, B, JURSA, S, BERGMAN, A, ATHANASIADOU, M, HOVANDER, L, GAŠPERÍKOVÁ, D, TRNOVEC, T, ŠEBÖKOVÁ, E & KLIMEŠ, I 2007. Possible

Effects of Persistent Organochlorinated Pollutants Cocktail on Thyroid Hormone Levels and Pituitary–Thyroid Interrelations. *Chemosphere,* 70, 110.
LANS, MC, KLASSON-WEHLER, E, WILLEMSEN, M, MEUSSEN, E, SAFE, S & BROUWER, A 1993. Structure-Dependent, Competitive Interaction of Hydroxy-Polychlorobiphenyls, -Dibenzo-p-Dioxins and -Dibenzofurans with Human Transthyretin. *Chemico-Biological Interactions,* 88, 7.
LANS, MC, SPIERTZ, C, BROUWER, A & KOEMAN, JH 1994. Different Competition of Thyroxine Binding to Transthyretin and Thyroxine-Binding Globulin by Hydroxy-PCBs, PCDDs and PCDFs. *European Journal of Pharmacology: Environmental Toxicology and Pharmacology,* 270, 129.
LEATHERLAND, JF & RONALD, K 1981. Plasma Concentrations of Thyroid Hormones in a Captive and Feral Polar Bear (*Ursus maritimus*). *Comparative Biochemistry and Physiology Part A: Physiology,* 70, 575.
LENTH, R 2015. Least-Square Means. R Package. *The R Development Core Team*.
LETCHER, RJ, BUSTNES, JO, DIETZ, R, JENSSEN, BM, JØRGENSEN, EH, SONNE, C, VERREAULT, J, VIJAYAN, MM & GABRIELSEN, GW 2010. Exposure and Effects Assessment of Persistent Organohalogen Contaminants in Arctic Wildlife and Fish. *Science of the Total Environment,* 408, 2995.
LYDERSEN, C, WOLKERS, H, SEVERINSEN, T, KLEIVANE, L, NORDØY, ES & SKAARE, JU 2002. Blood Is a Poor Substrate for Monitoring Pollution Burdens in Phocid Seals. *Science of the Total Environment,* 292, 193.
LYDERSEN, C 2014. Status and Biology of Ringed Seals (*Phoca hispida*) in Svalbard. *NAMMCO Scientific Publications,* 1, 46.
MA, J, HUNG, H, TIAN, C & KALLENBORN, R 2011. Revolatilization of Persistent Organic Pollutants in the Arctic Induced by Climate Change. *Nature Climate Change,* 1, 255.
MACDONALD, RW, HARNER, T, PYFE, J, LOENG, H & WEINGARTNER, T 2002. AMAP Assessment 2002: The Influence of Global Change on Contaminant Pathways to, within, and from the Artic. *Arctic Monitoring and Assessment Programme (AMAP)*.
MACDONALD, RW, MACKAY, D, LI, YF & HICKIE, B 2003. How Will Global Climate Change Affect Risks from Long-Range Transport of Persistent Organic Pollutants? *Human and Ecological Risk Assessment: An International Journal,* 9, 643.
MCKINNEY, MA, PEACOCK, E & LETCHER, RJ 2009. Sea Ice-Associated Diet Change Increases the Levels of Chlorinated and Brominated Contaminants in Polar Bears. *Environmental Science & Technology,* 43, 4334.
MCKINNEY, MA, LETCHER, RJ, AARS, J, BORN, EW, BRANIGAN, M, DIETZ, R, EVANS, TJ, GABRIELSEN, GW, PEACOCK, E & SONNE, C 2011. Flame Retardants and Legacy Contaminants in Polar Bears from Alaska, Canada, East Greenland and Svalbard, 2005–2008. *Environment International,* 37, 365.
MCKINNEY, MA, IVERSON, SJ, FISK, AT, SONNE, C, RIGÉT, FF, LETCHER, RJ, ARTS, MT, BORN, EW, ROSING-ASVID, A & DIETZ, R 2013. Global Change Effects on the Long-Term Feeding Ecology and Contaminant Exposures of East Greenland Polar Bears. *Global Change Biology,* 19, 2360.
MCKINNEY, MA, PEDRO, S, DIETZ, R, SONNE, C, FISK, AT, ROY, D, JENSSEN, BM & LETCHER, RJ 2016. A Review of Ecological Impacts of Global Climate Change on Persistent Organic Pollutant and Mercury Pathways and Exposures in Arctic Marine Ecosystems. *Current Zoology*.
MCNABB, FA 1992. *Thyroid Hormones*, Prentice Hall.

MCNABB, FMA 1995. Thyroid Hormones, Their Activation, Degradation and Effects on Metabolism. *The Journal of Nutrition,* 125, 1773.
MIYAZAKI, W, IWASAKI, T, TAKESHITA, A, TOHYAMA, C & KOIBUCHI, N 2008. Identification of the Functional Domain of Thyroid Hormone Receptor Responsible for Polychlorinated Biphenyl–Mediated Suppression of Its Action in Vitro. *Environmental Health Perspectives,* 116, 1231.
MOENTER, SM, WOODFILL, CJ & KARSCH, FJ 1991. Role of the Thyroid Gland in Seasonal Reproduction: Thyroidectomy Blocks Seasonal Suppression of Reproductive Neuroendocrine Activity in Ewes. *Endocrinology,* 128, 1337.
MORITZ, RE, BITZ, CM & STEIG, EJ 2002. Dynamics of Recent Climate Change in the Arctic. *Science,* 297, 1497.
NEGISHI, M, PEDERSEN, LG, PETROTCHENKO, E, SHEVTSOV, S, GOROKHOV, A, KAKUTA, Y & PEDERSEN, LC 2001. Structure and Function of Sulfotransferases. *Archives of Biochemistry and Biophysics,* 390, 149.
NORSTROM, R, BELIKOV, S, BORN, E, GARNER, G, MALONE, B, OLPINSKI, S, RAMSAY, M, SCHLIEBE, S, STIRLING, I & STISHOV, M 1998. Chlorinated Hydrocarbon Contaminants in Polar Bears from Eastern Russia, North America, Greenland, and Svalbard: Biomonitoring of Arctic Pollution. *Archives of Environmental Contamination and Toxicology,* 35, 354.
NOYES, PD, MCELWEE, MK, MILLER, HD, CLARK, BW, VAN TIEM, LA, WALCOTT, KC, ERWIN, KN & LEVIN, ED 2009. The Toxicology of Climate Change: Environmental Contaminants in a Warming World. *Environment International,* 35, 971.
NØST, TH, HELGASON, LB, HARJU, M, HEIMSTAD, ES, GABRIELSEN, GW & JENSSEN, BM 2012. Halogenated Organic Contaminants and Their Correlations with Circulating Thyroid Hormones in Developing Arctic Seabirds. *Science of the Total Environment,* 414, 248.
OKI, C & ATKINSON, S 2004. Diurnal Patterns of Cortisol and Thyroid Hormones in the Harbor Seal (*Phoca vitulina*) During Summer and Winter Seasons. *General and Comparative Endocrinology,* 136, 289.
ORTIZ, RM, HOUSER, DS, WADE, CE & ORTIZ, CL 2003. Hormonal Changes Associated with the Transition between Nursing and Natural Fasting in Northern Elephant Seals (*Mirounga angustirostris*). *General and Comparative Endocrinology,* 130, 78.
POLDER, A, GABRIELSEN, G, ODLAND, JØ, SAVINOVA, T, TKACHEV, A, LØKEN, K & SKAARE, J 2008. Spatial and Temporal Changes of Chlorinated Pesticides, PCBs, Dioxins (PCDDs/PCDFs) and Brominated Flame Retardants in Human Breast Milk from Northern Russia. *Science of the Total Environment,* 391, 41.
POLISCHUK, S, LETCHER, R, NORSTROM, R & RAMSAY, M 1995. Preliminary Results of Fasting on the Kinetics of Organochlorines in Polar Bears (*Ursus maritimus*). *Science of the Total Environment,* 160, 465.
POLISCHUK, S, NORSTROM, R & RAMSAY, M 2002. Body Burdens and Tissue Concentrations of Organochlorines in Polar Bears (*Ursus maritimus*) Vary During Seasonal Fasts. *Environmental Pollution,* 118, 29.
PROP, J, AARS, J, BÅRDSEN, B-J, HANSSEN, SA, BECH, C, BOURGEON, S, DE FOUW, J, GABRIELSEN, GW, LANG, J & NOREEN, E 2015. Climate Change and the Increasing Impact of Polar Bears on Bird Populations. *Frontiers in Ecology and Evolution,* 3, 33.
REGEHR, EV, LUNN, NJ, AMSTRUP, SC & STIRLING, IAN 2007. Effects of Earlier Sea Ice Breakup on Survival and Population Size of Polar Bears in Western Hudson Bay. *Journal of Wildlife Management,* 71, 2673.

RIGÉT, F, BIGNERT, A, BRAUNE, B, STOW, J & WILSON, S 2010. Temporal Trends of Legacy POPs in Arctic Biota, an Update. *Science of the Total Environment,* 408, 2874.
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.
ROBBINS, CT, LOPEZ-ALFARO, C, RODE, KD, TØIEN, Ø & NELSON, OL 2012. Hibernation and Seasonal Fasting in Bears: The Energetic Costs and Consequences for Polar Bears. *Journal of Mammalogy,* 93, 1493.
ROUTTI, H, JENSSEN, BM, LYDERSEN, C, BÄCKMAN, C, ARUKWE, A, NYMAN, M, KOVACS, KM & GABRIELSEN, GW 2010. Hormone, Vitamin and Contaminant Status During the Moulting/Fasting Period in Ringed Seals (*Pusa hispida*) from Svalbard. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 155, 70.
SCHUSSLER, GC & ORLANDO, J 1978. Fasting Decreases Triiodothyronine Receptor Capacity. *Science,* 199, 686.
SCHUUR, A, BERGMAN, Å, BROUWER, A & VISSER, T 1999. Effects of Pentachlorophenol and Hydroxylated Polychlorinated Biphenyls on Thyroid Hormone Conjugation in a Rat and a Human Hepatoma Cell Line. *Toxicology in Vitro,* 13, 417.
SCHUUR, AG, BROUWER, A, BERGMAN, Å, COUGHTRIE, MW & VISSER, TJ 1998a. Inhibition of Thyroid Hormone Sulfation by Hydroxylated Metabolites of Polychlorinated Biphenyls. *Chemico-Biological Interactions,* 109, 293.
SCHUUR, AG, LEGGER, FF, VAN MEETEREN, ME, MOONEN, MJ, VAN LEEUWEN-BOL, I, BERGMAN, Å, VISSER, TJ & BROUWER, A 1998b. In Vitro Inhibition of Thyroid Hormone Sulfation by Hydroxylated Metabolites of Halogenated Aromatic Hydrocarbons. *Chemical Research in Toxicology,* 11, 1075.
SIMON, E, BYTINGSVIK, J, JONKER, W, LEONARDS, PEG, DE BOER, J, JENSSEN, BM, LIE, E, AARS, J, HAMERS, T & LAMOREE, MH 2011. Blood Plasma Sample Preparation Method for the Assessment of Thyroid Hormone-Disrupting Potency in Effect-Directed Analysis. *Environmental Science & Technology,* 45, 7936.
SIMON, E, VAN VELZEN, M, BRANDSMA, SH, LIE, E, LØKEN, K, DE BOER, J, BYTINGSVIK, J, JENSSEN, BM, AARS, J, HAMERS, T & LAMOREE, MH 2013. Effect-Directed Analysis to Explore the Polar Bear Exposome: Identification of Thyroid Hormone Disrupting Compounds in Plasma. *Environmental Science & Technology,* 47, 8902.
SKAARE, JU, BERNHOFT, A, WIIG, Ø, NORUM, KR, HAUG, E, EIDE, DM & DEROCHER, AE 2001. Relationships between Plasma Levels of Organochlorines, Retinol and Thyroid Hormones from Polar Bears (*Ursus maritimus*) at Svalbard. *Journal of Toxicology and Environmental Health Part A,* 62, 227.
ST. AUBIN, D & GERACI, J 1988. Capture and Handling Stress Suppresses Circulating Levels of Thyroxine (T4) and Triiodothyronine (T3) in Beluga Whales (*Delphinapterus leucas*). *Physiological Zoology*, 170.
STIRLING, I, SPENCER, C & ANDRIASHEK, D 1989. Immobilization of Polar Bears (*Ursus maritimus*) with Telazol® in the Canadian Arctic. *Journal of Wildlife Diseases,* 25, 159.
STIRLING, I & DEROCHER, AE 2012. Effects of Climate Warming on Polar Bears: A Review of the Evidence. *Global Change Biology,* 18, 2694.
STRBAK, V, MACHO, L, UHERCIK, D & KLIMENT, V 1978. The Effect of Lactation on Thyroid Activity of Women. *Endokrinologie,* 72, 183.
SØRMO, EG, JUSSI, I, JUSSI, M, BRAATHEN, M, SKAARE, JU & JENSSEN, BM 2005. Thyroid Hormone Status in Gray Seal (*Halichoerus grypus*) Pups from the Baltic Sea

and the Atlantic Ocean in Relation to Organochlorine Pollutants. *Environmental Toxicology and Chemistry,* 24, 610.
TOMASI, TE, HELLGREN, EC & TUCKER, TJ 1998. Thyroid Hormone Concentrations in Black Bears (*Ursus americanus*): Hibernation and Pregnancy Effects. *General and Comparative Endocrinology,* 109, 192.
TORGET, V 2015. Relationships between Environmental and Biological Variables, Plasma Clinical-Chemical Parameters and Persistent Organic Pollutants (POPs) in Polar Bears (*Ursus maritimus*) from Svalbard, Norway. *NTNU. Master thesis.*
UCÁN-MARÍN, F, ARUKWE, A, MORTENSEN, A, GABRIELSEN, GW, FOX, GA & LETCHER, RJ 2009. Recombinant Transthyretin Purification and Competitive Binding with Organohalogen Compounds in Two Gull Species (*Larus argentatus* and *Larus hyperboreus*). *Toxicological Sciences,* 107, 440.
VAN RAAIJ, J, KAPTEIN, E, VISSER, T & VAN DEN BERG, K 1993. Increased Glucuronidation of Thyroid Hormone in Hexachlorobenzene-Treated Rats. *Biochemical Pharmacology,* 45, 627.
VERREAULT, J, SKAARE, JU, JENSSEN, BM & GABRIELSEN, GW 2004. Effects of Organochlorine Contaminants on Thyroid Hormone Levels in Arctic Breeding Glaucous Gulls (*Larus hyperboreus*). *Environmental Health Perspectives,* 112, 532.
VERREAULT, J, MUIR, DCG, NORSTROM, RJ, STIRLING, I, FISK, AT, GABRIELSEN, GW, DEROCHER, AE, EVANS, TJ, DIETZ, R, SONNE, C, SANDALA, GM, GEBBINK, W, RIGET, FF, BORN, EW, TAYLOR, MK, NAGY, J & LETCHER, RJ 2005. Chlorinated Hydrocarbon Contaminants and Metabolites in Polar Bears (*Ursus maritimus*) from Alaska, Canada, East Greenland, and Svalbard: 1996−2002. *Science of the Total Environment,* 351–352, 369.
VERREAULT, J, BECH, C, LETCHER, RJ, ROPSTAD, E, DAHL, E & GABRIELSEN, GW 2007. Organohalogen Contamination in Breeding Glaucous Gulls from the Norwegian Arctic: Associations with Basal Metabolism and Circulating Thyroid Hormones. *Environmental Pollution,* 145, 138.
VILLANGER, GD, JENSSEN, BM, FJELDBERG, RR, LETCHER, RJ, MUIR, DCG, 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*). *Environment International,* 37, 694.
VILLANGER, GD, LYDERSEN, C, KOVACS, KM, LIE, E, SKAARE, JU & JENSSEN, BM 2011b. Disruptive Effects of Persistent Organohalogen Contaminants on Thyroid Function in White Whales (*Delphinapterus leucas*) from Svalbard. *Science of the Total Environment,* 409, 2511.
VILLANGER, GD, GABRIELSEN, KM, KOVACS, KM, LYDERSEN, C, LIE, E, KARIMI, M, SØRMO, EG & JENSSEN, BM 2013. Effects of Complex Organohalogen Contaminant Mixtures on Thyroid Homeostasis in Hooded Seal (*Cystophora cristata*) Mother–Pup Pairs. *Chemosphere,* 92, 828.
VISSER, TJ, VAN BUUREN, JC, RUTGERS, M, ROODA, SJE & DE HERDER, WW 1990. The Role of Sulfation in Thyroid Hormone Metabolism. *Trends in Endocrinology and Metabolism,* 1, 211.
VISSER, TJ 1994. Role of Sulfation in Thyroid Hormone Metabolism. *Chemico-Biological Interactions,* 92, 293.
VISSER, TJ & PEETERS, RP 2000. Metabolism of Thyroid Hormone. *Endotext.* Accesed December 2015. [Online]. Available: http://europepmc.org/abstract/med/25905401
WADE, MG, PARENT, S, FINNSON, KW, FOSTER, W, YOUNGLAI, E, MCMAHON, A, CYR, DG & HUGHES, C 2002. Thyroid Toxicity Due to Subchronic Exposure to a

Complex Mixture of 16 Organochlorines, Lead, and Cadmium. *Toxicological Sciences,* 67, 207.
WEBSTER, JR, MOENTER, SM, BARRELL, GK, LEHMAN, MN & KARSCH, FJ 1991. Role of the Thyroid Gland in Seasonal Reproduction. III. Thyroidectomy Blocks Seasonal Suppression of Gonadotropin-Releasing Hormone Secretion in Sheep. *Endocrinology,* 129, 1635.
WEISS, JM, ANDERSSON, PL, LAMOREE, MH, LEONARDS, PE, VAN LEEUWEN, SP & HAMERS, T 2009. Competitive Binding of Poly- and Perfluorinated Compounds to the Thyroid Hormone Transport Protein Transthyretin. *Toxicological Sciences,* 109, 206.
WHITEMAN, J, HARLOW, H, DURNER, GM, ANDERSON-SPRECHER, R, ALBEKE, SE, REGEHR, EV, AMSTRUP, SC & BEN-DAVID, M 2015. Summer Declines in Activity and Body Temperature Offer Polar Bears Limited Energy Savings. *Science,* 349, 295.
WILBER, JF & UTIGER, RD 1969. The Effect of Glucocorticoids on Thyrotropin Secretion. *Journal of Clinical Investigation,* 48, 2096.
WINGFIELD, JC 2008. Comparative Endocrinology, Environment and Global Change. *General and Comparative Endocrinology,* 157, 207.
WU, S-Y, GREEN, WL, HUANG, W-S, HAYS, MT & CHOPRA, IJ 2005. Alternate Pathways of Thyroid Hormone Metabolism. *Thyroid,* 15, 943.
YU, WG, LIU, W & JIN, YH 2009. Effects of Perfluorooctane Sulfonate on Rat Thyroid Hormone Biosynthesis and Metabolism. *Environmental Toxicology and Chemistry,* 28, 990.
ZOELLER, RT, TAN, SW & TYL, RW 2007. General Background on the Hypothalamic-Pituitary-Thyroid (HPT) Axis. *Critical Reviews in Toxicology,* 37, 11.

7.0 APPENDICES

Appendix A

List of compounds analysed in plasma from free-ranging female polar bears from Svalbard.

Group	Acronym	Analyte
PCBs	PCB-99	2, 2', 4, 4', 5-Pentachlorobiphenyl
	PCB-105	2, 3, 3', 4, 4'-Pentachlorobiphenyl
	PCB-118	2, 3', 4, 4', 5-Pentachlorobiphenyl
	PCB-128	2, 2', 3, 3', 4, 4'-Hexachlorobiphenyl
	PCB-137	2, 2', 3, 4, 4', 5-Hexachlorobiphenyl
	PCB-138	2, 2', 3, 4, 4', 5'-Hexachlorobiphenyl
	PCB-153	2, 2', 4, 4', 5, 5'-Hexachlorobiphenyl
	PCB-156	2, 3, 3', 4, 4', 5-Hexachlorobiphenyl
	PCB-157	2, 3, 3', 4, 4', 5'-Hexachlorobiphenyl
	PCB-170	2, 2', 3, 3', 4, 4', 5-Heptachlorobiphenyl
	PCB-180	2, 2', 3, 4, 4', 5, 5'-Heptachlorobiphenyl
	PCB-183	2, 2', 3, 4, 4', 5', 6-Heptachlorobiphenyl
	PCB-187	2, 2', 3, 4', 5, 5', 6-Heptachlorobiphenyl
	PCB-189	2, 3, 3', 4, 4', 5, 5'-Heptachlorobiphenyl
	PCB-194	2, 2', 3, 3', 4, 4', 5, 5'-Octachlorobiphenyl
	PCB-196	2, 2', 3, 3', 4, 4', 5, 6'-Octachlorobiphenyl
	PCB-206	2, 2', 3, 3', 4, 4', 5, 5', 6-Nonachlorobiphenyl
	PCB-209	Decachlorobiphenyl
OCPs	a-HCH	1α ,2α ,3β ,4α ,5β ,6β -Hexachlorocyclohexane
	b-HCH	1α ,2β ,3α ,4β ,5α ,6β -Hexachlorocyclohexane
	p,p'-DDE	p, p,'-Dichloro-diphenyl-dichloroethylene
	HCB	Hexachlorobenzene
	Oxychlordane	Oxychlordane
	trans-Nonachlor	*trans*-Nonachlor
PBDEs	BDE-47	2, 2', 4, 4'-Tetrabromodiphenylether
	BDE-99	2, 2', 4, 4', 5-Pentabromodiphenylether
	BDE-100	2, 2', 4, 4', 6-Pentabromodiphenylether
	BDE-153	2, 2', 4, 4', 5, 5'-Hexabromodiphenylether
PFASs	PFHxS	Perfluorohexane sulfonate
	PFOS	Perfluorooctane sulfonate
	PFOA	Perfluorooctanoate
	PFNA	Perfluorononanoate
	PFDA	Perfluorodecanoate
	PFUnDA	Perfluoroundecanoate
	PFDoDA	Perfluorododecanoate
	PFTrDA,	Perfluorotridecanoate
OH-PCBs	4'-OH-CB106	4′-hydroxy-2, 3, 3′, 4, 5-Pentachlorobiphenyl
	4-OH-CB107	4-hydroxy-2, 3, 3', 4', 5-Pentachlorobiphenyl

	4’-OH-CB108	4’-hydroxy-2’, 3, 3’, 4’, 5-Pentachlorobiphenyl
	3-OH-CB118	3-hydroxy-2, 3’, 4, 4’, 5-Pentachlorobiphenyl
	4’-OH-CB130	4’-hydroxy-2, 2’, 3, 3’, 4, 5’-Hexachlorobiphenyl
	3’-OH-CB138	3-hydroxy-2, 2’, 3’, 4, 4’, 5-Hexachlorobiphenyl
	4-OH-CB146	4-hydroxy-2, 2’, 3, 4’, 5, 5’-Hexachlorobiphenyl
	4’-OH-CB159	4-hydroxy-2’, 3, 3’, 4’, 5, 5’-Hexachlorobiphenyl
	4’-OH-CB172	4-hydroxy-2, 2’, 3, 3’, 4’, 5, 5’-Heptaachlorobiphenyl
	3’-OH-CB180	3-hydroxy-2, 2’, 3’, 4, 4’, 5, 5’-Heptachlorobiphenyl
	4-OH-CB187	4-hydroxy-2, 2’, 3, 4’, 5, 5’, 6-Heptachlorobiphenyl
OH-PBDEs	4-OH-BDE42	4-hydroxy-2, 2, 3, 4-Tetrabromodiphenylether
	3-OH-BDE47	3-hydroxy-2, 2’, 4, 4’-Tetrabromodiphenylether
	6-OH-BDE47	6-hydroxy-2, 2’, 4, 4’-Tetrabromodiphenylether
	4’-OH-BDE49	4’-hydroxy-2, 2’, 4, 5’-Tetrabromodiphenylether
	2’-OH-BDE68	2'-hydroxy-2*,* 3′*,* 4*,* 5'-Tetrabromodiphenylether

Appendix E

List of the parsimonious models within each set of models. One set of 25 models was tested for each of the POP groups. Models marked with an asterix contain a significant POP variable.

TH	POP group	Parsimonious model	AICc	
TT3	∑PCB	sumPCB+Season+Status+Season:Status	23.81	
	∑PESTBDE	Season+Status+Season:Status	23.84	
	∑PFAS	Season+Status+Season:Status	23.84	
	∑OHPCB	Season+Status+Season:Status	23.84	
FT3	∑PCB	sumPCB+Season+Status+Season:Status	165.35	*
	∑PESTBDE	sumPESTBDE+Season+Season:Status	170.48	*
	∑PFAS	sumPFAS+Season+Status+Season:Status	169.75	*
	∑OHPCB	sumOHPCB+Season+Season:Status	173.94	
TT4	∑PCB	Season+Season:Status	698.28	
	∑PESTBDE	Season+Season:Status	698.28	
	∑PFAS	Season+Season:Status	698.28	
	∑OHPCB	Season+Season:Status	698.28	
FT4	∑PCB	Season	484.6	
	∑PESTBDE	Season	484.6	
	∑PFAS	Season	484.6	
	∑OHPCB	Season	484.6	
TT3:FT3	∑PCB	sumPCB+Season	385.55	*
	∑PESTBDE	Season	392.79	
	∑PFAS	sumPFAS+Season	389.3	*
	∑OHPCB	Season	392.79	
TT4:FT4	∑PCB	Season	149.07	
	∑PESTBDE	Season:sumPESTBDE	147.95	
	∑PFAS	sumPFAS+Season+Season:sumPFAS	138.36	*
	∑OHPCB	Season	149.07	
TT4:TT3	∑PCB	sumPCB+Season+Status	641.07	
	∑PESTBDE	sumPESTBDE +Status+Season:sumPESTBDE	637.93	*
	∑PFAS	sumPFAS+Status+Season:sumPFAS	646.67	*
	∑OHPCB	Season+Status	641.79	