NTNU – Trondheim
Norwegian University of
Science and Technology

Disruptive Effects of Organohalogenated Contaminants on Thyroid Hormone Levels in Glaucous Gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard

Marte Melnes

Environmental Toxicology and Chemistry
Submission date: May 2014
Supervisor: Bjørn Munro Jenssen, IBI
Co-supervisor: Geir Wing Gabrielsen, Norsk Polarinstitutt

Norwegian University of Science and Technology
Department of Biology

NTNU – Trondheim
Norwegian University of
Science and Technology

IN COLLABORATION WITH

ACKNOWLEDGEMENTS

I wish to thank my supervisors Bjørn Munro Jenssen and Geir Wing Gabrielsen for letting me take part in this project. Thank you for support, advice and feedback along the way. I wish to thank Geir Wing Gabrielsen for calling to check in when it turned out it was more challenging than first thought to capture glaucous gulls in Ny-Ålesund.

I am thankful to Kjetil Sagerup (Akvaplan-niva) for training in field. Thankful thoughts are also sent out to all the crew in Ny-Ålesund for help with the equipment, having a warm meal ready after a long day in field among angry birds, and making it a highly memorable time. Mostly, sincere thanks to my field partner, laboratory partner and dear friend Mari Løseth for the cooperation and great time even when weather was bad, the trap didn't work and calving glaciers caused severe situations.

Thank you to Dorte Herzke and Arntraut Götsch at NILU for help with chemical analysis. Grethe Stavik Eggen (NTNU) has given me great help in the thyroid hormone analysis and interpretation.

A challenge has been the choice of statistical treatment of the data and I wish to thank several people for valuable discussions and help. Eugen Sørmo (NTNU) has been a great support when I struggled with the modelling. Thank you to Kristin Møller Gabrielsen for discussion about how to treat field data and my brother-in-law to be Audun Brunes for help with SPSS.

I am thankful to Terje Hekkelstrand for technical support when computer issues were threatening to take all my time.

Finally, my family deserves to be acknowledged for supporting and helping me. A special thank goes to my sister Silje for proof reading.

I am thankful for my family and friends for always listening interested to my stories about birds in the Arctic.

Trondheim, May 2014

Marte Melnes

ABSTRACT

The glaucous gull (*Larus hyperboreus*) is a predator and scavenger occupying a high position in the arctic marine food web. This species is exposed to high levels of a variety of persistent anthropogenic pollutants thought to cause adverse health effects. Organohalogenated contaminants (OHCs) are possible endocrine disruptors, suggested to interfere with the thyroid hormone system among others. The aim of this project was to investigate the possible effects of the cocktail of OHCs to which glaucous gulls are exposed to on circulating thyroid hormone levels.

Blood from male and female glaucous gulls were sampled during the incubation periods of 2011, 2012 and 2013 in Kongsfjorden, Svalbard in the Norwegian Arctic. The plasma was analyzed for a variety of OHCs, including polychlorinated biphenyls (PCBs), persistent organochlorinated pesticides (OCPs), brominated flame retardants such as polybrominated diphenyl ethers (PBDEs) as well as perfluorinated alkylated substances (PFASs). Total and unbound fractions of thyroid hormones were also quantified. Multivariate data analysis was conducted to evaluate associations between contaminants and thyroid hormones.

Highly chlorinated PCBs, some persistent OCPs and the emerging perfluorooctane sulfonate (PFOS) were the compounds detected at the highest levels in the present study. Females seemed to be more susceptible to disruptive effects of OHCs on thyroid hormone levels than male glaucous gulls. The prevailing contaminants detected were also those shown to significantly affect unbound fractions of both T3 and T4 in female glaucous gulls. The results from the present study demonstrate complex combined effects on TH levels from the environmental mixtures of halogenated organic contaminants to which glaucous gulls are exposed to in the Arctic.

SAMMENDRAG

Polarmåker (*Larus hyperboreus*) er predatorer og åtseletere posisjonert på toppen av det arktiske marine næringsnettet. Denne arten er utsatt for høye nivåer av en rekke antropogene og persistente miljøgifter som er antatt å kunne medføre negative helseeffekter. Organiske halogenerte miljøgifter (OHCer) er mulige hormonforstyrrende stoffer, som blant annet er vist å kunne forstyrre tyroidhormonsystemet. Hensikten med dette prosjektet var å evaluere mulige effekter av blandinger av slike halogenerte miljøgifter på nivåer av tyroidhormoner i hekkende polarmåker.

Blodprøver ble tatt av hekkende polarmåker i Kongsfjorden, Svalbard under rugeperiodene i 2011, 2012 og 2013. Blodplasmaet ble analysert for en rekke OHCer inkludert polyklorerte bifenyler (PCBs), persistente organiske klorerte pesticider (OCPs), bromerte flammehemmere som polybromerte difenyl-etere (PBDEs) samt perfluor-alkylerte stoffer (PFASs). Total og frie fraksjoner av tyroidhormoner ble kvantifisert. Multivariat dataanalyse ble utført for å evaluere mulig virkning av OHCer på nivåer av tyroidhormonene.

Høyt klorerte PCBs, flere persistente OCPs og perfluoro-oktan-sulfonat (PFOS) var de forbindelsene påvist i høyest nivåer i denne studien. Hunner syntes å være mer utsatt for tyroidforstyrrende effekter av eksponering for OHCer enn hann-polarmåker. De dominerende stoffene detektert var også de som hadde størst effekter på tyroidhormoner i hunn-polarmåkene. Resultatene fra denne studien viser komplekse effekter på nivåer av tyroidhormoner fra blandingene av miljøgifter som polarmåker er utsatt for i Arktis.

ABBREVIATIONS

AhR	Aryl hydrocarbon receptor
AMAP	Arctic Monitoring and Assessment Program
ANOVA	Analysis of variance
BCI	Body condition index
BFR	Brominated flame retardant
CHL	Chlordane
Cpm	Counts per minute
CV	Coefficient of variation
CV-ANOVA	Cross validated analysis of variance
CYP450	Cytochrome P450
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyltrichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
EDC	Endocrine disrupting chemical
FC	Free cholesterol
FT3	Free triiodothyronine
FT4	Free thyroxine
GC	Gas chromatograph
HCB	Hexachlorobenzene
ID	Identification
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
MS	Mass spectrometer
N	Number of observations
NILU	Norwegian Institute for Air Research
NPI	Norwegian Polar Institute
NTNU	Norwegian University of Science and Technology
OC	Organochlorine
OCN	Octachloronaphtalene
OHC	Organohalogenated contaminant
OCP	Organochlorinated pesticide
OPLS	Orthogonal projection to latent structures
P	Probability of rejecting the hypothesis
PBDE	Polybrominated diphenyl ether
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PFAS	Perfluoroalkylated substance
PFBA	Perfluorobutanoate
PFBS	Perfluorobutane sulfonate
PFCA	Perfluoroalkyl carboxylates
PFDcA	Perfluorodecanoate
PFDcS	Perfluorodecane sulfonate

PFDoA	Perfluorododecanoate
PFHpS	Perfluoroheptane sulfonate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoate
PFOA	Perfluorooctanoate
PFOS	Perfluorooctane sulfonate
PFPA	Perfluoropentanoate
PFSA	Perfluoroalkyl sulfonates
PFTeA	Perfluorotetradecanoate
PFTrA	Perfluorotridecanoate
PL	Phospholipid
PLS	Projection to latent structures
POP	Persistent organic pollutant
POSF	Perfluorooctanesulfonyl fluoride
Q^2	Goodness of prediction coefficient
Qstd	Quantification standard
R	Pearson correlation coefficient
RIA	Radioimmuno assay
R^2X	Explained variance
R^2Y	Goodness of fit coefficient
Rpm	Rounds per minute
SD	Standard deviation
SRM	Standard reference material
TC	Total cholesterol
2,3,7,8-TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TDC	Thyroid disrupting chemical
TG	Triglyceride
TH	Thyroid hormone
T3	Triiodothyronine
T4	Thyroxine
TR	Thyroid receptor
TT3	Total triiodothyronine
TT4	Total thyroxine
TSH	Thyroid stimulating hormone
TTR	Transthyretin
UDP-GT	Uridine 5'-diphospho-glucuronosyltransferase
UHPLC	Ultra-high pressure liquid chromatography
UV	Unit variance
VIP	Variable importance in projection
Ww	Wet weight

CONTENTS

ACKNOWLEDGEMENTS i
ABSTRACT v
SAMMENDRAG vii
ABBREVIATIONS viii
CONTENTS x
1 INTRODUCTION 1
1.1 Organohalogenated contamination in the Arctic 1
1.2 Endocrine disruption 3
1.3 The thyroid hormone system 3
1.4 The glaucous gull 4
1.5 Aim of study 5
2 MATERIALS AND METHODS 7
2.1 Sampling area 7
2.2 Field procedures 8
2.3 OHC analyses 9
2.3.1 Analyses of chlorinated and brominated organic compounds 10
2.3.2 Analyses of perfluorinated compounds 12
2.4 Thyroid hormone analysis 14
2.4.1 Quality assurance and method validation 15
2.5 Lipid analysis 15
2.6 Sex determination 15
2.7 Statistical analysis 16
2.7.1 Data treatment of samples <LOD 17
2.7.2 Principal component analysis 17
2.7.3 OPLS 18
3 RESULTS 19
3.1 Biological variables 19
3.2 Contaminants 20
3.2.1 OHC levels 20
3.2.2 OHC patterns 21
3.3 Thyroid hormones 23

3.4 Associations between THs and OHCs 24
3.4.1 Principal component analysis 24
3.4.2 OPLS 29
3.4.3 Mixture correlation analysis 34
4 DISCUSSION 37
4.1 Contaminants: prevalence and levels 37
4.2 Thyroid hormones 41
4.3 Associations between body condition and OHCs 43
4.4 Associations between OHCs and THs 44
4.5 Future perspectives 51
5 CONCLUSION 53
REFERENCES 55
APPENDIX A: Detection limits of OHCs 67
APPENDIX B: Individual biometric measures 67
APPENDIX C: Individual OHC concentrations 69
APPENDIX D: Individual TH concentrations 72
APPENDIX E: OPLS-regression model (Y=BCI) 73

1 INTRODUCTION

1.1 Organohalogenated contamination in the Arctic

Despite little direct use and production of organohalogenated contaminants (OHCs) in the Arctic, such compounds of anthropogenic origin have extensively been reported in arctic biota for decades (Braestrup et al., 1974; Barrie et al., 1992; Muir and de Wit, 2010). High-latitude areas may act as sinks for anthropogenic contaminants due to atmospheric long-range transport, ocean and river currents in combination with a cold climate (Burkow and Kallenborn, 2000; Macdonald et al., 2002). The physical and chemical properties of OHCs make them resistant to degradation in the nature, and their persistence allow them to still be found in pristine areas several decades after being banned in use (El-Shahawi et al., 2010). There has been a decline in the levels of the legacy OHCs found in arctic biota since the 1970s and 80s due to regulation of production and use. The regulation of persistent organic pollutants (POPs) have mainly been through the Convention of Long-range Transboundary Air Pollution initiated in 1979 (UNECE, 1979) and the Stockholm Convention on POPs effective from 2004 (UNEP, 2001). However, concerns should be given to the increasing trends of emerging contaminants like brominated flame retardants (BFRs) (de Wit et al., 2010) and perfluorinated compounds (PFCs), also termed perfluorinated alkyl substances (PFASs) (Dietz et al., 2008) found in high latitude regions.

The lipid solubility and persistency of many OHCs occurring in the Arctic lead to a long half-life in biota and accumulation in the food webs. Hydrophobic contaminants are readily absorbed in organic matter. Transfer within the food web through ingestion of prey is the major source of uptake of OHCs and other POPs in animals occupying upper positions in the food web (De Wit et al., 2004). High bioconcentration at the first trophic levels in a marine food chain coupled with low excretion rates result in higher concentrations of OHCs in animals occupying apical positions in the food chain (Braune et al., 2005).

The OHCs found in the Arctic include persistent industrial compounds such as polychlorinated biphenyls (PCBs), -dibenzo-*p*-dioxins (PCDDs) and -dibenzofurans (PCDFs,) and metabolic products from abiotic or biotic break-down processes like hexachlorobenzen (HCB). Industrial chemicals are either produced intentionally or they are unintended compounds formed by the production of other organics. PCBs are one of the dominating contaminant groups found in the Arctic, and the number and positioning of chlorines on the biphenyl rings affect the persistency and behavior of these chemicals in the environment

(Jensen, 1991; Muir et al., 2000; De Wit et al., 2004). Organochlorinated pesticides (OCPs) such as the highly persistent dichlorodiphenyltrichloroethane (DDT) and its metabolites, as well as chlordanes and mirex have toxic properties by design (De Wit et al., 2004). PCBs and OCPs are all well-known pollutants among the initial contaminants listed under the Stockholm Convention (Buccini, 2003), and are by far the most studied classes of contaminants in the glaucous gull (*Larus hyperboreus*) (Verreault et al., 2005c).

More emerging classes of contaminants such as BFRs and PFASs have received increasing attention and concern the last decade (De Wit et al., 2004; Verreault et al., 2005b). Polybrominated diphenyl ethers (PBDEs) are used in a variety of products as flame retardants. Due to these contaminants' structural resemblance to PCBs, one may expect similar adverse effects in the environment (Manchester-Neesvig et al., 2001). In contrast to the lipophilic chlorinated and brominated compounds, PFASs are associated to proteins in organisms. The ampiphatic properties of PFASs make them able to repel both water and oils (Kelly et al., 2009). Thus, PFASs have been used as surfactants in a variety of manufactured and consumer products, and are common constitutes in stain repelling agents, lubricants, fire-fighting foams and paint (Buck et al., 2011). The unique chemical and biological stability of PFASs against degradation results in high persistency and potential for bioaccumulation in wildlife (Verreault et al., 2005b; Verreault et al., 2010), especially when the perfluorinated chain exceeds 6-7 carbons (Martin et al., 2004). The perfluoro sulfonate (PFSA) perfluorooctane sulfonate (PFOS) is the major PFAS identified in arctic biota (Giesy and Kannan, 2001; Verreault et al., 2005b; Bytingsvik et al., 2012b). However, long-chained perfluorocarboxylic acids (PFCAs) have also been detected (Verreault et al., 2005b; Bytingsvik et al., 2012b). Despite the agreement on the concerns about potential toxicological implications of PFOS and other perfluorinated compounds, and the resulting regulations on production and use, PFASs are still used for several industrial purposes (Prevedouros et al., 2006; Buck et al., 2011).

Free ranging Arctic mammals and birds are exposed to a complex mixture of OHCs as well as other contaminants. The result is a cocktail effect with many possible additive, synergistic and antagonistic effect combinations of contaminants (Sagerup et al., 2009a; Letcher et al., 2010). Thus, evaluation of combinations of contaminants in wildlife is more relevant in contrast to assessment of single compounds. However, the complex exposure mixtures complicate extrapolation from laboratory effect studies of POPs in animals and make it challenging to reveal effects of single toxic compounds (Bustnes, 2006).

1.2 Endocrine disruption

Exposure to OHCs may cause reproductive, behavioral and developmental stress by having potential to modulate actions of endocrine systems in animals (Bustnes et al., 2001a; Bustnes et al., 2002; Bustnes et al., 2003b). Endocrine-disrupting chemicals (EDCs) are compounds that are able to interfere with the actions of endogenous hormones due to similar structural properties (Colborn et al., 1993; Hotchkiss et al., 2008). Interaction may occur with hormone transport proteins, hormone metabolism or excretion, or by binding to hormone receptors and thereby mimicking or blocking the effects of the endogenous hormones (Jenssen, 2006). The thyroid hormone system is among the endocrine systems where the most profound relationships between disrupted function and exposure to EDCs have been found, and is thus subjected to further research (Colborn, 2002; Zoeller et al., 2002).

1.3 The thyroid hormone system

The avian thyroid hormone (TH) system controls body weight and growth, thermoregulation, lipid metabolism, moulting and development of several body systems like the central nervous system, the skeletal system and the musculature (Sturkie, 1965; McNabb, 2000). The presence of TH receptors in almost all cell types in the organism reveals the importance and widespread actions of THs. Thus, maintenance of normal functioning of THs is essential for sustaining good health (McNabb, 2000). Assessment of circulating levels of THs has been proposed to be a useful biomarker of effect of OHCs in wildlife (Fox, 1993; Rolland, 2000; Skaare et al., 2002). Actions of the hypothalamic-pituitary-thyroid (HPT) axis controls production and release of THs. The thyroid gland produces and secretes mainly thyroxine (T4) in response to thyroid-stimulating hormone (TSH) released by the pituitary. T4 enters the bloodstream bound to TH-binding proteins such as transthyretin (TTR) and albumin, and undergoes deiodination by peripheral enzymes in target tissues to become the biological active triiodothyronine (T3) (McNabb, 2007). Evidence that T3 accounts for most of the TH actions in vertebrates includes the much higher affinity of T3 than T4 to the thyroid hormone receptor (TR), which is a nuclear receptor where actions are mediated through (Sturkie, 1965; Zoeller et al., 2007). The major environmental factors causing natural variation in circulating THs include temperature changes and food availability (McNabb, 2000; McNabb, 2007). The nature of the food source, iodine disposal, season and age are more features influencing TH levels and function. Circulating T4 rise and peak during the dark phase of the diurnal cycle,

and decrease during the light phase. T3 patterns seem to be opposite (Wentworth and Ringer, 1986). Patterns and proportions of plasma T4 and T3 depend on hormone production and secretion by the thyroid gland and the deiodination enzymes present in peripheral tissues (McNabb, 2007).

Thyroid disruption may occur by interference of thyroid disrupting chemicals (TDCs) on several levels on the HPT axis. Interference may, for instance, occur by altering thyroid gland morphology and function or impairing TH synthesis, metabolism, transport and actions (Brouwer et al., 1998; Zoeller et al., 2009). The potency of blood accumulating compounds to bind to TH transport proteins is demonstrated in several studies and suggests a central mechanism of disruption (Van den Berg et al., 1991; Braathen et al., 2004; Weiss et al., 2009). Specifically, hydroxylated PCBs, and to a lesser extent PBDE congeners have shown to be effective competitive ligands in glaucous gull TTR in relation to both T3 and T4 (Ucán-Marín et al., 2009). Such competitive binding is thought to increase the biliary excretion rates of particularly T4, and thus represents a major mode of toxic actions of such compounds on the TH system (Boas et al., 2006). Despite indications that TDCs may decrease circulating THs, there is a lack of establishment of both TH baseline levels and lowest contaminant concentration causing effects (Boas et al., 2006; Simon et al., 2011).

1.4 The glaucous gull

The glaucous gull has a circumpolar distribution in the Arctic, and the populations breeding in the Barents Sea region winter mainly in the northern part of the Atlantic Ocean (Løvenskiold, 1964). The glaucous gull is a large bird occupying an apical position in the arctic marine food web (Anker-Nilssen et al., 2000). As an opportunistic predator and scavenger it utilizes a wide range of food items such as fish, molluscs, crustaceans, eggs, chicks and adults from other seabirds, as well as carrion, refuse and offal (Hoyo et al., 1992). Since the early 1970s, high levels of anthropogenic, long-transported contaminants have been reported in the glaucous gull (Bourne and Bogan, 1972). During the late 1980s, dead and dying glaucous gulls were reported on Bjørnøya, where the largest colony of these gulls in the Barents Sea is located (Anker-Nilssen et al., 2000). It was proposed that high levels of OCs contributed to the increased mortality (Gabrielsen et al., 1995). Occasionally high levels of OHCs in combination with reports on abnormal behavior of this top predator have extensively been reported since that time (Bustnes et al., 2000; Sagerup et al., 2009b; Verreault et al.,

2010). The exposure of OHCs through the diet, combined with a somewhat restricted capacity for biotransformation (Henriksen et al., 2000), make the birds susceptible for bioaccumulation and biomagnification of high levels of such compounds (Bustnes et al., 2003b; De Wit et al., 2004). Previous studies have assessed potential thyroid disruptive effects of OHCs in glaucous gulls (Verreault et al., 2004; Verreault et al., 2007; Verreault et al., 2010), and a growing body of evidence proposes that these birds are being adversely affected by their high body burden of complex mixtures of persistent anthropogenic chemicals to which they are exposed.

A wide range of *in vitro* assays are available for screening endocrine disruption, however they may be less relevant for wildlife toxicology. Endocrine disruption may occur via multiple mechanisms, and the endocrine effects of a specific chemical *in vitro* may not necessarily be predictive of its *in vivo* effects. Furthermore, there are numerous natural and anthropogenic factors that may influence the exposure and effects of OHCs in arctic wildlife, such as prey availability, competition, pathogens and a changing climate (Bustnes et al., 2008a). Furthermore, sex may affect chemical exposure pattern and levels, and the different physiology of males and females suggest that dissimilar TH disruptive effects from OHCs may be expected (Verreault et al., 2005c). The combined effects of natural stressors and anthropogenic stress like exposure to OHCs need to be considered in biomarker effect studies like the present. Despite the clear exposure patterns of OHCs in glaucous gulls, a causal link between contaminant exposure and adverse health impacts in Svalbard glaucous gulls remains to be established (Verreault et al., 2010).

1.5 Aim of study

The aim of the present study was to investigate levels and potential effects of mixtures of OHCs on thyroid hormone levels in male and female glaucous gulls breeding in Kongsfjorden, Svalbard.

It is hypothesized that high levels of persistent chlorinated, brominated and fluorinated compounds will be quantified, and that mixtures of such compound exert thyroid disruptive properties.

2 MATERIALS AND METHODS

2.1 Sampling area

Field sampling was conducted in Kongsfjorden, located in the vicinity of Ny-Ålesund, Svalbard (78°55′N 11°56′E) in the Norwegian Arctic. An estimate of 40 breeding pairs of glaucous gulls in Kongsfjorden was known from previous studies. Sampling was conducted between 7th and 23rd of June 2013, during the second part of the incubation period. In addition to 19 glaucous gulls sampled in the season of 2013, the individuals studied in this survey are also included 16 and 14 birds sampled in 2011 and 2012, respectively, making a total of 49 individuals included. The breeding conditions in Kongsfjorden are characterized by continuous daylight, low temperatures, and occasionally strong winds and snowfall. Within a breeding season there are pronounced variations in food availability in different part of Kongsfjorden. These distinctions may in part determine the spatial and temporal foraging distributions and thereby trophic level of top predators such as the glaucous gull (Hop et al., 2002).

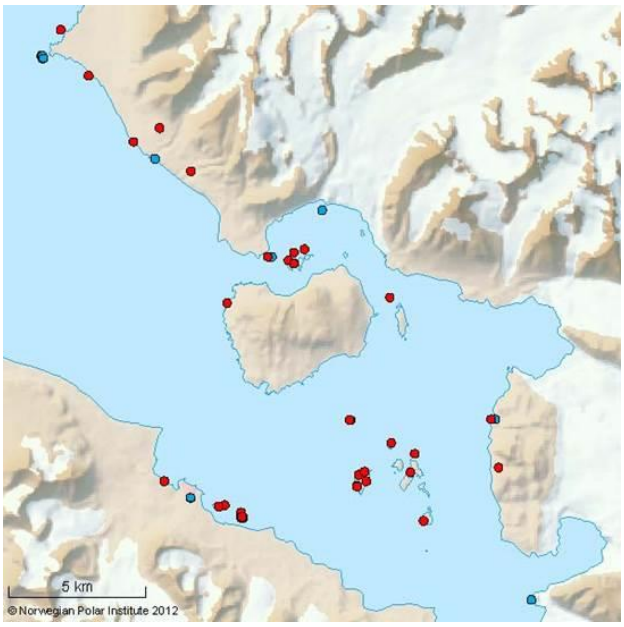

Figure 1. The sampling area in Kongsfjorden, located on northwestern Spitsbergen, Svalbard. The red dots represent breeding spots for glaucous gull (*Larus hyperboreus*) pairs through the summer of 2011, 2012 and 2013. The blue dots represent pairs not included in the monitoring programme. Map: Norwegian Polar Institute.

2.2 Field procedures

The birds were captured using an automatic triggered nest trap (Bustnes et al., 2001a) or a net canon. The nest trap was placed with a nylon snare around the nest bowl, leading to a releaser. When triggered by a radio transmitter, the trap was released when the bird entered the nest. Biometric data of the birds were recorded, including body mass, wing length, total head length, bill length and gonys height. All individuals were equipped with numbered rings from the Norwegian Ringing Centre, Stavanger Museum, as well as a letter coded ring for simple identification. A total of 10 mL blood was drawn from the branchial veins on the inside of the wings, using a heparinized syringe (Terumo syringe 5 and 10 mL with 2 drops of Heparin Leo ® 5000 IE/mL, Pharma AS) or heparinized vacutainer blood collection tube (Venosafe vacuette with Lithium Heparin, Terumo) using a 23G fine-jet one-time cannula (0.6x30 mm, BD Microlance) or 22G fine-jet one-time needles (Vacuette®, Greiner Bio-One). The blood samples were kept cool and dark in field, and centrifuged (10 000 rmp, 10 minutes) when returned to the lab facilities in Ny-Ålesund within 8 hours. The plasma samples for thyroid hormone analysis were kept in 1.2 mL cryogenic tubes, and plasma for contaminant analysis in 5 mL cryogenic tubes (Nalgene© cryoware cryogenic vials, USA). Red blood cells for sex determination were kept in 1.5 mL Eppendorf tubes. All samples were kept frozen at -20°C and transported to laboratories at NILU, Tromsø and NTNU, Trondheim for analysis at the end of the field work. All glaucous gulls sampled were breeding, thus the age of the birds were assumed to be four years or older (Gaston et al., 2009).

The current project was approved by the Governor of Svalbard (reference: 2013/00050-28), and the project RIS-ID was 5267. Sampling and handling of the birds were in accordance with the regulations of the Norwegian Animal Welfare Act.

2.3 OHC analyses

The OHC analyses were performed in the laboratories at the Norwegian Institute for Air Research (NILU) in Tromsø, and were funded by the Norwegian Polar Institute (NPI). The blood samples were analyzed for 12 PCB congeners, 6 PBDE congeners, 16 chlorinated pesticides and 16 PFASs listed in Table 1.

Table 1. The chlorinated, brominated and fluorinated contaminants analyzed in plasma from glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. Congeners of PCBs and PBDEs are numbered by the IUPAC system (International Union of Pure and Applied Chemistry).

Group	Acronym	Analyte
CHLs	*t*-chlordane	*trans*-chlordane
	c-chlordane	*cis*-chlordane
	oxy-chlordane	*oxy*-chlordane
	t-nonachlor	*trans*-nonachlor
	c-nonachlor	*cis*-nonachlor
	mirex	1,1α,2,2,3,3α,4,5,5,5α,5β,6-dodecachlorooctahydro-1*H*-1,3,4-(methanetriyl)cyclobuta[*cd*]pentalene
HCHs	α-HCH	1α,2α,3β,4α,5β,6β-Hexachlorocyclohexane
	β-HCH	1α,2β,3α,4β,5α,6β-Hexachlorocyclohexane
	γ-HCH	1α,2α,3β,4α,5α,6β-Hexachlorocyclohexane
HCB	HCB	Hexachlorobenzene
DDT's	*o,p'*-DDT	*o,p,'*-Dichloro-α,α-diphenyl-β,β,β-trichloroethane
	p,p'-DDT	*p,p,'*-Dichloro-α,α-diphenyl-β,β,β-trichloroethane
	o,p'-DDD	*o,p,'*-Dichloro-diphenyl-dichloroethane
	p,p'-DDD	*p,p,'*-Dichloro-diphenyl-dichloroethane
	o,p'-DDE	*o,p,'*-Dichloro-diphenyl-dichloroethylene
	P,p'-DDE	*p,p,'*-Dichloro-diphenyl-dichloroethylene
PCBs	PCB-28	2,4,4'-Trichlorobiphenyl
	PCB-52	2,2',5,5'-Tetrachlorobiphenyl
	PCB-99	2,2',4,4',5-Pentachlorobiphenyl
	PCB-101	2,2',4,5,5'-Pentachlorobiphenyl
	PCB-105	2,3,3',4,4'-Pentachlorobiphenyl
	PCB-118	2,3',4,4',5-Pentachlorobiphenyl
	PCB-138	2,2',3,4,4',5'-Hexachlorobiphenyl
	PCB-153	2,2',4,4',5,5'-Hexachlorobiphenyl
	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-194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
PBDEs	BDE-28	2',4,4'-Tribromodiphenyl ether
	PBDE-47	2,2',4,4'-Tetrabromodiphenyl ether
	PBDE-66	2',3',4,4'-Tetrabromodiphenyl ether
	PBDE-85	2,2',3',4,4'-Pentabromodiphenyl ether
	PBDE-99	2,2',4,4',5'-Pentabromodiphenyl ether
	PBDE-100	2,2′,4,4′,6'-Penta-bromodiphenyl ether
	PBDE-138	2,2',3',4,4',5'-Hexabromodiphenyl ether
	PBDE-153	2,2',4,4',5,5'-Hexabromobiphenyl ether
	PBDE-154	2,2',4,4',5,6'-Hexabromodiphenyl ether
	PBDE-183	2,2',3',4,4',5',6'-Heptabromodiphenyl ether
PFSAs	PFBS	Perfluorobutane sulfonate
	PFHxS	Perfluorohexane sulfonate
	PFOS	Perfluorooctane sulfonate
	Iso-PFOS	Perfluorodecan sulfonate
	PFD	Perfluorodecan sulfonate

Table 1 continued. The chlorinated, brominated and fluorinated contaminants analyzed in plasma from glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. Congeners of PCBs and PBDEs are numbered by the IUPAC system (International Union of Pure and Applied Chemistry).

	PFOSA	Perfluoroctane sulfonamide
PFCAs	PFHxA	Perfluorohexanoate
	PFHpA	Perfluoroheptanoate
	PFOA	Perfluorooctanoate
	PFNA	Perfluorononanoate
	PFDcA	Perfluorodecanoate
	PFUnA	Perfluoroundecanoate
	PFDoA	Perfluorododecanoate
	PFTrA	Perfluorotridecanoate
	PFTeA	Perfluorotetradecanoate
FTSs	6:2 FTS	6:2 fluorotelomer sulfonate

2.3.1 Analyses of chlorinated and brominated organic compounds

Extraction

The method for analysis is previously described (Bustnes et al., 2008b). The glaucous gull plasma was unfrozen before extraction. Plasma (500 µL) was transferred to 15 mL glass vials and spiked with internal standard (^{13}C-labelled POPs solution, 20 µL, 250 pg/µL). Ammonium sulfate saturated deionized water (2 mL) and ethanol (2 mL) was added to ensure denaturation of proteins and enhance phase separation. Extraction was conducted twice by mixing *n*-hexane (6 mL) thoroughly into samples. After phase separation for approximately 20 minutes, the hexane extract was carefully transferred to new 15 mL glass vials and further concentrated to 500 µL using Rapidvap (Rapid Vap; Labconco Corp., Kansas City, MO, USA). Samples were capped and kept cool overnight.

Clean-up

Clean-up by Florisil (magnesium silicate, particle size 150-250 µm, heated at 600°C for 8 hours) was conducted to separate the analytes from interfering compounds such as biological matrices. Columns were packed with Florisil (1 ± 0.03 g) between two glass fiber frits (Isolute SPE Accessories Frits, 10 µm, 3 mL). Each extract was passed through a Florisil column using a RapidTrace SPE Workstation (Caliper Life Sciences, Hopkinton, USA), and further diluted with dichloromethane in *n*-hexane (10 %). A small amount of *iso*-octane was added to each sample after clean up, to prevent evaporation of volatile chemicals. The extracts were concentrated to 200 µL using RapidVap, followed by transfer to 1.8 mL glass vials with insert, and further concentration to approximately 30 µL using a N_2 evaporation unit (N_2 purity of 99,995 %, quality 5.0, Hydrogas, Porsgrunn, Norway). ^{13}C PCB-159 (10 µL, 213

pg/µl, 25.09.12) was added as a recovery standard to each vial and samples were kept cool until time of contaminant quantification by gas chromatograph-mass spectrometry (GC/MS).

All labelled and internal standards were purchased from Cambridge Isotope Laboratories (Woburn, MA, USA). The solvents applied were provided by Merck (Darmstadt, Germany). The standard reference material (SRM, 1958 Human Serum) was provided by National institute of standards and technology (NIST), MD, USA).

Instrumental analysis

Chlorinated and brominated contaminants were quantified as described by Bustnes et al. 2008b. A sample from each extract (50 µL) was transferred to GC/MS vials. PCBs, PBDEs and DDT with metabolites were analyzed by an Agilent 7890 gas chromatograph equipped with a triple quadrupole mass spectrometer, Quattro Micro GC (Waters Corporation, Manchester UK). Analysis of other OCPs were carried out by an Agiland 7890A gas chromatograph equipped with a 5975C mass spectrometer (Agilent Technology, Böblingen, Germany). For separation, a DB-5MS column (30m; 0.25 µm film thickness, 0.25 mm inner diameter; J & W Scientific, Folsom, USA) with helium as carrier gas (flow rate 1 ml/min) was used. The enhancement of separation was facilitated by a temperature gradient (70°C for 2 minutes followed by a steady increase of 15°C/min until 180°C, before an incline of 5°C/min up to 280°C where the temperature was stable for 10 minutes).

Quantification

Quantification of individual compounds was conducted by the internal standard method. Standards with known concentrations of ^{12}C and ^{13}C labelled equivalents representing all PCBs, DDT and metabolites, HCHs, HCB, PBDEs and chlordanes, were measured together with the samples of unknown concentration. POP I in *iso*-octane (250 pg/µL, glass 7, 20.11) was applied to all samples. BROM I (250 pg/µL, glass 3, 44.09) was applied to samples from 2011 and 2012, while BROM I (glass 5, 25.07) was applied for samples from 2013. The peak areas of the standards in the chromatograph produced a standard curve used to calculate concentrations of the sample extracts using Equation 1.

$$C_{sample} = Rf(C_{std} \times Area_{sample}) / Area_{std} \qquad \text{Equation 1}$$

Where C_{sample} is the concentration of unknown sample, C_{std} is the concentration of known standard, $Area_{sample}$ is known area from the GC/MS chromatogram of the sample and $Area_{std}$ is known area of the internal standard on the GC/MS graph of the sample. Rf represents the

response factor calculated from the areas and concentrations of the ^{12}C and ^{13}C labelled equivalents acquired in the standard chromatograms.

Quality assurance

One blank and one SRM were analyzed for every fifteenth sample for quality control of the method and validation of repeatability. The reference samples were treated equally as the plasma samples. In cases where contamination of the blank by specific compounds was observed (applicable for γ-HCH, HCB, *trans*-chlordane, *cis*-chlordane, *oxy*-chlordane, *trans*-nonachlor, *cis*-nonachlor, *p,p'*-DDE and PCB-28) , the limit of detection (LOD) was set to three times the blank signal. Otherwise, LOD was set to threefold the chromatographic noise in the sample run. For PBDE-28, -47, -99 and -154, LOD was set to two times the noise of the sample due to little matrix disturbances in the MS/MS experiment. LODs of the individual compounds can be found in Appendix A. The results from the reference samples were within the given limits of accuracy. The recovery standard (^{13}C PCB-159) was added prior to instrumental analysis for both quantification standard and samples as an assessment of the analytical method performance.

2.3.2 Analyses of perfluorinated compounds

Extraction

The Powley method was performed for analysis of perfluorinated compounds (Powley et al., 2005). The samples were analyzed for a total of 16 PFASs, including five perfluoro sulfonic acids (PFSAs), nine perfluoro carboxylic acids (PFCAs) and one fluorotelomer sulfonate (FTS), included in Table 1. PFASs with carbon chains containing four to 14 carbons were included in the analysis. The method for analysis is previously described in Bustnes et al.2008b. Each plasma sample (200 µL) was spiked with internal standards containing mass-labeled PFASs (20 µL, 0.1 ng/µL ^{13}C PFC mix, 14.06.13) in Eppendorf tubes (1.5 mL). Liquid-liquid extraction was performed adding methanol (1 mL) followed by 3x10 minutes in ultrasonic bath to ensure removal of interfering compounds such as biological matrices. Phase separation was enhanced by centrifugation (10 000 rpm, 10 minutes).

Clean-up

Clean-up was conducted adding the methanol supernatant to new Eppendorf tubes (1.5 mL) containing superclean ENVI-Carb 120/400 (25 mg, Supelco 57210-U, Bellefonte, PA, USA) in glacial acetic acid (50 µL). After mixing by vortexing and centrifugation (10 000 rpm, 10

min), samples of each supernatant (500 µL) were transferred to new glass vials. Recovery standard (20 µL, 0.1 ng/µg RSTD in methanol (3,7-diMeo-PFOA, 23.07.2013)) was added to allow recoveries of the internal standards for quantification, and the samples were kept cool prior to instrumental quantification. An aliquot of each extracts (100 µL) were transferred to an autosampler vial with insert as well as NH_4OAc in water (100 µL, 2mM) at time of analysis.

All labelled and internal standards were provided by NILU (IRMM-427, ID 0119). All solvents applied were provided by Merck (Darmstadt, Germany). The standard reference material (SRM, 1957 Human Serum) was provided by National institute of standards and technology (NIST), MD, USA).

Instrumental analysis

Quantification of PFASs was performed by ultra-high pressure liquid chromatography triple-quadropole mass spectrometry (UHPLC-MS/MS). Instrumental analysis was performed on a Thermo Scientific quaternary Accela 1250 pump (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a PAL Sample Manager (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a Thermo Scientific Vantage MS/MS (Vantage TSQ) (Thermo Fisher Scientific Inc., Waltham, MA, USA). Injection of sample (10 µL) on a Waters Acquity UPLC HSS 3 T column (2.1 * 100 mm, 1.8 µm) equipped with a Waters van guard HSS T3 guard column (2.1 * 5 mm, 1.8 µm) (Waters Corporation, Milford, MA, USA) was performed following separation of the compounds by using NH_4OAc (2 mM) in 90:10 MeOH:water and methanolic NH_4OAc (2 mM) as the mobile phases. Further details on the instrumental performance is given in (Hanssen et al., 2013).

Quantification

Quantification was conducted using the LCQuan software from Thermo Scientific (Version 2.6) (Thermo Fisher Scientific Inc., Waltham, MA, USA). Standards with known concentrations of ^{12}C and ^{13}C labelled equivalents representing all PFSAs and PFCAs were measured together with the samples of unknown concentration. The obtained standard curve from concentrations of the internal standards was used for quantification of the samples using Equation 1.

Quality assurance

One blank and one standard reference material (SRM, 1957 Human Serum) were analyzed for every fifteenth sample for quality control of the method and validation of repeatability. The

reference samples were treated equally as the plasma samples. No contamination of the blanks was observed, and LOD was set to threefold the instrumental noise. The results from the reference samples were within the given limits of accuracy. The recovery standard was added prior to instrumental analysis for both quantification standard and samples as an assessment of the analytical method performance.

Contaminant concentrations were given in blood wet weight due to this measure is being considered most relevant when assessing toxic effects (Henriksen et al., 1996).

2.4 Thyroid hormone analysis

Quantification of THs in glaucous gull plasma was performed using radioimmunoassay (RIA) Coat-A-Count® TT3, FT3, TT4 and FT4 kits (Siemens medical solution, Diagnostics, Los Angeles, CA, USA) at NTNU, Trondheim. RIA is based on competitive binding between I-125 labelled THs and sample THs for sites on TH-specific antibody-coated tubes. Counting the test tube in a gamma counter yields a number inversely related to the concentration of THs present in the plasma. The method is a well-established and sensitive technique (McNabb, 2000), and the RIA kits have extensively been used for TH analysis in avian plasma including glaucous gulls (Verreault et al., 2004; Verreault et al., 2007).

Plasma samples were analyzed for four fractions of THs: free and total T3 (FT3 and TT3), and free and total T4 (FT4 and TT4) with respective detection limits (LOD) of 0.153 pmol/L, 0.044 nmol/L, 0.015pmol/L and 0.336 nmol/L. Samples were kept on ice during thawing prior to analysis. Plasma and radioactive labelled THs were added to the test tubes and were allowed to compete for binding sites of the pre-coated antibody tubes. After thoroughly decanting, the bound radioactive labelled THs were counted using gamma scintillation counter (Cobra Auto Gamma, model 5003, Packard Instrument Company, Dowers grove, IL, USA). 5 standards provided in each kit with known TH concentrations were used to obtain a calibration curve from which the TH levels in the samples could be calculated. The concentration range of the standards was for TT3 0 - 622 ng/dL, for TT4 0-24 µg/dL, for FT3 0-23 pg/mL and for FT4 0-5 ng/dL.

2.4.1 Quality assurance and method validation

The repeatability of the replicates and assay accuracy was tested for each TH kit by analyzing standard reference material (SRM) controls (human serum, Immunoassay Plus Control level 1, 2, 3, Biorad Laboratories, CA, USA) for every twentieth sample, and the laboratory's own quality control, bovine (*Bos primigenius*) serum once per kit. The reference samples were treated equally as the plasma samples. Plasma samples were run in duplicates for TT3 and FT3, and triplicates for TT4 and FT4. Coefficients of variations (CV) were calculated as statistical measures of dispersion of data. A %CV less than 15 % was considered acceptable, and samples with a %CV over 15 % were analyzed twice. All blanks were below LOD, and the range of results met the obligations set by the laboratory.

2.5 Lipid analysis

Lipid quantification was performed by Unilab Analysis AS, Tromsø, Norway. Analysis of triglycerides (TG), free cholesterol (FC), total cholesterol (TC) and phospholipids (PL) were determined enzymatically, and a summed lipid level was calculated using Equation 2 (Akins et al., 1989). The total lipid concentration was converted to plasma lipid weight percentage for further treatment as a covariable.

$$\text{Total lipid} = 1.677 * (TC - FC) + FC + TG + PL \qquad \text{Equation 2}$$

2.6 Sex determination

Determination of sex was mainly conducted biometrically, with male being larger than female glaucous gulls. A commonly used assumption that birds with bill longer than 61.5 mm and total head (head + bill) longer than 142 mm were males, were practiced (Coulson et al., 1983; Bustnes et al., 2000). For individuals with indistinguishable sizes, molecular sexing was performed at NTNU, Trondheim. The analysis was conducted by chelex extraction polymerase chain reaction (PCR) and gel electrophoresis, and is described by (Griffiths et al., 1998). The principle of sexing by PCR is detection of the specific female W-chromosome, visualized as two bands (ZW) in the agarose gel, separated from one band (ZZ) for males.

A droplet of blood cells was added together with chelex solution (5 %, 200 µL) to Eppendorf tubes prior to incubation (20 min, 56°C) and mixing by vortex. A second incubation was

conducted (8 min, 98°C) following vortexing and centrifugation (12 000 rpm, 3 min). The supernatant from each sample (20 µL) was transferred to new tubes. The prepared PCR stock solution (8 µL) together with the DNA supernatant (2 µL) were transferred to PCR tubes following PCR (30 cycles with thermal profile 94°C : 51°C : 70 °C) (Stratagene MX3000).

2.7 Statistical analysis

Of the sample size of initially 49 glaucous gulls, 10 individuals were sampled twice during the three years of captures. For the individuals captured twice, only data from a randomly picked capture were included for further statistical analysis to ensure that all observations were independent. The final sample size was thus 39 glaucous gulls; 15 males and 24 females. Univariate statistical analyses were conducted using software SPSS (version 21.0, IBM, SPSS Inc., Chicago, IL, USA). Multivariate data analyses were performed using SIMCA P+ (version 12.0.0.0, Umetrics, Umeå, Sweden).

The data were tested for normality using Shapiro-Wilk's W-test. Variables that were not normal distributed were log 10-transformed, including all OHCs and FT4, for approximating normality.

Statistical significance was set to $p \leq 0.05$, and p values were two-tailed. The Levene's test was used to test for equality of variance. Pearson correlation analysis was performed to check for bivariate correlations, and correlations are given as p (significance level) and r_p (pearson correlation coefficient). Independent samples T-tests were conducted to test for differences in variables between male and female glaucous gulls. Bonferroni correction was not applied when comparing associations between multiple variables because of the increased probability of producing false negatives (Moran, 2003).

A one-way ANOVA (Analysis of variance) with Scheffe *post hoc* test revealed that there were no significant differences in chlorinated compounds or TH levels between gulls captured in 2011, 2012 and 2013, respectively. There were however statistically significant different levels of some fluorinated and brominated compounds between the years. This applied for PFNA and PFUnA in males, and PFDcA, PFUnA, PFDoA, PFTrA, PFTeA and PBDE-47 in females. However, OHC data from the three years were pooled to obtain a larger variation in contaminant concentrations which is advantageous in correlation analyses.

2.7.1 Data treatment of samples <LOD

OHCs detected in minimum 60 % of the individuals were included in the statistical analysis. On this background, the following compounds were excluded from statistical analysis: α-HCH, γ-HCH, *t*-chlordane, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, PCB-101, PFBS, *iso*-PFOS, PFDS, PFHxA, PFHpA, 6:2FTS, PBDE-28, PBDE-66, PBDE-85, PBDE-99, PBDE-100, PBDE-138, PBDE-153, PBDE 154 and PBDE-183. Individual samples with compounds quantified to be under limit of detection (LOD) were assigned a random number between 0 and the compound specific LOD to avoid missing values in the dataset (Verreault et al., 2007). This was the case for *c*-chlordane, PCB-52, PFHxS, PFOA, PFTeA and PFOSA. Limit of quantification (LOQ) was defined as 3xLOD, and samples measured <LOQ was treated equally as other samples. LOD for the different compounds are presented in Appendix A.

2.7.2 Principal component analysis

Principal component analysis (PCA) was conducted for visualization of grouping and correlation among variables. Based on eigenvalues > 1, the two most significant principal components (PC1 and PC2) were used as x and y axis, respectively, in the PCA plots. PCA reduces the number of descriptive variables to a smaller set which can account for the systematic variation in the data set (Kemsley, 1996). Loading plots were used to interpret the relation among the variables. Variables with a loading value close to zero indicate little influence on the variation in the X matrix. Variables grouped close to each other are positively correlated, while variables positioned on the opposite of the origo have negative correlation.

A body condition index (BCI) was estimated for each individual to account for individual difference in size and body mass, and was calculated separately for male and females since glaucous gulls are sexually dimorphic (Sagerup et al., 2009a). A single measure of size was calculated from total head length, bill length, wing length and gonys height obtained from PCA (Jolicoeur and Mosimann, 1960). A linear regression model was performed with body mass as the dependent variable and the first principal component (PC1) from the size measure as the independent variable. The BCI was defined as the standardized residuals of the regression (Jakob et al., 1996; Sagerup et al., 2009a). A negative BCI indicates a below-average body condition based upon the regression model for a bird of that sex and size (Fox et al., 2007).

2.7.3 OPLS

Orthogonal partial least-square to latent structures (OPLS) regression was performed to assess which variables that were most predictive for the levels of THs, and possible combined effects of OHCs on THs. Ordinary multivariate regression is insufficient when the number of observations are somewhat limited and there is a large number of variables which are collinear with each other (Trygg and Wold, 2002). Such high correlations among the independent variables may be a problem as some predictors may incorrectly be found insignificant in predicting the response variable (Mac Nally, 2000). OPLS differs from the precursor model partial least squares (PLS) in separating the systematic variation in the independent variables X into one part which is linearly related to Y, and one part which is orthogonal to Y. Such separation increases the interpretability of the results compared to traditional PLS (Wiklund et al., 2008; Sørmo et al., 2011). For each OPLS model, a calculated R^2Y shows the dispersion of the data from the model, and Q^2 shows the cross-validation of the model. An $R^2Y>0,7$ and $Q^2>0,4$ characterize an acceptable, or good model for biological data (Lundstedt et al., 1998). OPLS modelling provides a coefficient plot which shows the relationships between the X variables and the Y variable (positive or negative), and a variable importance in projection (VIP) plot, which reflects the relative importance of each X variable in explaining the Y. Thus, OPLS modelling provides information about important and significant variables in the model. Predictor variables with VIP value >1 have the most explanatory power of Y (Sørmo et al., 2011). An ANOVA of the cross-validated residuals (CV-ANOVA) was conducted for each OPLS model to test the significance (Eriksson et al., 2008).

The data was scaled and centered before modelling, and skewed variables were log transformed. The OPLS models were step-wise optimized by removing X-variables with lowest VIP values, as these have low importance in explaining the Y (Sørmo et al., 2011). Each time an X-variable was removed and a new OPLS model was created, the explained variation in the X-matrix (R^2X), the goodness of fit (R^2Y) and the goodness of prediction (Q^2) was evaluated until achieving a significant model.

3 RESULTS

3.1 Biological variables

Mean body weight was significantly higher in male than in female glaucous gulls (18 %, $p<0.001$). There were no significant differences in body condition index (BCI) and lipid % between males and females (p = 0.867 and 0.735, respectively). Biometric measurements, BCI and lipid % are presented in Table 2 as mean ± standard deviation, median, minimum and maximum levels. Individual biometric measures can be found in Appendix B.

Table 2. Mean ± standard deviation (SD), median, minimum and maximum measures of biological variables of male (n=15) and female (n=24) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. Sex differences in mean values (p <0.001) are denoted with *.

	Males			Females		
	Mean ± SD	Median	Range	Mean ± SD	Median	Range
Body mass (g) *	1760 ± 109	1740	1535 – 2030	1442 ± 86	1437	1315 - 1700
Total head length (mm) *	151.2 ± 2.4	151.0	147.6 – 156.0	136.8 ± 2.5	136.0	133.6 – 141.5
Bill length (mm) *	64.8 ± 1.8	64.9	61.0 – 67.3	57.7 ± 1.8	57.7	55.0 – 63.1
Wing length (mm) *	482.7 ± 15.9	487.0	454.0 – 502.0	463.8 ± 10.1	461.5	441.0 – 485.0
Gonys height (mm) *	23.5 ± 0.9	23.3	22.2 – 26.0	21.0 ± 0.8	20.9	19.5 – 23.0
BCI	0 ± 1	-0,19	-1.77 – 2.26	0 ± 1	-0.73	-1.34 – 2.24
Lipid %	1.44 ± 0.16	1.46	1.17 – 1.69	1.47 ± 0.22	1.48	0.97 – 1.98

3.2 Contaminants

3.2.1 OHC levels

30 individual OHCs were sufficiently quantified and included in the results. The mean, standard deviation, median, minimum and maximum values are reported in Table 3. The OHC concentrations in each individual are presented in Appendix C.

Table 3. Mean ± standard deviation (SD), median, minimum and maximum concentrations (ng/g ww) of OHCs quantified in plasma from male (n=15) and female (n=24) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. The sums of OCPs PCBs, PBDEs, PFSAs, PFCAs and total PFASs are included, denoted with ∑. Sex differences in mean OHC levels are denoted with * (p<0.05) and **(p<0.01). *N* denotes the number of observations per variable. Congeners of PCBs and PBDEs are numbered by the IUPAC system (International Union of Pure and Applied Chemistry).

	Males				Females			
Compound	N	Mean ± SD	Median	Range	N	Mean ± SD	Median	Range
β-HCH**	15	0.56 ± 0.30	0.45	0.17 – 0.99	24	0.28 ± 0.17	0.24	0.08 – 0.69
HCB*	15	10.04 ± 3.78	9.93	4.36 – 15.90	24	7.49 ± 2.40	7.45	3.97 – 15.10
c-chlordane	15	0.02 ± 0.01	0.02	0.01 – 0.33	22	0.02 ± 0.01	0.02	0.01 – 0.08
Oxy-chlordane*	15	17.26 ± 12.56	11.40	4.74 – 41.90	24	8.68 ± 6.19	5.32	1.95 – 25.20
t-nonachlor	15	0.99 ± 0.72	0.87	0.07 – 2.67	24	1.15 ± 1.08	0.68	0.26 – 4.82
c-nonachlor*	15	0.66 ± 0.38	0.54	0.14 – 1.41	24	1.16 ± 0.91	0.99	0.34 – 4.74
mirex**	15	8.01 ± 5.44	5.41	2.79 – 19.40	24	3.59 ± 2.84	2.29	0.85 – 10.20
p,p'-DDE**	15	6.00 ± 3.52	4.77	2.13 – 11.90	24	2.81 ± 2.08	1.82	0.74 – 9.00
∑OCPs*	15	43.53 ± 25.24	33.14	17.91 – 89.96	24	25.12 ± 13.71	19.68	8.72 – 60.92
PCB-28**	15	0.24 ± 0.10	0.24	0.09 – 0.41	24	0.15 ± 0.07	0.14	0.05 – 0.33
PCB-52	12	0.16 ± 0.14	0.16	0.04 – 0.50	18	0.29 ± 0.23	0.24	0.03 – 0.75
PCB-99**	15	7.52 ± 4.29	5.80	2.40 – 13.90	24	3.49 ± 2.62	2.33	0.51 – 11.10
PCB-105*	15	2.98 ± 1.86	2.36	0.31 – 6.15	24	1.68 ± 1.11	1.19	0.35 – 4.56
PCB-118**	15	13.97 ± 8.35	10.60	4.14 – 30.2	24	6.51 ± 4.53	4.55	1.39 – 17.20
PCB-138**	15	31.71 ± 19.36	23.90	9.68 – 66.40	24	14.07 ± 9.41	9.34	2.87 – 37.10
PCB-153**	15	68.12 ± 47.92	45.80	20.10 – 162.00	24	28.51 ± 21.91	19.10	6.53 – 87.00
PCB-180*	15	32.21 ± 24.41	21.80	9.78 – 82,40	24	14.29 ± 13.81	8.31	2.63 – 52.80
PCB-183**	15	4.60 ± 3.03	3.49	1.41 – 9.95	24	2.05 ± 1.68	1.28	0.44 – 6.59
PCB-187**	15	5.41 ± 2.59	4.62	2.24 – 9.35	24	2.62 ± 1.99	1.93	0.82 – 9.72
PCB-194*	15	4.40 ± 3.42	2.90	1.17 – 12.20	24	1.99 ± 2.04	1.13	0.32 – 7.57
∑PCBs**	15	171.30 ± 113.36	122.35	52.48 – 378.27	24	75.57 ± 56.11	47.79	16.61 – 219.55
PBDE-47	15	2.50 ± 1.29	1.97	1.26 – 4.47	24	1.90 ± 1.56	1.33	0.47 – 6.68
∑PBDEs	15	2.50 ± 1.29	1.97	1.26 – 4.47	24	1.90 ± 1.56	1.33	0.47 – 6.68
PFHxS	14	0.80 ± 0.52	0.58	0.20 – 1.89	20	2.34 ± 4.40	0.63	0.15 – 18.72
PFOS	15	14.26 ± 8.01	12.23	5.99 – 33.34	24	47.22 ± 110.60	10.09	2.56 – 507.66
PFOSA	13	1.03 ± 0.89	0.78	0.35 – 3.66	19	0.74 ± 0.33	0.72	0.33 – 1.81
∑PFSAs	15	15.87 ± 8.90	12.71	6.62 – 35.77	24	49.69 ± 114.64	11.16	2.78 – 526.95
PFOA**	9	0.13 ± 0.09	0.12	0.03 – 0.34	19	0.30 ± 0.17	0.27	0.09 – 0.75
PFNA	15	2.67 ± 1.68	2.23	0.95 – 6,25	24	2.27 ± 1.04	2.20	0.30 – 4.42
PFDcA	15	1.06 ± 0.59	0.83	0.44 – 2.56	24	0.86 ± 0.43	0.72	0.31 – 2.09
PFUnA	15	4.42 ± 1.59	3.81	2.47 – 7.76	24	3.76 ± 2.09	3.27	1.28 – 10.60
PFDoA*	15	1.16 ± 0.37	1.05	0.65 – 1.89	24	0.80 ± 0.48	0.74	0.21 – 2.49
PFTrA**	15	3.96 ± 1.31	3.68	2.52 – 7.73	24	2.69 ± 1.23	2.48	0.98 – 5.88
PFTeA**	15	0.57 ± 0.26	0.52	0.35 – 1.36	18	0.35 ± 0.12	0.29	0.16 – 0.62
∑PFCAs	15	13.91 ± 5.00	11.50	8.50 – 23.20	24	10.90 ± 5.09	9.93	3.28 – 26.04
∑PFASs	15	29.79 ±12.68	22.86	17.58 – 58.97	24	60.58 ±113.86	25.49	6.87 – 533.22

3.2.2 OHC patterns

Differences between male and female glaucous gulls were evident for both contaminant levels and patterns. Among the OHCs where sex differences in levels were apparent, PFOA was the only contaminant in which females had significantly higher concentrations than males (p= 0.02). The differences in contaminant group levels among the sexes are depicted in Figure 2.

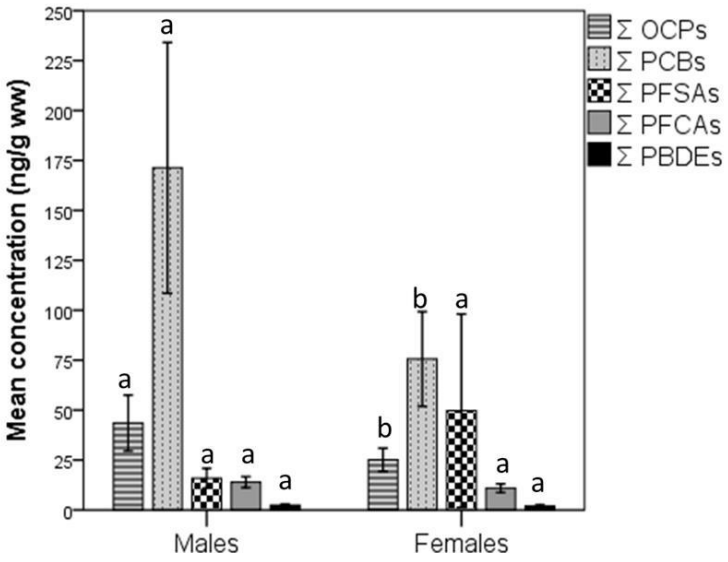

Figure 2. Differences in mean levels of contaminant groups between male (n=15) and female (n=24) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. The error bars represent the 95 % confidence interval. Different letters between the sexes indicates significant differences in the mean contaminant group levels. ∑OCPs denotes β-HCH, HCB, *c*-chlordane, *c*-nonachlor, *t*-nonachlor, *oxy*-chlordane, mirex and *p,p'*-DDE. ∑PCBs denotes the PCB congeners 28, 52, 99, 105, 118, 138, 153, 180, 183, 187 and 194. ∑PFSAs denotes PFHxS, PFOS and PFOSA. ∑PFCAs denotes PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTrA and PFTeA. ∑PBDE denotes PBDE-47.

The patterns of contaminant group contribution to the total OHC burden is presented in Figure 3A for males, and 3B for females.

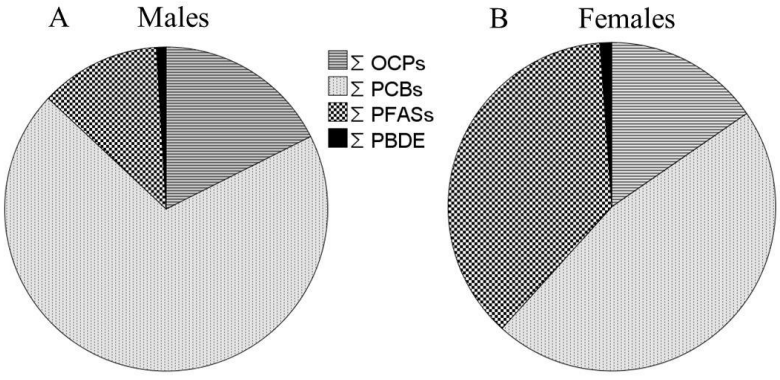

Figure 3. Contribution of the contaminant groups to the total OHC burden in male (A) and female (B) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. ∑OCPs denotes β-HCH, HCB, *c*-chlordane, *c*-nonachlor, *t*-nonachlor, *oxy*-chlordane, mirex and *p,p'*-DDE. ∑PCBs denotes the PCB congeners 28, 52, 99, 105, 118, 138, 153, 180, 183, 187 and 194. ∑PFASs denotes PfHxS, PFOS, PFOSA, PFOA PFNA, PFDcA, PFUnA, PFDoA, PFTrA, and PFTeA. ∑PBDE denotes PBDE-47.

The contaminant burden in male glaucous gulls was as follows: ∑PCBs (69.35%) > ∑OCPs (17.62 %) > ∑PFASs (12.06 %) > ∑PBDE (0.97 %).

For female glaucous gulls, the contaminant burden was as follows: ∑PCBs (46.31 %) > ∑PFASs (37.13 %) > ∑OCPs (15.40 %) > ∑PBDE (1.16 %).

For both sexes, PCBs were the dominant contaminant group. The most abundant PCB was PCB-153 (39.77 % and 37.72 % of ∑PCBs in males and females, respectively). The second and third most contributing PCB for males and females were PCB-180 (18.80 % and 18.91 %) and PCB-138 (18.51 % and 18.62 %), respectively. The dioxin-like PCB-118 was the fourth most abundant PCB in both sexes (approximately 8 % of total PCB fraction).

PFASs were the second major contaminant group in female glaucous gulls, and the third major group in males (Figure 3). The mean levels of PFASs were 49 % higher in females than in males. However this was not a significant difference, as the PFSA levels in female glaucous gulls were skewed due to great dispersion of the individual PFOS levels. Thus, comparing the median levels would be more appropriate in comparison of PFASs between sexes (Table 3). Thus, there was a considerable larger burden of mean PFSA levels in females, however not significant. Of the PFASs, PFOS was absolutely dominating in both sexes (47.88 % in males and 78.14 % in females). The PFSAs contributed significantly more than the PFCAs ($p<0.05$) for both males and females (6.43 % PFSAs and 5.63 % PFCAs in males versus 30.45 % PFSAs and 6.68 % PFCAs in females).

The OCPs were the second most contributing contaminant group in males, and the third in female glaucous gulls. In both sexes, *oxy*-chlordane was the major pesticide (39.66 % in males and 34.54 % in females), followed by HCB (23.06 % in males and 29.61 % in females), mirex (18.40 % in males and 14.29 % in females) and *p,p'*-DDE (13.78 % in males and 11.17 % in females).

P,p'-DDE was the only DDT-metabolite detected in the glaucous gull plasma, and the only PBDE detected was PBDE-47. The levels of PBDE-47 contributed to less than 1.5 % of the total contaminant burden in both male and female gulls.

Based on the substantial variance in OHC levels among the sexes, results on relationships between levels of OHC and THs are presented separately for male and female glaucous gulls.

3.3 Thyroid hormones

Average %CV between replicates were calculated for each TH kit. Average %CV was 2.88 % for TT3, 5.05 % for FT3, 5.54 % for TT4 and 6.17 % for FT4. For FT3, two values had %CV exceeding 15 % (17.75 % -19.79 %). One reason for this may be that FT3 analysis was only conducted in duplicates. Furthermore, due to the low concentration range of FT3, small variations in the readings may lead to a large %CV. However the concentrations in these two individuals did not differ significantly from the pool of samples. For FT4, the %CV value exceeded 15 % in only one sample (17.79 %). This individual had considerably higher FT4 levels than the other samples. When obtaining the same high values in a second run, the individual was however still included in statistical analysis, representing individual variations in TH levels. The %CV values for intra-assay precision test for the reference material were <7.56 % for FT3, <7.21 % for FT4, <9.96 % for TT4 and <8.09 % for TT3.

There were no significant differences in thyroid hormone levels between female and male glaucous gulls (p=0.594, 0.079, 0.654 and 0.619 for TT3, TT4, FT3 and FT4, respectively). The mean ± standard deviation, median, minimum and maximum TH plasma levels are presented in Table 4. Individual TH concentrations are found in Appendix D.

Table 4. Mean concentrations of total and free T3 (TT3 and FT3) and T4 (TT4 and FT4), standard deviation (SD), median, minimum and maximum measured in plasma of male (n=15) and female (n=24) glaucous gull (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013.

	Males			Females		
	Mean ± SD	Median	Range	Mean ± SD	Median	Range
TT3 (nmol/L)	2.21 ± 1.02	1.95	1.72 – 4.38	2.38 ± 0.97	2.33	0.82 – 4.59
TT4 (nmol/L)	24.52 ± 11.53	22.18	13.16 – 43.71	30.69 ± 9.59	29.36	10.62 – 50.90
FT3 (pmol/L)	3.23 ± 1.83	3.45	0.80 – 6.12	2.97 ± 1.61	2.98	0.55 – 6.57
FT4 (pmol/L)	30.32 ± 41.35	14.83	4.94 – 133.15	22.64 ± 9.37	20.62	6.26 – 41.47

3.4 Associations between THs and OHCs

3.4.1 Principal component analysis

Principal component analysis (PCA) was performed for initial visualization of possible relationships between all THs, OHCs and biological variables. The PCA including both male and female glaucous gulls (R^2X=0.649, Q^2=0.496) resulted in 3 principal components with eigenvalues > 1, explaining 40.61 %, 16.45 % and 7.82 %, respectively, of the variation in the individuals. The score plot (Figure 4) reveal a clear difference in grouping between males and females. Based on this indication, in combination with the finding that the major contaminant groups had significant different levels between the sexes, PCA was performed separately for males and females. This approach was applied to avoid masking results or eliminate potential confounding factors related to sex differences.

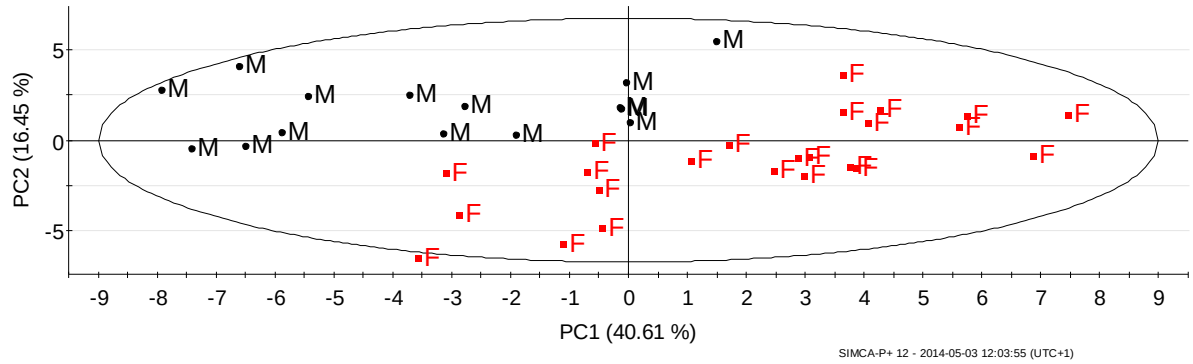

Figure 4. Principal component analysis biplot scores based on OHC concentrations, TH concentrations, biological measurements and lipid % in plasma from glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. Males (n=15) and females (n=24) are assigned "M" and "F", respectively, and marked with different colors.

The PCA including only male glaucous gulls (R^2X=0.634, Q^2=0.399) resulted in two significant PCs where PC1 explained 46.36 % and PC2 explained 17.07 % of the variation (Figure 5). The principal components with highest explanatory power (PC1 and PC2) were plotted against each other in a loading plot.

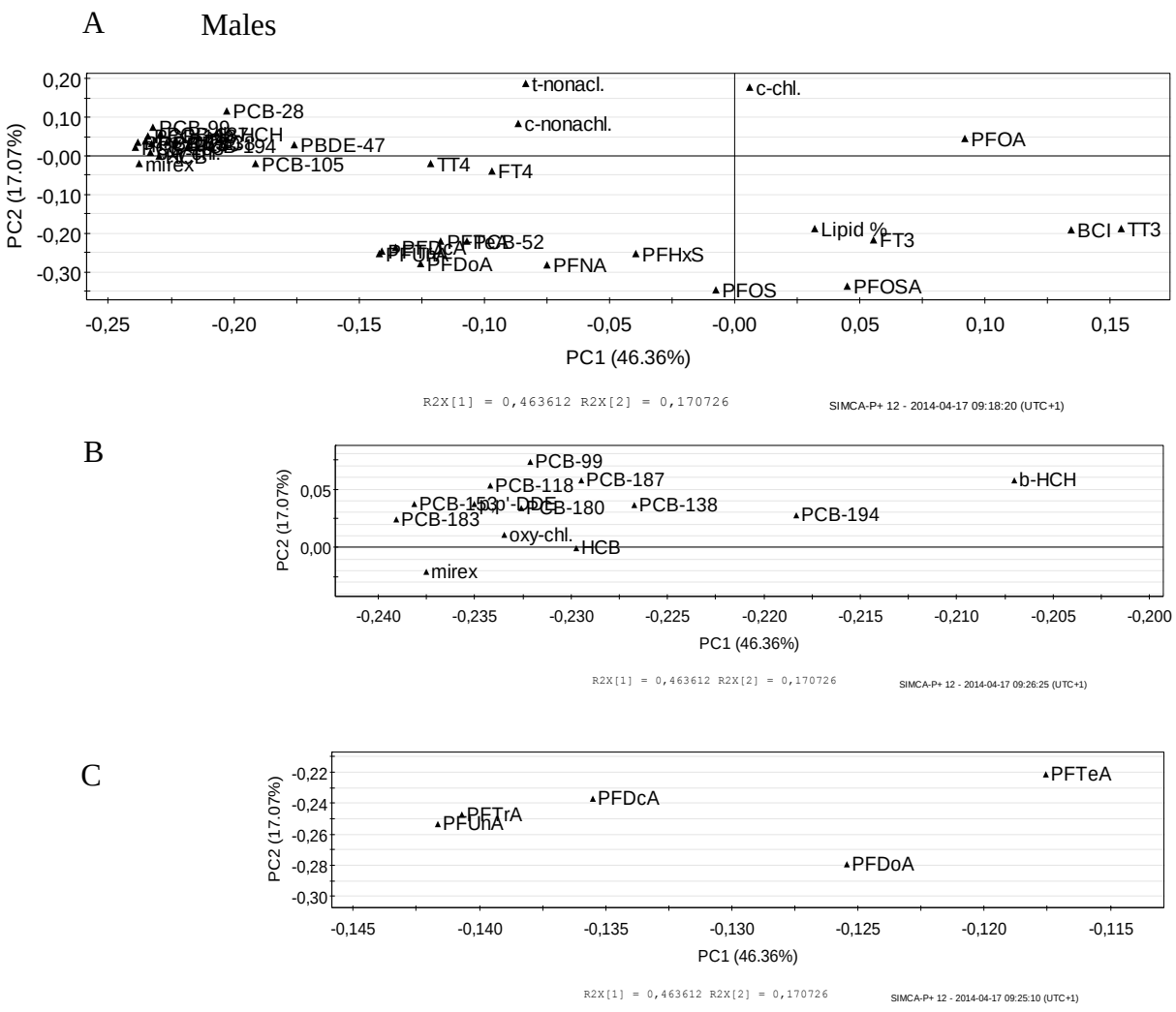

Figure 5. Principal component analysis loading plot with (A) all TH variables (TT3, FT3, TT4, FT4), all OHCs (explained in the text), body condition index (BCI) and plasma lipid % in male glaucous gulls (*Larus hyperboreus*) (n=15) breeding in Kongsfjorden, Svalbard, during the summer of 2011, 2012 and 2013. *B* shows an enlargement of the OHCs clustering at PC1~ -0.23. *C* shows an enlargement of the OHCs clustering at PC1~ -0.13.

The PCA indicated relationships among the THs in males (Figure 5A). TT4 and FT4 were located closed to each other along the PC1 axis and this strong positive correlation was confirmed by correlation analysis (p<0.001, r_p=0.836). FT3 and TT3 were located on the opposite side of PC2, also positively correlated to each other (p=0.031, r_p=0.556). Furthermore, all the OCs except for *c*-nonachlor, *t*-nonachlor and *c*-chlordane grouped along the PC1 and were strongly positively correlated (p<0.05, r_p>0.944), as shown in Figure 5B. These displayed a separation from the PFCAs PFDcA, PFDoA, PFUnA, PFTrA and PFTeA (Figure 5C), which were positively correlated to each other (p <0.05).

The plot furthermore indicated a positive relationship between BCI and TT3 in males. This was confirmed in correlation analysis (p=0.002, r_p=0.727). Furthermore, inverse associations were indicated between both TT3 and the OCs, and BCI and the OCs. Correlations between TT3 and the OCs are summarized in Table 5. Correlation analysis also confirmed that BCI correlated negatively with PCB-28, 99, 118, 138, 153, 180, 183, 187, β-HCH and *p,p'*-DDE (p<0.05, r_p> -0,505).

TT3 was the only TH where clear relationships to explanatory variables were indicated in the PCA. Lipid %, FT3, FT4, TT4, *c*-nonachlor, *c*-chlordane and *t*-nonachlor were positioned close to origin and thus, no correlations with these variables were indicated by the PCA.

A PCA performed for female glaucous gulls (R^2X=0.594, Q^2=0.456) resulted in two significant components explaining 45.62 % and 13.79 % of the variation among the individuals, respectively (Figure 6).

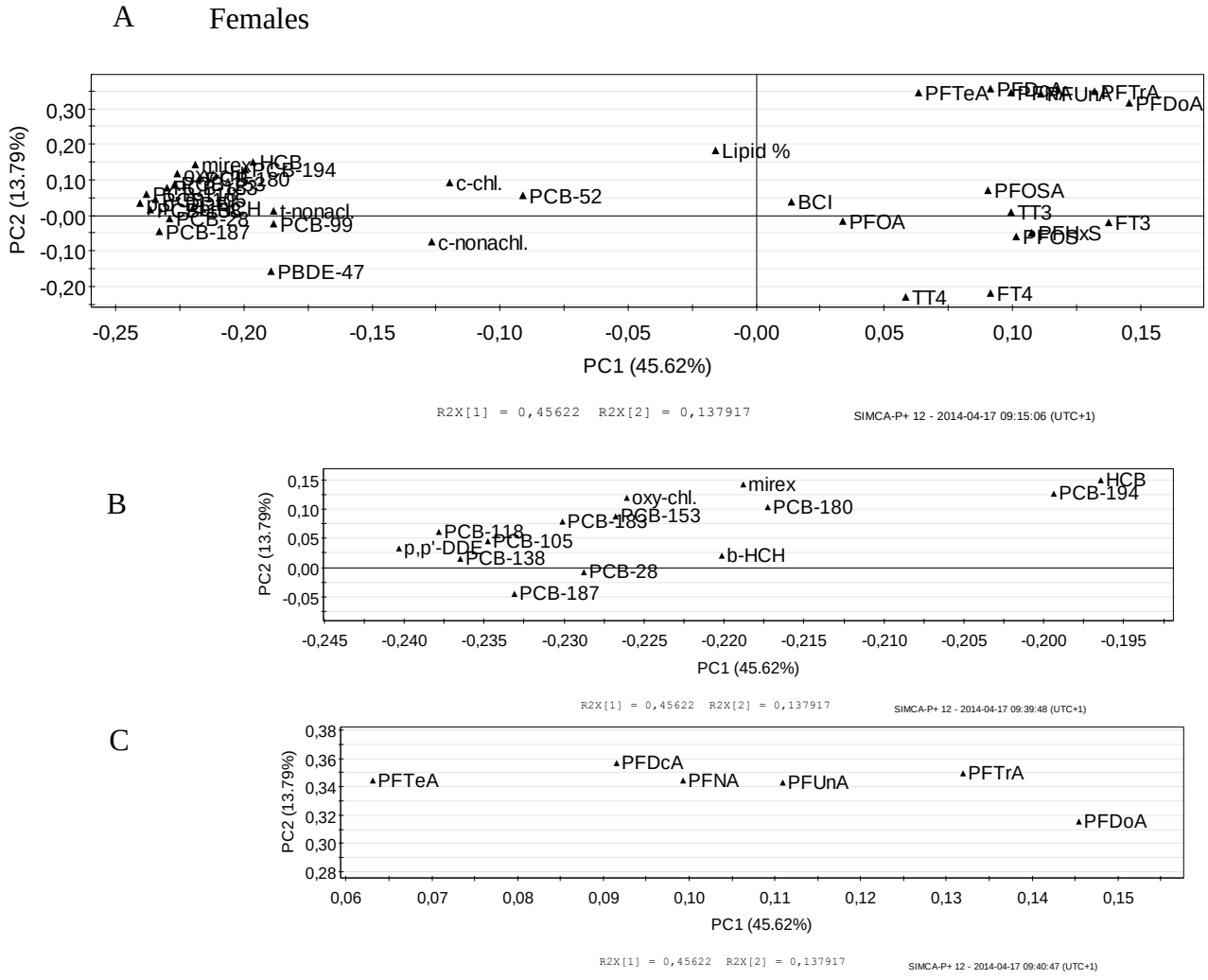

Figure 6. Principal component analysis loading plot with (*A*) TH variables (TT3, FT3, TT4, FT4), all OHCs (explained in the text), body condition index (BCI and plasma lipid % in female glaucous gulls (*Larus hyperboreus*) (n=24) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. *B* shows an enlargement of the cluster of OHCs grouping at PC1~ -0.22. *C* shows the cluster of OHCs grouping at PC2~ 0.35.

As the patterns shown in the PCA conducted for male glaucous gulls, the PCA for females indicated a similar grouping of most of the OCs along the PC1, with exceptions of *c*-nonachlor, *t*-nonachlor, PCB-52, PCB-194, and *c*-chlordane (Figure 6B). These displayed, relative to the males, a more distinct separation from the PFCAs PFTra, PFTeA, PFDoA, PFUnA, PFNA and PFDcA, which clustered together on the opposite site of PC1 (Figure 6C). This indication of negative relationship between PFCAs and OCs was confirmed by correlation analysis. Furthermore, PBDE-47 also showed a strong inverse correlation with the PFCAs (p=<0.001, r_p= -0.686).

FT3 and the cluster of OCs were positioned on the opposite sides of PC1, displaying a clear negative relationship between these (Figure 6A), confirmed by correlation analysis (Table 5). The PCA also indicated a negative relationship between FT4 and the OCs grouped on the negative side of PC1, also supported by correlation tests (Table 5).

FT4 and FT3 were the two THs in which effects from explanatory variables could be indicated by the PCA for female glaucous gulls. Even though TT3 and FT3 were positively correlated (p<0.001, r_p=0.918), and FT4 and TT4 are positively correlated (p<0.001, r_p=0.857), only relationships between OHCs and FT4 or FT3 were confirmed by correlation tests (Table 5). The variables located close to origo in the PCA loading plot were not well explained in the model. This was the case for BCI, lipid %, *c*-nonachlor, *c*-chlordane, PFOS, PFHxS and PFOA. However, a significant relationship was found between FT4 and lipid % (p = 0.015, r_p = -0.492).

Table 5: Significant associations between THs and OHCs in female (F, n=24) and male (M, n=15) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013, as tested by pearson correlations. Significance levels (p) and correlation coefficient (r) are included. Borderline significance are marked in italic when $0.05 < p \leq 0.07$.

	FT3 (**F**)		FT4 (**F**)		TT3 (**F**)		TT3 (**M**)	
Compound	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*
β-HCH	0.021	-0.468	*0.067*	*-0.388*	-	-	0.014	-0.620
HCB	*0.070*	*-0.377*	0.004	-0.574	-	-	-	-
oxy-chlordane	0.016	-0.487	0.020	-0.480	-	-	-	-
t-nonachlor	-	-	0.053	-0.408	-	-	-	-
mirex	0.013	-0.500	0.009	-0.531	-	-	0.042	-0.530
p,p'-DDE	0.014	-0.495	0.023	-0.471	-	-	0.012	-0.630
PCB-28	0.046	-0.411	*0.052*	*-0.409*	-	-	0.016	-0.607
PCB-99	-	-	-	-	-	-	0.015	-0.614
PCB-105	0.034	-0.435	0.038	-0.435	-	-	-	-
PCB-118	0.018	-0.479	0.027	-0.461	-	-	0.021	-0.587
PCB-138	0.022	-0.464	0.032	-0.447	-	-	0.014	-0.619
PCB-153	0.023	-0.461	0.025	-0.465	-	-	0.022	-0.584
PCB-180	0.028	-0.450	0.044	-0.414	-	-	0.026	-0.570
PCB-183	0.020	-0.472	0.029	-0.454	-	-	0.020	-0.591
PCB-187	0.012	-0.504	*0.064*	*-0.384*	-	-	0.005	-0.684
PCB-194	0.045	-0.413	0.037	-0.438	-	-	*0.054*	*-0.506*
PFOS	0.019	0.476	-	-	0.008	0.525	-	-

3.4.2 OPLS

OPLS regression was performed with different THs as Y variables and all OHCs, BCI, year of capture and lipid % as X variables. No significant models were obtained when including both sexes in the models, thus separate OPLS models for male and female glaucous gulls were created. After stepwise removing X variables of low importance in the model (low VIP), significant OPLS-models could be obtained for TT3 in male and for FT4 in female glaucous gulls. The Y=TT3 (males) model resulted in a CV-ANOVA; p=0.042, R^2X=0.789, R^2Y=0.745 and Q^2=0.598. Hence, R^2X and Q^2 were above the values that define an acceptable or good model for biological data (Lundstedt et al., 1998). The information from the variable importance plot (VIP-plot) should be supplemented with the information from the coefficient plot to determine the variables having the greatest explanatory power of TT3, and whether these are contributing in a positive or negative manner on TT3 levels. The VIP plot (Y=TT3, males) estimated the following variables of having the greatest absolute importance in describing the variations of TT3 levels in male glaucous gulls (in descending order): BCI > PCB-187 > *p,p'*-DDE > β-HCH > PBDE-47 > PCB-138 > PCB-99 > PCB-183 > PCB-153 > *oxy*-chlordane > *c*-nonachlor > PCB-180 > PCB-28 > PFOSA > *t*-nonachlor > HCB > PCB-105 (Figure 7). The variables with VIP-value > 1 were considered most important for the variation of TT3 in males. This applied for BCI, PCB-187, *p,p'*-DDE, β-HCH, PBDE-47, PCB-138, PCB-99, PCB-183 and PCB-153. None of the explanatory variables in the VIP-model had error bars crossing the 0-lines, which confirms importance of these variables in predicting Y (TT3, males).

The coefficient plot showed the magnitude and direction of the relationships between the X variables and TT3 in male glaucous gulls (Figure 8). The variables with greatest reliability were characterized by the error bars not crossing the 0-line in the plot. This was the case for BCI and PFOSA (positive relationship with Y) and PCB-187, PBDE-47, *t*-nonachlor and *c*-nonachlor (negative relationship with Y).

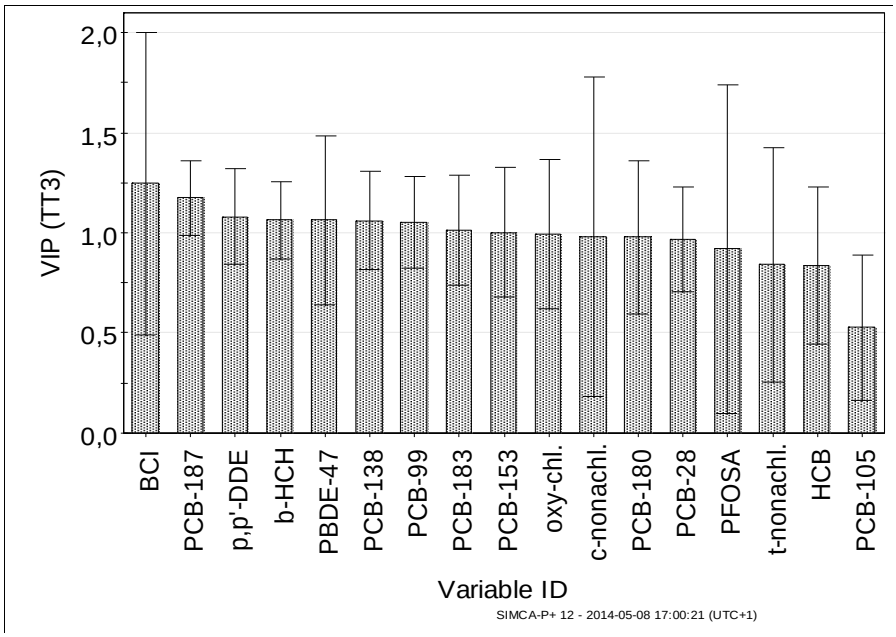

Figure 7. OPLS VIP plot organizing the X-variables according to their explanatory power of TT3 levels in male glacous gulls (*Larus hyperboreus*) (n=15) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. Variables with VIP-values over 1.0 are considered most important in explaining TT3.

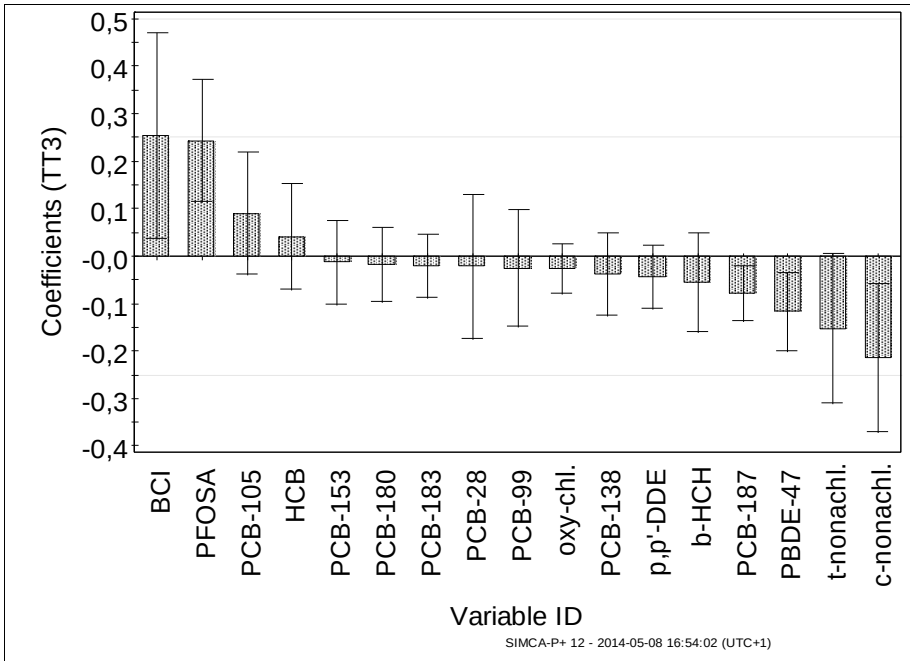

Figure 8. OPLS coefficient plot describing the direction of relationships between X-variables and TT3 levels in male glaucous gulls (*Larus hyperboreus*) (n=15) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. The error bars represent the 95% confidence interval.

The PCA (Figure 5) indicated that BCI was positively correlated with TT3 and negatively correlated with OCs in male glaucous gulls. The results from the OPLS-regression (Y=TT3, males) indicated that BCI was the variable influencing TT3 in males the most (Figure 7). A second OPLS model with BCI as Y variable was created to assess how OHCs affect the condition of these male glaucous gulls. The (Y=BCI, males) OPLS regression resulted in a significant model (p=0.049) and can be found in Appendix E. A significant OPLS model with BCI as Y in female glaucous gulls could not be obtained.

In order to assess how the contaminants affect TT3 in males without the effect of BCI, partial correlation (2-tailed) was performed when controlling for BCI. However, due to the correlations among the OHCs included in the OPLS model, multicolinearity may confound the outcome from a partial correlation analysis including all the individual compounds. Based on a PCA comprising all the OHCs included in the (Y=TT3, males) OPLS model, three clusterings were revealed. *P,p'*-DDE, β-HCH, HCB, PBDE-47, *oxy*-chlordane, PCB-28, -99, -105, -138, -153, -180, -183 and -187 grouped together at around PC1=0.9. *T*-nonachlor and *c*-nonachlor grouped separated from these, and PFOSA had a distinct separation from the two other clusters. PC1 scores from the two groups of intracorrelated contaminants, and the original PFOSA, were used further to assess the effect of these on TT3 levels in males when controlling for BCI. In this way the multicolinearity problem could be avoided. Significance levels dropped considerably, resulting in no significant relationships between any of the contaminant groups from the OPLS regression and TT3 in males when controlling for the effect of BCI (Table 6). The PC1 (OCPs + PBDE) and PFOSA had the greatest effect of the partial correlation analysis to TT3. The PC1 (nonachlores) retained a borderline significant negative relationship to TT3 in males after removal of the effect of BCI, indicating that these were the most important contaminants explaining the variation in TT3 in males. The initial pearson correlations between TT3 and the three groups of contaminants and results from the partial correlations after controlling for BCI are summarized in Table 6.

Table 6. Pearson correlations and partial correlations after controlling for BCI between TT3 levels and OHCs in male (n=15) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. Included are the significance levels (*p*) and correlation coefficient (*r)*. Only OHCs included in the OPLS-model and significantly correlated ($p<0.05$) with TT3 from pearson correlation analysis are included.

	Pearson correlation		Partial correlation	
Compounds	*p*	*r*	*p*	*r*
PC1 (OCPs + PBDE)[a]	0.012	-0.627	0.145	-0.410
PC1 (nonachlores)[b]	0.028	-0.566	0.051	-0.530
PFOSA	0.040	0.535	0.557	0.172

[a] PC1 (OCPs + PBDE) include *p,p'*-DDE, β-HCH, HCB, PBDE-47, *oxy*-chlordane, and the PCB congeners (PCB)-28, 99, 105, 138, 153, 180, 183 and 187.
[b] PC1 (nonachlores) include *c*-nonachlor and *t*-nonachlor.

The Y=FT4 (females) OPLS model resulted in a CV-ANOVA; $p=0.022$, $R^2X=0.781$, $R^2Y=0.621$ and $Q^2=0.437$, and was thus considered an acceptable model (Lundstedt et al., 1998). The following X-variables was predicted to be of greatest importance for FT4 levels (in descending order): HCB > mirex > lipid% > *p,p'*-DDE > PCB-118 > PCB-138 > *oxy*-chlordane > PCB-153 > PCB-183 > *t*-nonachlor > PCB-28 > PCB-180 > PCB-194 > PCB-99 > PFNA > PFOA (Figure 9). The following variables had VIP-values > 1.0 and thus greatest predictive force on FT4: HCB, mirex, lipid%, *p,p'*-DDE, PCB-118, PCB-138 and *oxy*-chlordane. The coefficient plot from the model (Y=FT4 females) indicated that all the variables included in the model had a negative effect on FT4 levels in females (Figure 10). PFNA, lipid%, PFOA and mirex were the four variables of greatest reliability in explaining FT4 levels in the model, as these had error bars not crossing the 0-line (Figure 10).

BCI did not influence neither THs nor OHC levels in females, indicated by the PCA (Figure 6A). Possible confounding effects of lipid% on the relationships between THs and OHCs in females were investigated by correlation analysis (pearson and partial). However, lipid% was not shown to influence or confound these associations.

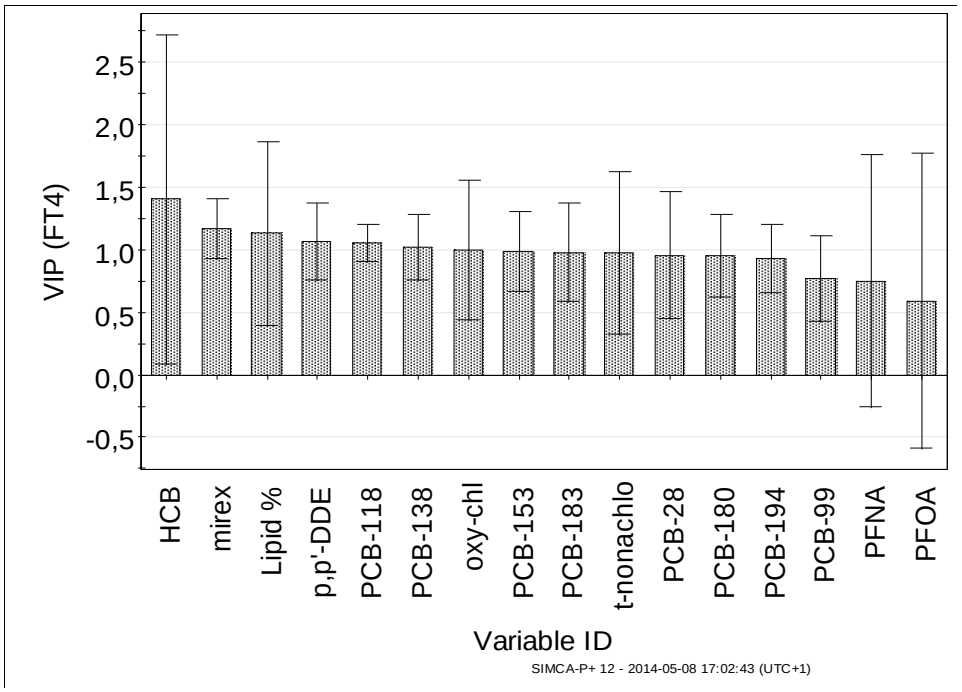

Figure 9. OPLS VIP plot organizing the X-variables according to their explanatory power of FT4 levels in female glacous gulls (*Larus hyperboreus*) (n=24) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. Variables with VIP-values over 1.0 are considered most important in explaining FT4. The error bars represent the 95 % confidence interval.

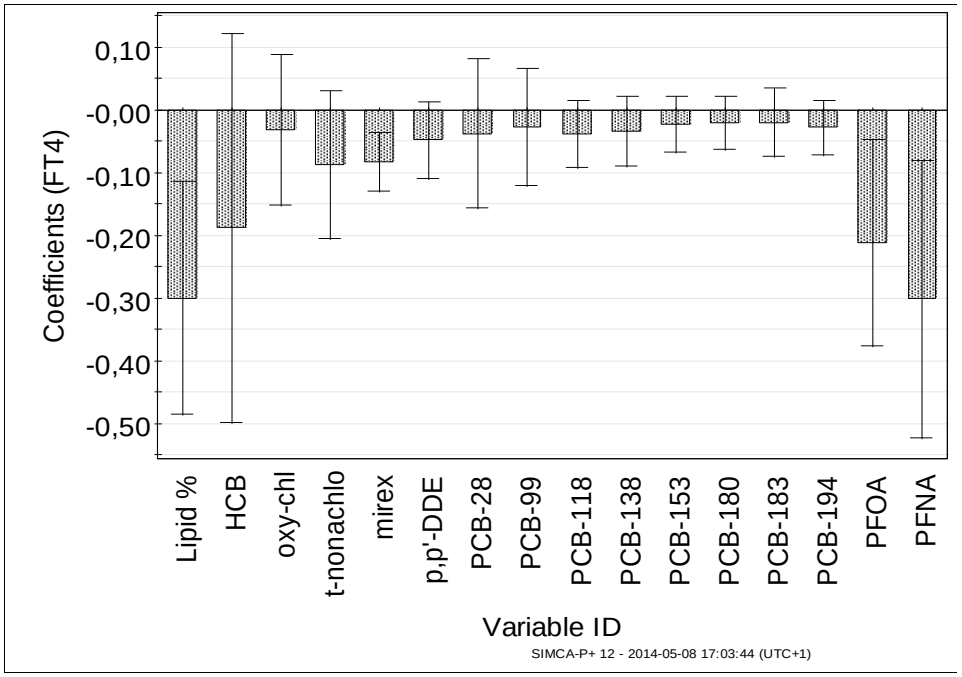

Figure 10. OPLS coefficient plot describing the direction of relationships between X-variables and FT4 levels in female glaucous gulls (*Larus hyperboreus*) (n=24) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. The error bars represent the 95% confidence interval.

Weaker, non-significant OPLS models could be obtained for FT3 and TT4, these were accordingly not included in the results.

3.4.3 Mixture correlation analysis

A central aim of the present study was to evaluate combined effects of mixtures of OHCs on THs in glaucous gulls. Thus, all the OHCs included in the significant Y= FT4 OPLS model in females were summed and plotted against FT4 to demonstrate the overall effect of this mixture of OHCs predicted to mostly affect the FT4 levels in female glaucous gulls (Figure 11). Since a significant OPLS model with FT3 as Y could not be obtained, the OHCs significantly correlated with FT3 in females (Table 5) were summed and plotted against FT3 in Figure 12. Figure 12A shows the relationship between the sums of OHCs only negatively correlated with FT3 (p = 0.017, r_p = -0.481). Figure 12B shows the relationship between FT3 and PFOS; the only OHC positively correlated to FT3 in females (p =0.019, r_p =0.476). Figure 12C shows the combined effects of the plots in *A* and *B*; that is the relationship between FT3 and the sum of all contaminants, both positively and negatively correlated with FT3 (from Table 5), in female glaucous gulls (p =0.567, r_p = -0.123).

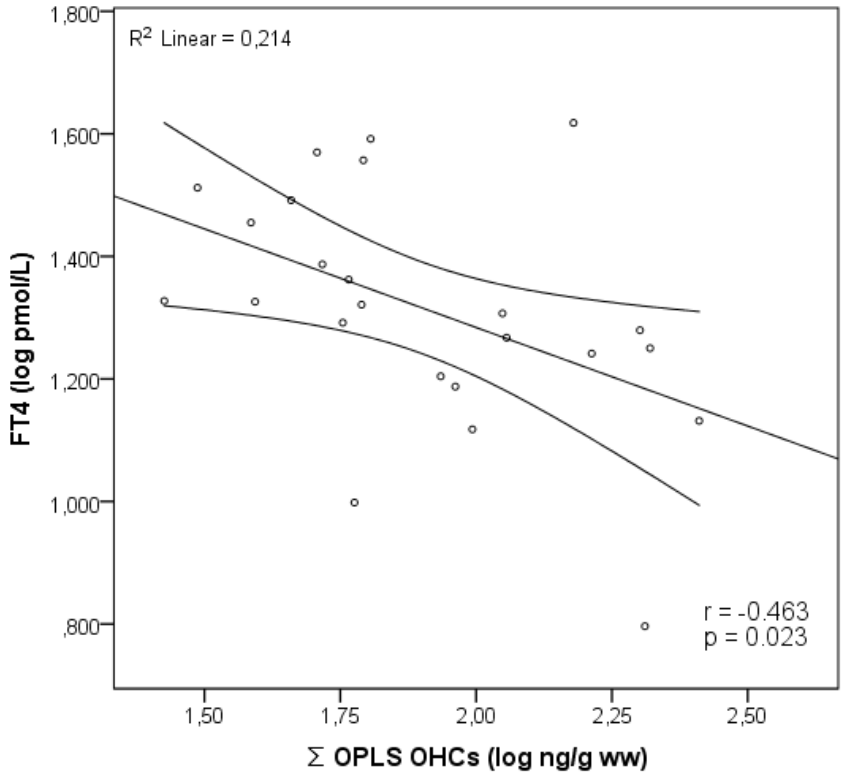

Figure 11. The linear relationships (± 95% confidence interval) between FT4 levels and the summed concentration of plasma OHCs included in the Y=FT4 OPLS model in female glaucous gulls (*Larus hyperboreus*) (n=24) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. The R^2, pearson correlation coefficient (r) and significance level (p) are shown in the plot. ∑ OPLS OHCs denotes HCB, *oxy*-chlordane, *t*-nonachlor, mirex, *p,p'*-DDE, PFOA, PFNA and PCB-28, -99, -118, -138, - 153, -180, -183 and -194.

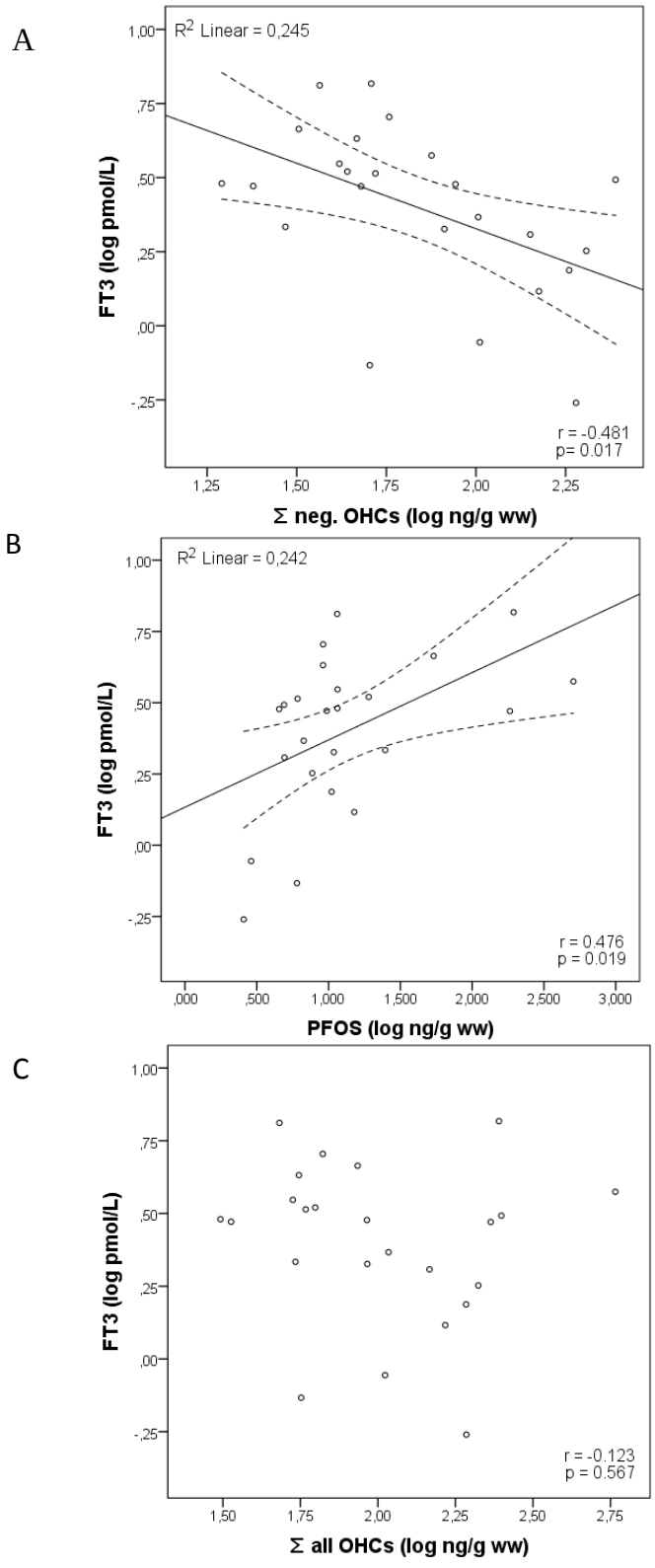

Figure 12. The linear relationships (± 95% confidence interval) between FT3 levels and (*A*) the concentration sum of plasma OHCs negatively correlated with FT3, (*B*) PFOS and (*C*) the concentration sum of all OHCs correlating with FT3 (both positively and negatively) in female glaucous gulls (*Larus hyperboreus*) (n=24) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. The R^2, pearson correlation coefficient (*r*) and significance level (*p*) are shown in the plots. ∑ neg. OHCs denotes β-HCH, HCB, *oxy*-chlordane, mirex, *p,p'*-DDE and PCB-28, -105, -118, -138, -153, -180, -183, -187 and -194. ∑ all OHCs denotes β-HCH, HCB, *oxy*-chlordane, mirex, *p,p'*-DDE and PCB-28, -105, -118, -138, -153, -180, -183, -187, -194 and PFOS.

4 DISCUSSION

4.1 Contaminants: prevalence and levels

The present study reports levels of organohalogens in glaucous gull plasma, and aimed to assess effects of complex mixtures of these on TH levels in this arctic top predator. PCBs and OCPs were the predominating contaminant groups, where PCB-153 and *oxy*-chlordane, respectively, were the compounds found in highest concentrations. The pattern of general high levels of PCBs and OCPs presented in this study is similar to what is previously reported in arctic seabird species (Letcher et al., 2010). PFOS was consistently the most prominent PFAS reported in the present study, which is in line with other findings in arctic avian wildlife (Gabrielsen et al., 2005; Verreault et al., 2005b; Nøst et al., 2012). Concentrations of PFASs in male glaucous gulls from the Kongsfjorden area and demonstration of combined effects of OHCs differently affecting TH levels in glaucous gulls are presented for the first time.

The OHC concentrations presented in this study are somewhat lower than what is reported in glaucous gulls from Bjørnøya (Verreault et al., 2004; Verreault et al., 2005b; Verreault et al., 2007); the pattern being most evident for legacy OCs. The present PFAS levels measured are in accordance with concentrations measured in a master project about female glaucous gulls from the same research area of Kongsfjorden in 2010 (Haugerud, 2011). PCBs were the predominant contaminant group detected in this study, reflecting the apical position of glaucous gulls when comparing with the lower PCB levels in other arctic seabird species (Wold et al., 2011).

There are often high correlations among persistent long transported contaminants (Jones and De Voogt, 1999). Thus, glaucous gulls with high levels of PCBs generally also have high levels of other persistent compounds (Bustnes et al., 2001a; Bustnes et al., 2001b). PCB-153 is thought to be one of the most persistent PCBs (Norstrom et al., 1988; Wolkers et al., 2004), which is in line with this congener being the most predominant PCB found in this and others studies on seabirds and mammals in the Arctic (Bustnes et al., 2004; Verreault et al., 2004; Bytingsvik et al., 2012a; Nøst et al., 2012). The OCs and PBDEs have physicochemical properties differing from the PFASs, visualized by different positioning in the PCA plots (Figure 5 and 6). The high protein affinity of PFASs results in accumulation of these in blood and liver, in contrast to the lipophilic chlorinated and brominated compounds partitioning in lipid rich compartments (Bossi et al., 2005; Butt et al., 2007).

Several of the major OCPs detected in glaucous gull plasma in the present study have extensively been identified in previous studies of glaucous gulls. The high levels of especially *oxy*-chlordane and HCB in this study is in accordance with previous findings in this seabird (Bustnes et al., 2003b; Bustnes et al., 2004; Verreault et al., 2005c). The use of the insecticide DDT was banned in most industrialized countries in the 1970s (Pacyna et al., 2003). The metabolite *p,p'*-DDE was the only DDT related compound detected in the present study. This finding was not unexpected, and is in accordance with other studies reporting this metabolite to be the major DDT compound detected in the Arctic (Gabrielsen et al., 1995; Verreault et al., 2004; Letcher et al., 2010; Bustnes et al., 2012). These findings reflect the great persistence of *p,p'*-DDE in animals (Hayes, 1982).

PBDE-47 was the only PBDE sufficiently detected in the present study. PBDE-47 being the major PBDE congener is in line with other studies in glaucous gulls (Herzke et al., 2003; Verreault et al., 2005a; Haugerud, 2011), polar bears (*Ursus maritimus*) (Verreault et al., 2005a; Sørmo et al., 2006) and black guillemot (*Cepphus grylle*) (Haukås et al., 2007). The mean concentrations of PBDE-47 were however 3-fold lower in males and 5-fold lower in females than the levels measured in glaucous gulls on Bjørnøya by Verreault et al. (2005b). Nevertheless, the consistent abundance of PBDE-47 suggests physicochemical factors favoring accumulation and exposure of this tetra-BDE compared to penta- and hexa-BDEs.

Toxicological implications of PBDEs and PCBs have widely been concerned endocrine disrupting properties, especially encompassing thyroid hormones. However, it is possible that observed effects of these contaminant groups are caused by break-down products rather than the parent product. Hydroxylated and methoxylated PBDEs (OH-PBDEs and MeOH-PBDEs, respectively) and PCBs (OH-PCBs and OH-PCBs) have been reported in several wildlife species (Letcher et al., 2000; Hakk and Letcher, 2003; Verreault et al., 2005c; de Wit et al., 2010). These metabolites are thought to originate from either natural formation by microorganisms, or by xenobiotic biotransformation in the liver or intestines (Wolkers et al., 2004). For instance, the cytochrome P450 (CYP450) enzyme system have shown to metabolize PBDE-47 in rodents (Örn, 1997; Orn and Klasson-Wehler, 1998). Wolkers et al. (2004) suggested possible capacity of polar bears for biotransformation of this congener. Formation of OH-PCBs is also thought to be mediated by the CYP450 enzyme complex (Letcher et al., 2000). Hydroxylation of both PBDEs and PCBs may make these compounds more potent thyroid disrupting chemicals (TDCs) as structural similarities to thyroid hormones increase (Boas et al., 2006). Glaucous gulls are thought to have a limited metabolic

capacity (Henriksen et al., 1998a; Henriksen et al., 2000). However feeding on marine mammals containing high levels of OH-metabolites of both PCBs and PBDEs may represent a major route of uptake in the glaucous gull.

Levels of PFASs in glaucous gulls from Kongsfjorden have only been reported once before (Haugerud, 2011). However, in that study they only included female glauocus gulls. PFOS was the dominating PFAS found in the present study, followed by the long-chained carboxylic acids PFUnA, PFTrA and PFNA. The pattern of these prevailing PFASs is in accordance with the patterns reported in plasma and eggs of Bjørnøya glaucous gulls (Verreault et al., 2005b), ivory gulls (*Pagophila eburnean*) in the Russian and Norwegian Arctic (Miljeteig et al., 2009), snow buntings (*Plectrophenax nivalis*) at Svalbard (Kristoffersen, 2012), Brünnich guillemot (*Uria lomvia*) eggs from Kongsfjorden (Miljeteig and Gabrielsen, 2010) and Svalbard polar bears (Bytingsvik et al., 2012b).

Manufacture of PFOS containing products was substantially reduced in 2001 (UNEP, 2008). PFOS and related compounds were added to the Stockholm Convention list of POPs in 2009 (Stockholm Convention, 2010). There are however some exemptions such as for use in production of stain repellent surface coatings and in firefighting foams (Muir and de Wit, 2010). The widespread environmental distribution and high levels of PFOS are supported in the present study. However, it is unclear whether the burden of PFOS in the environment is mainly originated from direct emissions or by conversion of precursor compounds. Perfluorooctanesulfonyl fluoride (POSF)-based compounds ultimately degrade to PFOS via the PFOSA intermediate, thus supporting the abundance of PFOS in the environment (Giesy and Kannan, 2002). Laboratory studies with rodents have shown ability of liver cells to biotransform PFOSA to PFOS (Xu et al., 2004). However, in glaucous gulls, research on metabolic activity is required to conclude upon this species' ability to biotransform PFASs.

It is suggested that bioconcentration and bioaccumulation of PFASs are directly related to the fluorinated carbon chain length (Martin et al., 2003), just as the bioaccumulation of persistent OCs and PBDEs can be related to hydrophobicity (Conder et al., 2008). The longer chained PFASs possess the highest bioaccumulation potential; however the ability to bioaccumulate is proposed to be limited by molecular size when the chain is longer than 12 carbons (Martin et al., 2003). PFSAs are generally more bioaccumulative than PFCAs of the same chain length (Ohmori et al., 2003; Houde et al., 2006). Despite the low bioaccumulating potential for PFCAs with carbon chain shorter than 7 carbons, the presence of these compounds in animals

at high trophic positions such as the glaucous gull demonstrates the ability of biomagnification of these compounds in food webs (Giesy and Kannan, 2002; Muir and Howard, 2006).

PFASs with a carbon chain longer than 5 carbons are thought to be more potent to bind to proteins in avian serum than shorter chained PFASs (Jones et al., 2003). Circulating levels of plasma proteins are thought to be an index of total protein reserves in an animal. Studies have shown positive correlations between body condition and plasma protein levels in birds (De le Court et al., 1995; Dawson and Bortolotti, 1997). Total plasma protein levels may increase with increasing dietary protein levels. Thus, levels of PFASs in plasma may be dependent on feeding ecology. A study on captive American kestrels (*Falco sparverius*) showed that plasma protein levels were higher in females than in males both before egg laying and during incubation (Dawson and Bortolotti, 1997). It may therefore be suggested that incubating female birds may have higher potential for retention of protein binding OHCs like PFASs than males. However; this suggestion cannot be confirmed in the present study, as PFOA was the only compound in which female glaucous gulls had significantly higher plasma levels than males.

The clear pattern of males having generally higher levels of OCs than females is consistent with other studies where glaucous gulls were sampled for blood subsequent to egg laying (Bustnes et al., 2003a; Verreault et al., 2004; Verreault et al., 2005c). The ability of females to incorporate lipid associated contaminants in egg yolk during egg formation is a wide accepted explanation for the lower body burden of such contaminants in females than males post egg laying (Bargar et al., 2001; Drouillard and Norstrom, 2001; Verreault et al., 2006). No clear sex difference in the levels of the protein associated PFASs were found in the present study, supporting the assumption that lipid soluble contaminants are most subjected to maternal transfer.

There are several factors influencing levels of OHCs in individuals in the wild. The limited opportunity to control physiological and biological condition variables influencing the toxicokinetics of OHCs may be the primary confounding aspect in assessing contaminant levels and effects in glaucous gulls and other wildlife species. In the present study only birds of reproductive age, thus more than four years old (Gaston et al., 2009) are included, minimizing confounding effects of hormone levels in juvenile birds in the pool of samples. However, feeding on different trophic levels and food item specialization may cause great

variations in OHC levels in blood between the individuals. Also, migration south during winter is thought to increase exposure of glaucous gulls to anthropogenic pollutants (Borgå et al., 2005). Glaucous gulls studied on Bjørnøya generally have higher body burden of OHCs than levels presented here. The indications that the diet of these gulls from Bjørnøya to a larger extent consists of eggs and chicks from other seabirds, are thought to some degree explain the different contaminant exposure between these populations (Bustnes et al., 2000). Furthermore, the fact that the OHC levels in the present study are lower than what is reported in glaucous gulls from Bjørnøya may also reflect either a spatial contaminant exposure difference, or it may reflect the pattern of the suggested slow decline in contamination of legacy pollutants in the Arctic (De Wit et al., 2004; Laender et al., 2011; Bytingsvik et al., 2012a).

Body fat content variations are thought to cause great fluctuations in body mass of breeding seabirds, and glaucous gulls generally have a lower body mass during the nestling period than prior to breeding (Gabrielsen and Ryg, 1992; Gabrielsen et al., 1995). Incubation, or heating of the eggs have an energetic cost (Thomson et al., 1998). Thus, the period of incubation may result in a reduction of energy reserves (body fat), especially in female glaucous gulls which lay the eggs (Bustnes et al., 2001a). Energy scarcity causes remobilization of fat reserves and associated lipid soluble contaminants. This is particularly evident in low temperatures, as many arctic species depend on usage of the stored body fat reserves (Bustnes et al., 2012). A study on the fasting common eider (*Somateria mollissima*) found that fluctuating blood lipid contents were closely related to circulating levels of lipid soluble POPs during incubation, and that the high arctic eiders experienced the highest concentration increase of such compounds (Bustnes et al., 2012). Thus, glaucous gulls during the incubation period may also be susceptible for increased blood levels of OHCs (Henriksen et al., 1996; Henriksen et al., 1998c).

4.2 Thyroid hormones

The thyroid hormone (TH) levels measured in the present study are comparable to levels previously reported in glaucous gulls from Kongsfjorden (Haugerud, 2011) and Bjørnøya (Verreault et al., 2004; Verreault et al., 2007). The quantification of THs in these three studies is conducted by RIA kits from the same producer as the present (Siemens medical solution, Diagnostics). Thus, comparison of TH levels between these studies and the present is appropriate. However, the many environmental and physiological factors influencing

circulating TH concentrations, in combination with no well-established baseline TH levels in glaucous gulls make it challenging to conclude if TH levels measured in the present study are in normal range for the species.

TT3 levels in males were found to strongly positively correlate with BCI (Figure 5). *C*-nonachlor and *t*-nonachlor were the OHCs suggested to have greatest influence on TT3 levels after removal of the effect of BCI (Table 6). No associations were found between THs and BCI in female glaucous gulls (Figure 6). Body condition is generally thought to be a factor influencing TH levels in birds (McNabb, 2000). However, Verreault et al. (2004, 2007) found that body condition of both male and female glaucous gulls from Bjørnøya did not influence TH levels statistically. Nonetheless, positive relationships were found between levels of TT4 and FT4 and body condition in Northern fulmar (*Fulmarus glacialis*) chicks (Nøst et al., 2012). Moreover, body condition was found to be positively associated with TT3 and TT4 variations in juvenile gray seals (*Halichoerus grypus*) (Hall et al., 2003), and TT3 was positively related to condition in free-ranging brown trout (*Salmo trutta*) (Mulder et al., 2012). Mulder et al. (2012) found that TT3, but not FT3, was positively related to body condition of the trout, which is in accordance with findings from the present study. FT3 levels remain constant independent on thyroid transport protein levels. However, in normal thyroid function, as levels of the major TH transport protein TTR change, TT3 also change (Mendel, 1989; Palha et al., 1994). The great influence of BCI on male TT3 levels in the present study may indicate that birds in a poor condition may have lower levels of transport proteins, and the ability to bind THs could be reduced. It is suggested that TH transport proteins such as TTR are down-regulated during periods of energy deprivation in mammals (Ingenbleek and Young, 1994; Jahoor et al., 1996) and fish (Power et al., 2000). The glaucous gulls in the present study were sampled during the incubation period, which is thought to be a period of energy scarcity, as previously discussed (see above). A down-regulation of TH carrier proteins may thus be possible, which may influence circulating TH levels. Hence, TT3 levels may be too dependent on levels of carrier proteins and the body condition, and FT3 may be more related to actual thyroid status. Thus, it is possible that FT3 may act as a more reliable biomarker than TT3 for effect of anthropogenic contamination. These suggestions are in line with the free hormone hypothesis, stating that the biological activity of a hormone is dependent on its unbound fraction (Mendel, 1989). The influence of BCI on plasma TT3 levels designates the importance in accounting for such confounders when discussing effects of contaminants on circulating THs.

4.3 Associations between body condition and OHCs

It has long been thought that body condition may be related to OHC levels in glaucous gulls and other avian wildlife species (Henriksen et al., 1998b). In the present study, BCI correlated negatively with OCs in males. However, contaminants were not found to significantly influence BCI in females, and no significant OPLS model with BCI as Y could be obtained for female glaucous gulls. The OPLS model (Y=BCI, males) was made to support the correlation observed between BCI and OHCs in males and to assess which variables influenced BCI the most (Appendix E). The model predicted that TT3 was the variable with greatest effect on BCI in males, followed by PFOSA and the highly chlorinated PCB congeners (PCB)-153, -138, -187 and -183 (Figure E1). Several other studies of wild bird species have reported relationships between condition and body contaminant burden. Poor body condition was found to not relate to pollutant levels in Belgian predatory birds (Jaspers et al., 2006). Furthermore, Verreault et al. (2004, 2007) found no association between body condition and OHCs in plasma of Bjørnøya glaucous gulls. However, another Bjørnøya study reported that nine of 14 OCs analyzed in liver of glaucous gulls were found to significantly negatively correlate with body condition (Sagerup et al., 2000). Moreover, a study on the subarctic top predator great black-backed gull (*Larus marinus*) from northern Norway reported significant negative associations between body condition and nearly all OHCs analyzed in female birds, however no associations were found between body condition and pollutants in males (Helberg et al., 2005). Henriksen et al. (1998) found that a 100 g decrease in body mass of glaucous gulls was associated with a doubling of OHC concentration in blood (Henriksen et al., 1998c). However studies on Bjørnøya glaucous gulls indicated relatively stable blood wet weight PCB-153 concentrations despite considerable fluctuations in body mass (Bustnes et al., 2001b). The highly varying findings on influence of body condition on pollutant loads in birds elucidate the importance in assessing the condition of the animals in contaminant effect studies. The present study thus adds to the number of equivocal results on relationships between OHCs and body condition in birds. Moreover, it is appropriate to compare these discussed studies with the present, due to the similar approach for establishing a measure of body condition in the birds. In the present study, OHC levels in males seem to be more influenced by BCI than in female glaucous gulls. Circulating OHCs fluctuating with BCI could imply that also effects of the contaminants may be altered by the influence of body condition. Body condition is highly related to nutritional status. It is thus possible that the adverse effects of OHCs would be less prominent if feeding conditions are optimal, since

birds with good body condition may better buffer for negative effects from OHC exposure (Helberg et al., 2005).

4.4 Associations between OHCs and THs

A considerable sex difference in contaminant effects on THs in glaucous gulls is revealed in the present study. Significant negative associations were found between THs and both individual compounds and mixtures of OHCs in females which are here reported to be more susceptible to thyroid disruptive effects from OHCs than male glaucous gulls from Kongsfjorden. Verreault et al. (2004) reported thyroid disruptive effects of OHCs only in male glaucous gulls. Captive American kestrels exposed to PCBs had depressed T3 levels regardless of sex (Smits et al., 2002). Thus, patterns in sex related differences in TH response to OHCs in birds are inconsistent. Sex differences in TH disruption by OHCs have also been reported in mammals. A study on Svalbard polar bears found a greater disruptive effect of PCBs on THs in females than in males (Braathen et al., 2004). In humans, hypothyroidism is more common in women than in men (Chiovato et al., 1993). The suggested higher susceptibility of contaminant induced TH imbalance in female humans and mammals may arise speculations whether this is applicable in birds as well. Nevertheless, the present study indicates greater thyroid disruptive effects of OHCs in female than in male glaucous gulls.

The legacy OCPs and PCBs were the major TDCs in this study, found to disrupt both FT3 and FT4 levels in female glaucous gulls. Effects of the highly chlorinated PCBs such as (PCB)-138, 153, 183 and 187, and the persistent pesticides HCB, mirex and *p,p'*-DDE were the most prominent. The fact that these contaminant groups also were the most abundant, illustrates their continued environmental prominence several decades after being banned in production and use.

Thyroid disruptive effects of PFASs were less protruding than effects of PCBs and OCPs. PFOS was the only compound positively correlated with free and total T3 in females (Table 5). The study conducted on female glaucous gulls from Kongsfjorden in 2010 also found significant positive relationships between PFOS and TT3 (Haugerud, 2011). Furthermore, that particular study reported positive relationships between TT3 and several long-chained PFCAs, namely PFDcA, PFTrA, PFUnA and PFTeA. None of these PFCAs were found to affect TH levels in the present study. PFOA and PFNA were included in the OPLS-model as contributors to the variations in FT4 levels in female glaucous gulls (Figure 9 and 10). However, among the other variables included in the model, PFOA and PFNA were those with

least explanatory power. This was established by lower VIP-values in addition to error bars crossing the 0-line, meaning these were less reliable predictors for FT4 levels in female glaucous gulls.

FT4 correlated negatively with lipid% in females. Also, the OPLS regression indicates that lipid% was the third most important variable predicting FT4 levels in females (Figure 9). This may suggest that FT4 levels in female glaucous gulls are influenced by feeding ecology, since the amount of lipids in plasma reflects choice of prey (Bustnes et al., 2000; Bustnes et al., 2012). Some glaucous gulls specialize on fish, zooplankton and molluscs, while those feeding at a higher trophic level forage on eggs and other seabirds, which may contain higher OHC levels. Eggs and chicks from other seabird species represent a lipid rich food source for glaucous gulls during the incubation period (Bustnes et al., 2000). Feeding on higher trophic levels may increase the plasma fat content, and thus circulating OHCs. The present study indicates reduced FT4 levels with increased circulating OHCs in females (Figure 11). Thus, a higher plasma lipid percentage may be associated with reduced FT4 levels in glaucous gulls.

HCB was the variable mostly affecting FT4 levels in females. However, its confidence interval reveals great variations and thereby suggests that HCB concentrations are somewhat less reliable as a predictor for FT4 levels than other variables (Figure 9). Mirex, *p,p'*-DDE and PCB-118 are other variables suggested to be important in the model (Y=FT4, females). The effect of PCB-118 was especially clear, as shown by the short error bars, suggesting little variations and high reliability (Figure 9). PCB-118 is a non-*ortho*-substituted PCB, and this coplanar structure resembles that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, frequently referred to as dioxin). The toxicological implications of dioxin-like PCBs are commonly concerned a receptor-mediated mechanism of action, as they have potential to bind to the aryl-hydrocarbon receptor (AhR) (Giesy and Kannan, 1998). An experimental study on mink (*Mustela vision*) exposed to PCB contaminated food indicated decreased T3 levels and increased T4 levels with increasing toxic equivalency factor (TEQ, in terms of 2,3,7,8-TCDD toxicity) levels in the food (Heaton et al., 1995). A study on gray seal pups from the Baltic Sea showed that PCB-118 was the OC that best explained variations in FT3 levels (Sørmo et al., 2005). In the present study, both FT3 and FT4 were shown to be influenced by PCB-118 in female glaucous gulls. Thus, it is possible that effects of PCB-118 and other dioxin-like contaminants on the TH system could be mediated through the AhR. Also, PCB-118 is known to be metabolized to 4-OH-2,3,39,49,5-pentachlorobiphenyl (4-OH-PCB-107) in mammals,

which has been reported to be one of the most potent OH-PCBs influencing different TH parameters (Brouwer et al., 1998).

Mechanisms by which dioxin-like chemicals may affect the TH system by interfering with gene transcription regulated by the AhR remain to be fully understood. Disruption of the HPT axis by dioxins could be through interference with the enzymes controlling TH metabolism. One such suggested mechanism is hepatic induction of Uridine 5'-diphospho-glucuronosyltransferase (UDP-GT), which may enhance glucuronidation and excretion of T4 (Van Birgelen et al., 1995). Interference with the iodothyronine deiodinases may disturb activation and deactivation of THs, and regulation of circulating free THs may also be disrupted by potential of OHCs to interfere with sulfotransferases (Brouwer et al., 1998). Pocar et al. (2006) investigated the effect of dioxin-like PCBs on the transcriptional regulation of genes related to functioning of the thyroid gland in mammals. They reported a significant decreased expression of a Na^+/I^- symporter, which may represent a negative interference with such compounds on iodine uptake by the thyroid gland (Pocar et al., 2006). The present study however only evaluates levels of THs as biomarkers of TH disruption by OHC exposure. Thus, it is not appropriate to conclude upon possible effects of OHCs on the TH system mediated through interference with regulatory functions involved in the HPT axis.

Thyroid disruption in vertebrates is to a great extent thought to be mediated through structural resemblance of specific chemicals to the endogenous THs. Analogous molecular structure may result in competitive binding to TH-binding proteins and thus, displacement of THs. Such displacement is suggested to facilitate excretion of the unbound THs (Ucán-Marín et al., 2009). TTR functions as a circulating reservoir for THs, buffering changes in TH levels (Weiss et al., 2009). Competitive binding affinity of OHCs to TTR have been reported in numerous *in vitro* and *in vivo* studies (Hakk and Letcher, 2003; Legler and Brouwer, 2003; Ucán-Marín et al., 2009; Weiss et al., 2009).

PCBs have long been recognized as potential TDCs in birds (Smits et al., 2002; Verreault et al., 2004), mammals (Collins Jr et al., 1977; Braathen et al., 2004) and fish (LeRoy et al., 2006). PCBs, PBDEs and their hydroxylated metabolites (OH-PCBs and OH-PBDEs) are structurally similar to T4 (Boas et al., 2006), and these have been shown to displace T4 from human TTR, releasing FT4 which may enhance T4 metabolism and excretion (Brouwer et al., 1998; Meerts et al., 2000). Competitive binding affinities of PCBs and other OHCs to TTR in relation to natural T4 and T3 ligands are however less studied in birds than in mammals. It

should be realized that structural differences in TTR exist between species. Thus, different binding affinities may be expected. One feature demonstrating this species and taxa difference is that avian TTR bind T3 with higher affinity than T4, in contrast to mammalian TT3 (Chang et al., 1999). Furthermore, the study by Ucan Marin et al. (2009) indicated that competitive displacement of T4 from TTR by TDCs is more prone than displacement of T3 in glaucous gulls. This suggests that a reduction in circulating T4 is more expected than T3 from exposure to TTR binding contaminants. Reduced T4 levels present in target tissues may however subsequently result in reduced T3 levels, as T4 is subjected to conversion to the more active T3 by deiodinase enzymes (Verhoelst et al., 2005). The results in the present study confirm that FT4 may be subjected to competitive displacement from TTR by OHCs in female glaucous gulls, as demonstrated by the negative relationships between FT4 and both individual and mixtures of OCs (Table 5 and Figure 11).

Analogous molecular structure of OHCs to THs could also imply potency of these to bind to the thyroid receptor (TR), which represents another mechanism of disruption on the HPT axis. Chemicals interfering with TR may produce varying effects on TH levels and signaling (Freitas et al., 2011). For instance, PCBs have been shown to interact directly with the TR, but it is less clear whether they act as agonists of antagonists (Zoeller, 2005). A master project with liver samples from glaucous gull as test matrix indicated that long-chained PFCAs possess abilities to bind to the TR at the same affinity range as endogenous THs (Mæhre, 2012). Thus, contaminant binding and potential agonistic or antagonistic effects may represent an important mechanism of action which should be considered when evaluating TH disruption.

PFOS was found to alter FT3 and TT3 in females in the present study. No effects of PFOS on T4 levels were observed. This highly environmentally relevant compound has been thought to alter TH function by interfering with transcriptions of genes constituting major parts of the HPT axis (Shi et al., 2009). It has further been proposed that PFOS not necessarily affects the regulations of the TH system itself, but may also competitively bind to the TH transport proteins (Lau et al., 2007; Chang et al., 2008). Weiss et al. (2009) found that binding potencies for PFASs to human TTR varied with carbon chain length and degree of fluorination of the alkyl chain. Furthermore, PFSAs were found to have higher TTR binding potencies than the PFCAs of the same carbon chain length (Weiss et al., 2009). However, PFASs are shown to have lower TTR binding affinity than OH-PCBs, and an order of one-tenth of the TTR binding affinity of natural T4 (Simon et al., 2011). A study on TTR binding

abilities of PCBs, OH-PCBs, hexabromocyclododecane (HBCD), PBDEs, OH-PBDEs and PFASs in polar bear cubs from 2008 found that OH-PCBs explained 54 ± 4 % of the TTR binding activity, and that PFASs explained ≤1.2 % (Bytingsvik et al., 2013). Since almost half of the TTR binding activity could not be explained by the contaminants investigated, further identification of contaminants that are able to competitively bind TTR is appropriate.

The present study confirms the toxicological implications of particularly highly chlorinated PCBs on the TH system. The fact that both free T3 and T4 levels in females decreased with increased PCB and OCP levels demonstrates that there may be several modes of action of thyroid disruption. Furthermore, the unbound fractions of THs were most sensitive to variations with OHC levels in the present study. Free T4 and T3 may be more applicable for actual biological function of the TH system, and thus, investigating these may be more relevant when evaluating the clinical TH status (Nauman et al., 1967; Mendel, 1989). The summed effects of the OHCs included in the OPLS model (Y=FT4, females) were a significant reduction in FT4 levels (Figure 11). The given OHCs were included in the model because these were important in describing FT4 levels. The fact that these OHCs mainly consisted of highly chlorinated PCBs and persistent OCPs stresses the thyroid disrupting properties of such contaminants.

The incidence of both positive and negative associations between OHCs and THs in this study emphasizes the complex actions of thyroid disruption. The physicochemical properties of the chlorinated and brominated contaminants differ from the perfluorinated compounds, resulting in dissimilar partitioning, actions and effects in an organism. OCs and BFRs have shown to be associated with reduced TH levels in both birds (Smits et al., 2002; Verreault et al., 2004), mammals (Skaare et al., 2001; Braathen et al., 2004; Villanger et al., 2011) and fish (Leatherland and Sonstegard, 1980). Also, when attention was given to effects of PFASs in the environment, these were commonly reported to have positive associations with TH levels in wildlife studies of birds (Haugerud, 2011; Nøst et al., 2012), and laboratory studies with fish larvae (Shi et al., 2009) and rats (Chang et al., 2008). The overall effect is thus a complex mode of TH disruption. The effects of different OHCs on levels of FT3 in females in the present study demonstrate this complexity. The combined effect of the sum of all OHCs negatively affecting FT3 levels was as expected, a highly significant inverse correlation (Figure 12A). The individual positive effect of PFOS on FT3 levels is depicted in Figure 12B. Reporting effects from different compounds individually is a common approach in toxicology studies; however this does not attempt to reflect the overall disruptive effects from the

cocktail of compounds to which free-ranging animals are exposed to. The present study aimed to assess effects of mixtures of OHCs. One such approach is further complicated when both positive and negative effects are predicted from the individual compounds. The overall effect of the sum of all OHCs affecting FT3 in female glaucous gulls is illustrated in Figure 12C, and the result was a non-significant relationship with no clear pattern. This result demonstrates the complication in discussing effects on FT3 levels in female glaucous gull of the present study. It is possible that the positive relationship between PFOS and FT3 may outweigh some of the negative relationships between OCs and FT3. However, due to the potential of the different compounds to act via several dissimilar mechanisms, and the many possible sites of action in the HPT axis, it is challenging to determine the effects of the combination of both OCs and PFASs on female FT3 levels in this study. These results stress the difficulties in concluding upon the combined effects of pollutant stress on TH levels in wildlife studies.

As demonstrated in the present study, it is challenging to evaluate endocrine disrupting potential of mixtures of chemicals due to the possible additive, synergistic or antagonistic effects of a contaminant cocktail (Daston et al., 2003; Miller et al., 2012). Extracting effects of specific compounds in a mixture is very challenging when the compounds are highly correlated. It is possible that a significant correlation between the response variable and one compound may only result from high correlation with the compounds actually causing effects. One should further realize that there may exist factors not measured or controlled for, that may be important for the study outcome (Bustnes, 2006). Also, effects observed may arise from the combined actions of multiple OHCs, and by different mechanisms of disruption, as proposed in this study (Zoeller et al., 2009). There is a lack of research reporting effects of mixtures of TDCs compared to individual compounds in avian wildlife. Both statistical approaches, ability to test for additivity and general study design represent limitations to this (Wade et al., 2002; Desaulniers et al., 2003). Crofton et al. (2005) tested if a mixture of TDCs affected serum TT4 levels in a dose-additive manner in female rats. The results indicated a greater-than-additive effect at the highest mixture doses, suggesting synergistic mechanisms for TDCs at high doses. In wildlife studies, it is widely established that studying effects of a cocktail of contaminants is more relevant than studying effects from single compounds. Thus, cumulative risk approaches should be considered and focus should be given on the lack of current modeling methodologies in assessing mixtures. Furthermore, both mixture and individual compound effect studies are most useful when the biomarker of interest have been

causally linked to the effect. Interpreting an effect study is easier when exposure to a chemical is only associated with one toxic effect. Causality is however challenging in wildlife toxicology studies, since adverse outcomes of contaminants and especially mixtures are often caused by several mechanisms. Establishing the molecular mechanisms for toxicity may nonetheless facilitate the evaluation of glaucous gull health in light of anthropogenic pollutant exposure.

Experimental studies linking molecular mechanisms to toxicological outcome are necessary for supporting findings in wildlife studies. However, such studies are often lacking. Therefore, causal associations between OHC exposure and TH system effects are speculative (Rolland, 2000). The altered TH levels observed in this study could be a direct result of OHC toxicity. However, natural fluctuations in TH levels are expected from individual differences in age, diet, nutritional status, season, time of day, and general physiological condition, which are factors that are challenging to control in wildlife studies. Also, a larger sample size would be advantageous for greater reliability of the results. Furthermore, individuals in the wild may respond differently to the same contaminant exposure, and healthy animals may be able to compensate to contaminant stress to a larger degree than individuals already at the limits of their tolerance (Beyers et al., 1999). Adverse health effects may be expected if TH levels are altered beyond the individual's capacity of homeostatic compensation (Rolland, 2000). Moreover, a great challenge comes with extrapolation between biomarker responses measured in individuals and effects on population level (AMAP, 2004).

There are ambiguous reports on how exposure to TDCs affect TH levels in birds, and the overall findings do not appear to provide any clear information about actions and mechanisms of thyroid disruption (Smits et al., 2002; Scanes and McNabb, 2003; Verreault et al., 2004; Nøst et al., 2012). In such studies as well as the present, it is thus challenging to assess whether a disrupted TH system is directly due to contaminant exposure or whether the effects are obscured by natural variations in hormone levels or other physiological features (McNabb, 2007). Moreover, comparison in OHC levels and effects between different studies should generally be done in caution due to differences in contaminant quantification methods and standards between different laboratories.

4.5 Future perspectives

It is challenging to conclude upon thyroid disruptive effects of the cocktail of OHCs to which glaucous gulls in Kongsfjorden are exposed to. The causal relationships between TH levels and different compounds have not been established, and the possible mechanisms are numerous and not well understood. However; evaluating effects of mixtures of contaminants rather than individual compounds on the TH system is more relevant in assessing the actual exposure and effect scenario in free-ranging glaucous gulls. Furthermore, use of non-destructive sample methods such as blood sampling should be upheld.

To obtain a comprehensive overview of thyroid disruptive effects of OHCs in glaucous gulls, it may be necessary to include assessment of more factors than the variables analyzed in this study. The present study stresses the importance in accounting for the individual body condition for evaluation of thyroid status. Furthermore, it is suggested that metabolites of PCBs and PBDEs may be potent thyroid disruptors, thus quantification of such compounds should be included in future studies investigating contaminant effects on the TH system. Due to the proteinophilic nature of both such hydroxylated metabolites and PFASs, an evaluation of protein content in plasma could be appropriate. Moreover, analyzing TTR expression and levels in the organisms could be highly interesting due to the central role this transport protein seems to play in thyroid disruption by competitively binding contaminants. The necessity of further research on the ability on other compounds to bind to TH transport proteins is elucidated in the present study.

Further research is required to determine if chemically altered TH levels in glaucous gulls are causally related to adverse health effects on both individual and population levels. There may be several adverse outcomes form chemically disrupted TH levels due to the key role this endocrine system plays in free ranging glauocus gulls. Thus, TH disruption should continue to be subject to research.

Most likely, the combination of natural and anthropogenic stressors is causing the greatest health concern in arctic species. A changing arctic climate is likely to pose additional stress in species already living in challenging environments. Future studies may reveal possible influence of a warming climate and changing sea ice cover on dynamics and kinetics of toxicants in the environment. Also, the consecutively replacement of POPs with known toxic actions could imply that new chemicals are emerging with unknown hazardous potential, to which focus on toxicological implications should be given.

5 CONCLUSION

In the present study, high levels of circulating persistent OHCs were quantified in plasma from glaucous gulls breeding in Kongsfjorden, demonstrating a continued high exposure to these anthropogenic contaminants in this circumpolar top predator. The predominant compounds analyzed were highly chlorinated PCBs, OCPs and PFOS.

Relationships between OHCs and TH levels in this study indicate a thyroid disruptive effect of exposure to a cocktail of persistent halogenated pollutants in glaucous gulls. The present attempt to study combined effects of the mixture of different OHCs in glaucous gulls demonstrated a complex overall TH disruption scenario. It is suggested that female glaucous gulls are more susceptible to thyroid disruptive effects caused by exposure to OHCs, as both FT3 and FT4 levels were modeled to be altered by a variety of compounds. It was demonstrated that TT3 levels in male gulls were influenced by the body condition of the individuals, which confounded the thyroid disruptive effect of OHCs in males. It is thus proposed that the free fractions of T3 (FT3) and T4 (FT4) are better biomarkers of effects of thyroid disrupting contaminants than the total fractions of these two hormones (TT3 and TT3) in both male and females glaucous gulls.

REFERENCES

Akins, J.R., Waldrep, K., Bernert Jr, J.T., 1989. The estimation of total serum lipids by a completely enzymatic 'summation'method. Clinica chimica acta 184, 219-226.

AMAP, 2004. AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xvi+310 pp.

Anker-Nilssen, T., Bakken, V., Strøm, H., Golovkin, A.N., Bianki, V.V., Tatarinkova, I.P., 2000. The status of marine birds breeding in the Barents Sea region. *Report series, Norwegian Polar Institute Report nr 113.* .

Bargar, T.A., Scott, G.I., Cobb, G.P., 2001. Maternal transfer of contaminants: Case study of the excretion of three polychlorinated biphenyl congeners and technical-grade endosulfan into eggs by white leghorn chickens (*Gallus domesticus*). Environmental Toxicology and Chemistry 20, 61-67.

Barrie, L., 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, 1-74.

Beyers, D.W., Rice, J.A., Clements, W.H., Henry, C.J., 1999. Estimating physiological cost of chemical exposure: integrating energetics and stress to quantify toxic effects in fish. Canadian Journal of Fisheries and Aquatic Sciences 56, 814-822.

Boas, M., Feldt-Rasmussen, U., Skakkebæk, N.E., Main, K.M., 2006. Environmental chemicals and thyroid function. European Journal of Endocrinology 154, 599-611.

Borgå, K., Gabrielsen, G., Kleivane, L., Skaare, J., Norstrom, R., Fisk, A., 2005. Why do semi-circumpolar trends of OCs differ among Arctic trophic levels. Environ Sci Technol (accepted).

Bossi, R., Riget, F.F., Dietz, R., Sonne, C., Fauser, P., Dam, M., Vorkamp, K., 2005. Preliminary screening of perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish, birds and marine mammals from Greenland and the Faroe Islands. Environmental Pollution 136, 323-329.

Bourne, W., Bogan, J., 1972. Polychlorinated biphenyls in North Atlantic seabirds. Marine Pollution Bulletin 3, 171-175.

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

Braestrup, L., Clausen, J., Berg, O., 1974. DDE, PCB and aldrin levels in Arctic birds of Greenland. Bulletin of environmental contamination and toxicology 11, 326-332.

Braune, B., Outridge, P., Fisk, A., Muir, D., Helm, P., Hobbs, K., Hoekstra, P., Kuzyk, Z., Kwan, M., Letcher, R., 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, 4-56.

Brouwer, A., Morse, D.C., Lans, M.C., Schuur, A.G., Murk, A.J., Klasson-Wehler, E., Bergman, Å., Visser, T.J., 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicology and industrial health 14, 59-84.

Buccini, J., 2003. The development of a global treaty on persistent organic pollutants (POPs), Persistent Organic Pollutants, Springer, pp. 13-30.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integrated environmental assessment and management 7, 513-541.

Burkow, I.C., Kallenborn, R., 2000. Sources and transport of persistent pollutants to the Arctic. Toxicology letters 112, 87-92.

Bustnes, J., Bakken, V., Erikstad, K., Mehlum, F., Skaare, J., 2001a. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. Journal of Applied Ecology 38, 791-801.

Bustnes, J., Erikstad, K., Bakken, V., Mehlum, F., Skaare, J., 2000. Feeding ecology and the concentration of organochlorines in glaucous gulls. Ecotoxicology 9, 179-186.

Bustnes, J., Fauchald, P., Tveraa, T., Helberg, M., Skaare, J., 2008a. The potential impact of environmental variation on the concentrations and ecological effects of pollutants in a marine avian top predator. Environment international 34, 193-201.

Bustnes, J., Folstad, I., Erikstad, K., Fjeld, M., Miland, Ø., Skaare, J., 2002. Blood concentration of organochlorine pollutants and wing feather asymmetry in glaucous gulls. Functional Ecology 16, 617-622.

Bustnes, J., Hanssen, S., Folstad, I., Erikstad, K., Hasselquist, D., Skaare, J., 2004. Immune function and organochlorine pollutants in Arctic breeding glaucous gulls. Archives of environmental contamination and toxicology 47, 530-541.

Bustnes, J.O., 2006. Pinpointing potential causative agents in mixtures of persistent organic pollutants in observational field studies: a review of glaucous gull studies. Journal of Toxicology and Environmental Health, Part A 69, 97-108.

Bustnes, J.O., Bakken, V., Skaare, J.U., Erikstad, K.E., 2003a. Age and accumulation of persistent organochlorines: A study of arctic-breeding glaucous gulls *(Larus hyperboreus*). Environmental toxicology and chemistry 22, 2173-2179.

Bustnes, J.O., Borgå, K., Erikstad, K.E., Lorentsen, S.H., Herzke, D., 2008b. Perfluorinated, brominated, and chlorinated contaminants in a population of lesser black-backed gulls (*Larus fuscus*). Environmental Toxicology and Chemistry 27, 1383-1392.

Bustnes, J.O., Erikstad, K.E., Skaare, J.U., Bakken, V., Mehlum, F., 2003b. Ecological effects of organochlorine pollutants in the Arctic: a study of the glaucous gull. Ecological Applications 13, 504-515.

Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Fenstad, A.A., Nordstad, T., Borgå, K., Gabrielsen, G.W., 2012. Temporal dynamics of circulating persistent organic pollutants in a fasting seabird under different environmental conditions. Environmental science & technology 46, 10287-10294.

Bustnes, J.O., Skaare, J.U., Erikstad, K.E., Bakken, V., Mehlum, F., 2001b. Whole blood concentrations of organochlorines as a dose metric for studies of the glaucous gull *(Larus hyperboreus*). Environmental Toxicology and Chemistry 20, 1046-1052.

Butt, C.M., Mabury, S.A., Muir, D.C., Braune, B.M., 2007. Prevalence of long-chained perfluorinated carboxylates in seabirds from the Canadian Arctic between 1975 and 2004. Environmental science & technology 41, 3521-3528.

Bytingsvik, J., Lie, E., Aars, J., Derocher, A.E., Wiig, Ø., Jenssen, B.M., 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, 117-128.

Bytingsvik, J., Simon, E., Leonards, P.E., Lamoree, M., Lie, E., Aars, J., Derocher, A.E., Wiig, Ø., Jenssen, B.M., Hamers, T., 2013. Transthyretin-binding activity of contaminants in blood from polar bear (*Ursus maritimus*) cubs. Environmental science & technology 47, 4778-4786.

Bytingsvik, J., van Leeuwen, S.P., Hamers, T., Swart, K., Aars, J., Lie, E., Nilsen, E.M.E., Wiig, Ø., Derocher, A.E., Jenssen, B.M., 2012b. Perfluoroalkyl substances in polar bear mother–cub pairs: A comparative study based on plasma levels from 1998 and 2008. Environment international 49, 92-99.

Chang, L., Munro, S.L., Richardson, S.J., Schreiber, G., 1999. Evolution of thyroid hormone binding by transthyretins in birds and mammals. European journal of biochemistry 259, 534-542.

Chang, S.-C., Thibodeaux, J.R., Eastvold, M.L., Ehresman, D.J., Bjork, J.A., Froehlich, J.W., Lau, C., Singh, R.J., Wallace, K.B., Butenhoff, J.L., 2008. Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). Toxicology 243, 330-339.

Chiovato, L., Lapi, P., Fiore, E., Tonacchera, M., Pinchera, A., 1993. Thyroid autoimmunity and female gender. Journal of endocrinological investigation 16, 384.

Colborn, T., 2002. Clues from wildlife to create an assay for thyroid system disruption. Environmental Health Perspectives 110, 363.

Colborn, T., vom Saal, F.S., Soto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental health perspectives 101, 378.

Collins Jr, W.T., Capen, C.C., Kasza, L., Carter, C., Dailey, R.E., 1977. Effect of polychlorinated biphenyl (PCB) on the thyroid gland of rats: ultrastructural and biochemical investigations. The American journal of pathology 89, 119.

Conder, J.M., Hoke, R.A., Wolf, W.d., Russell, M.H., Buck, R.C., 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environmental science & technology 42, 995-1003.

Coulson, J., Thomas, C., Butterfield, J., Duncan, N., Monaghan, P., Shedden, C., 1983. The use of head and bill length to sex live gulls Laridae. Ibis 125, 549-557.

Daston, G.P., Cook, J.C., Kavlock, R.J., 2003. Uncertainties for endocrine disrupters: our view on progress. Toxicological Sciences 74, 245-252.

Dawson, R.D., Bortolotti, G.R., 1997. Total plasma protein level as an indicator of condition in wild American kestrels (*Falco sparverius*). Canadian Journal of Zoology 75, 680-686.

De le Court, C., Aguilera, E., Recio, F., 1995. Plasma chemistry values of free-living white spoonbills (*Platalea leucorodia*). Comparative Biochemistry and Physiology Part A: Physiology 112, 137-141.

De Wit, C., Fisk, A., Hobbs, K., Muir, D., Gabrielsen, G., Kallenborn, R., Krahn, M., Norstrom, R., Skaare, J., 2004. AMAP assessment 2002: Persistant Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo,Norway, pp. 1e309.

de Wit, C.A., Herzke, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment—trends and new candidates. Science of the Total Environment 408, 2885-2918.

Desaulniers, D., Leingartner, K., Musicki, B., Yagminas, A., Xiao, G.-H., Cole, J., Marro, L., Charbonneau, M., Tsang, B., 2003. Effects of postnatal exposure to mixtures of non-ortho-PCBs, PCDDs, and PCDFs in prepubertal female rats. Toxicological Sciences 75, 468-480.

Dietz, R., Bossi, R., Riget, F.F., 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, 2701-2707.

Drouillard, K.G., Norstrom, R.J., 2001. Quantifying maternal and dietary sources of 2, 2′, 4, 4′, 5, 5′-hexachlorobiphenyl deposited in eggs of the ring dove (*Streptopelia risoria*). Environmental toxicology and chemistry 20, 561-567.

El-Shahawi, M., Hamza, A., Bashammakh, A., Al-Saggaf, W., 2010. An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. Talanta 80, 1587-1597.

Eriksson, L., Trygg, J., Wold, S., 2008. CV-ANOVA for significance testing of PLS and OPLS® models. Journal of Chemometrics 22, 594-600.

Fox, G.A., 1993. What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. Journal of Great Lakes Research 19, 722-736.

Fox, G.A., Jeffrey, D.A., Williams, K.S., Kennedy, S.W., Grasman, K.A., 2007. Health of herring gulls (*Larus argentatus*) in relation to breeding location in the early 1990s. I. Biochemical measures. Journal of Toxicology and Environmental Health, Part A 70, 1443-1470.

Freitas, J., Cano, P., Craig-Veit, C., Goodson, M.L., David Furlow, J., Murk, A.J., 2011. Detection of thyroid hormone receptor disruptors by a novel stable in *vitro* reporter gene assay. Toxicology in Vitro 25, 257-266.

Gabrielsen, G., Ryg, M., 1992. Fugl og pattedyr. Økosystem Barentshavet. Mesna-Trykk A/S, Lillehammer, 203-229.

Gabrielsen, G.W., Knudsen, L.B., Schlabach, M., 2005. Organic pollutants in northern fulmars (Fulmarus glacialis) from Bjørnøya. *Report 922, Statens Forurensningstilsyn*.

Gabrielsen, G.W., Skaare, J.U., Polder, A., Bakken, V., 1995. Chlorinated hydrocarbons in glaucous gulls (*Larus hyperboreus*) in the southern part of Svalbard. Science of the Total Environment 160, 337-346.

Gaston, A.J., Descamps, S., Gilchrist, H.G., 2009. Reproduction and survival of Glaucous Gulls breeding in an Arctic seabird colony. Journal of Field Ornithology 80, 135-145.

Giesy, J.P., Kannan, K., 1998. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. CRC Critical Reviews in Toxicology 28, 511-569.

Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environmental science & technology 35, 1339-1342.

Giesy, J.P., Kannan, K., 2002. Peer reviewed: perfluorochemical surfactants in the environment. Environmental science & technology 36, 146A-152A.

Griffiths, R., Double, M.C., Orr, K., Dawson, R.J., 1998. A DNA test to sex most birds. Molecular ecology 7, 1071-1075.

Hakk, H., Letcher, R.J., 2003. Metabolism in the toxicokinetics and fate of brominated flame retardants—a review. Environment international 29, 801-828.

Hall, A.J., Kalantzi, O.I., Thomas, G.O., 2003. Polybrominated diphenyl ethers (PBDEs) in grey seals during their first year of life—are they thyroid hormone endocrine disrupters? Environmental Pollution 126, 29-37.

Hanssen, L., Dudarev, A.A., Huber, S., Odland, J.Ø., Nieboer, E., Sandanger, T.M., 2013. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. Science of the Total Environment 447, 430-437.

Haugerud, A.J., 2011. Levels and Effects of Organohalogenated Contaminants on Thyroid Hormone Levels in Glaucous Gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard, unpublished Master project, NTNU, Trondheim, Norway.

Haukås, M., Berger, U., Hop, H., Gulliksen, B., Gabrielsen, G.W., 2007. Bioaccumulation of per-and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. Environmental Pollution 148, 360-371.

Hayes, W.J., 1982. Pesticides studied in man. Williams & Wilkins, Baltimore, MD, Chap. 6, pp. 179-205.

Heaton, S., Bursian, S., Giesy, J., Tillitt, D., Render, J., Jones, P., Verbrugge, D., Kubiak, T., Aulerich, R., 1995. Dietary exposure of mink to carp from Saginaw Bay, Michigan. 1. Effects on reproduction and survival, and the potential risks to wild mink populations. Archives of Environmental Contamination and Toxicology 28, 334-343.

Helberg, M., Bustnes, J.O., Erikstad, K.E., Kristiansen, K.O., Skaare, J.U., 2005. Relationships between reproductive performance and organochlorine contaminants in great black-backed gulls (*Larus marinus*). Environmental pollution 134, 475-483.

Henriksen, E., Brunström, B., Skaare, J., Gabrielsen, G., 1998a. Bioassay-derived 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin equivalents and mono-ortho polychlorinated biphenyl concentrations in liver of glaucous gulls (*Larus hyperboreus*) from Svalbard. Organohalogen compounds 39, 415-418.

Henriksen, E., Gabrielsen, G., Skaare, J., Skjegstad, N., Jenssen, B., 1998b. Relationships between PCB levels, hepatic EROD activity and plasma retinol in glaucous gulls (*Larus hyperboreus*). Marine environmental research 46, 45-49.

Henriksen, E., Gabrielsen, G., Trudeau, S., Wolkers, J., Sagerup, K., Skaare, J., 2000. Organochlorines and possible biochemical effects in glaucous gulls (*Larus hyperboreus*) from Bjørnøya, the Barents Sea. Archives of Environmental Contamination and Toxicology 38, 234-243.

Henriksen, E.O., Gabrielsen, G.W., Skaare, J.U., 1996. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (*Rissa tridactyla*), in relation to mobilization of body-lipids associated with reproduction. Environmental Pollution 92, 27-37.

Henriksen, E.O., Gabrielsen, G.W., Utne Skaare, J., 1998c. Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gulls (*Larus hyperboreus*). Chemosphere 37, 2627-2643.

Herzke, D., Gabrielsen, G.W., Evenset, A., Burkow, I.C., 2003. Polychlorinated camphenes (toxaphenes), polybrominated diphenylethers and other halogenated organic pollutants in glaucous gull *(Larus hyperboreus*) from Svalbard and Bjørnøya (Bear Island). Environmental Pollution 121, 293-300.

Hop, H., Pearson, T., Hegseth, E.N., Kovacs, K.M., Wiencke, C., Kwasniewski, S., Eiane, K., Mehlum, F., Gulliksen, B., Wlodarska-Kowalczuk, M., 2002. The marine ecosystem of Kongsfjorden, Svalbard. Polar Research 21, 167-208.

Hotchkiss, A.K., Rider, C.V., Blystone, C.R., Wilson, V.S., Hartig, P.C., Ankley, G.T., Foster, P.M., Gray, C.L., Gray, L.E., 2008. Fifteen years after "Wingspread"—environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. Toxicological Sciences 105, 235-259.

Houde, M., Martin, J.W., Letcher, R.J., Solomon, K.R., Muir, D.C., 2006. Biological monitoring of polyfluoroalkyl substances: a review. Environmental science & technology 40, 3463-3473.

Hoyo, J.D., Elliott, A., Sargatal, J., Cabot, J., 1992. Handbook of the Birds of the World. Lynx Edicions, Barcelona Vol. 1. Ostrich to Ducks.

Ingenbleek, Y., Young, V., 1994. Transthyretin (prealbumin) in health and disease: nutritional implications. Annual review of nutrition 14, 495-533.

Jahoor, F., Bhattiprolu, S., Del Rosario, M., Burrin, D., Wykes, L., Frazer, M., 1996. Chronic protein deficiency differentially affects the kinetics of plasma proteins in young pigs. The Journal of nutrition 126, 1489-1495.

Jakob, E.M., Marshall, S.D., Uetz, G.W., 1996. Estimating fitness: a comparison of body condition indices. Oikos, 61-67.

Jaspers, V., Covaci, A., Voorspoels, S., Dauwe, T., Eens, M., Schepens, P., 2006. Brominated flame retardants and organochlorine pollutants in aquatic and terrestrial predatory birds of Belgium: levels, patterns, tissue distribution and condition factors. Environmental Pollution 139, 340-352.

Jensen, J., 1991. Report on organochlorines. The State of the Arctic Environment Reports, Actic Centre Publications 2. ISBN 951-634-231-0, University of Lapland, Rovaniemi, Finland, pp. 325-384., 325-384.

Jenssen, B.M., 2006. Endocrine-disrupting chemicals and climate change: a worst-case combination for arctic marine mammals and seabirds? Environmental health perspectives 114, 76.

Jolicoeur, P., Mosimann, J.E., 1960. Size and shape variation in the painted turtle. A principal component analysis. Growth 24, 339-354.

Jones, K.C., De Voogt, P., 1999. Persistent organic pollutants (POPs): state of the science. Environmental Pollution 100, 209-221.

Jones, P.D., Hu, W., De Coen, W., Newsted, J.L., Giesy, J.P., 2003. Binding of perfluorinated fatty acids to serum proteins. Environmental Toxicology and Chemistry 22, 2639-2649.

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Surridge, B., Hoover, D., Grace, R., Gobas, F.A., 2009. Perfluoroalkyl contaminants in an Arctic marine food web: trophic magnification and wildlife exposure. Environmental science & technology 43, 4037-4043.

Kemsley, E., 1996. Discriminant analysis of high-dimensional data: a comparison of principal components analysis and partial least squares data reduction methods. Chemometrics and intelligent laboratory systems 33, 47-61.

Kristoffersen, S., 2012. Organohalogenated Contaminants in Eggs of Snow Buntings (*Plectrophenax nivalis*) from Human Settlements in Svalbard, unpublished Master project, NTNU, Trondheim, Norway.

Laender, F.D., Hammer, J., Hendriks, A.J., Soetaert, K., Janssen, C., 2011. Combining monitoring data and modeling identifies PAHs as emerging contaminants in the Arctic. Environmental science & technology 45, 9024-9029.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicological sciences 99, 366-394.

Leatherland, J., Sonstegard, R., 1980. Effect of dietary polychlorinated biphenyls (PCBs) or mirex in combination with food deprivation and testosterone administration on serum thyroid hormone concentration and bioaccumulation of organochlorines in rainbow trout, *Salmo gairdneri*. Journal of Fish Diseases 3, 115-124.

Legler, J., Brouwer, A., 2003. Are brominated flame retardants endocrine disruptors? Environment International 29, 879-885.

LeRoy, K.D., Thomas, P., Khan, I.A., 2006. Thyroid hormone status of Atlantic croaker exposed to Aroclor 1254 and selected PCB congeners. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 144, 263-271.

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

Letcher, R.J., Klasson-Wehler, E., Bergman, A., 2000. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls, Volume 3 Anthropogenic Compounds Part K, Springer, pp. 315-359.

Lundstedt, T., Seifert, E., Abramo, L., Thelin, B., Nyström, Å., Pettersen, J., Bergman, R., 1998. Experimental design and optimization. Chemometrics and Intelligent Laboratory Systems 42, 3-40.

Løvenskiold, H.L., 1964. Avifauna Svalbardensis: with a discussion on the geographical distribution of the birds in Spitsbergen and adjacent islands. Norsk Polarinstiutts Skrifter, 129:1-460

Mac Nally, R., 2000. Regression and model-building in conservation biology, biogeography and ecology: the distinction between–and reconciliation of–'predictive'and 'explanatory'models. Biodiversity & Conservation 9, 655-671.

Macdonald, R.W., 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), Oslo, Norway (2003) xii + 65 pp.

Manchester-Neesvig, J.B., Valters, K., Sonzogni, W.C., 2001. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Michigan salmonids. Environmental science & technology 35, 1072-1077.

Martin, J.W., Mabury, S.A., Solomon, K.R., Muir, D.C., 2003. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss)*. Environmental Toxicology and Chemistry 22, 196-204.

Martin, J.W., Smithwick, M.M., Braune, B.M., Hoekstra, P.F., Muir, D.C., Mabury, S.A., 2004. Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. Environmental science & technology 38, 373-380.

McNabb, F., 2000. Thyroids. Avian physiology. Academic Press, San Diego, 461-472.

McNabb, F.A., 2007. The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. CRC Critical Reviews in Toxicology 37, 163-193.

Meerts, I.A., Van Zanden, J.J., Luijks, E.A., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, Å., Brouwer, A., 2000. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. Toxicological Sciences 56, 95-104.

Mendel, C.M., 1989. The Free Hormone Hypothesis: A Physiologically Based Mathematical Model. Endocrine Reviews 10, 232-274.

Miljeteig, C., Gabrielsen, G.W., 2010. Contaminants in Brünnich's guillemots from Kongsfjorden and Bjørnøya in the period from 1993 to 2007.

Miljeteig, C., Strøm, H., Gavrilo, M.V., Volkov, A., Jenssen, B.M., Gabrielsen, G.W., 2009. High levels of contaminants in ivory gull Pagophila eburnea eggs from the Russian and Norwegian Arctic. Environmental science & technology 43, 5521-5528.

Miller, V.M., Sanchez-Morrissey, S., Brosch, K.O., Seegal, R.F., 2012. Developmental coexposure to polychlorinated biphenyls and polybrominated diphenyl ethers has additive effects on circulating thyroxine levels in rats. Toxicological Sciences 127, 76-83.

Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos, 403-405.

Muir, D., Riget, F., Cleemann, M., Skaare, J., Kleivane, L., Nakata, H., Dietz, R., Severinsen, T., Tanabe, S., 2000. Circumpolar trends of PCBs and organochlorine pesticides in the Arctic marine environment inferred from levels in ringed seals. Environmental science & technology 34, 2431-2438.

Muir, D.C., de Wit, C.A., 2010. Trends of legacy and new persistent organic pollutants in the circumpolar arctic: Overview, conclusions, and recommendations. Science of the total environment 408, 3044-3051.

Muir, D.C., Howard, P.H., 2006. Are there other persistent organic pollutants? A challenge for environmental chemists. Environmental science & technology 40, 7157-7166.

Mulder, P.J., Lie, E., Eggen, G.S., Ciesielski, T.M., Berg, T., Skaare, J.U., Jenssen, B.M., Sørmo, E.G., 2012. Mercury in molar excess of selenium interferes with thyroid hormone function in free-ranging freshwater fish. Environmental science & technology 46, 9027-9037.

Mæhre, S., 2012. Binding of Persistent Organic Pollutants to Thyroid Hormone Receptors in Glaucous Gull (*Larus hyperboreus*), unpublished Master project, UiT, Tromsø, Norway.

Nauman, J.A., Nauman, A., Werner, S.C., 1967. Total and free triiodothyronine in human serum. Journal of Clinical Investigation 46, 1346.

Norstrom, R.J., Simon, M., Muir, D.C., Schweinsburg, R.E., 1988. Organochlorine contaminants in Arctic marine food chains: identification, geographical distribution and temporal trends in polar bears. Environmental science & technology 22, 1063-1071.

Nøst, T.H., Helgason, L.B., Harju, M., Heimstad, E.S., Gabrielsen, G.W., Jenssen, B.M., 2012. Halogenated organic contaminants and their correlations with circulating thyroid hormones in developing Arctic seabirds. science of the total environment 414, 248-256.

Ohmori, K., Kudo, N., Katayama, K., Kawashima, Y., 2003. Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology 184, 135-140.

Orn, U., Klasson-Wehler, E., 1998. Metabolism of 2, 2, 4, 4-tetrabromodiphenyl ether in rat and mouse. Xenobiotica 28, 199-211.

Pacyna, J.M., Breivik, K., Münch, J., Fudala, J., 2003. European atmospheric emissions of selected persistent organic pollutants, 1970–1995. Atmospheric Environment 37, 119-131.

Palha, J.A., Episkopou, V., Maeda, S., Shimada, K., Gottesman, M.E., Saraiva, M., 1994. Thyroid hormone metabolism in a transthyretin-null mouse strain. Journal of Biological Chemistry 269, 33135-33139.

Pocar, P., Klonisch, T., Brandsch, C., Eder, K., Fröhlich, C., Hoang-Vu, C., Hombach-Klonisch, S., 2006. AhR-agonist-induced transcriptional changes of genes involved in thyroid function in primary porcine thyrocytes. Toxicological sciences 89, 408-414.

Power, D., Melo, J., Santos, C., 2000. The effect of food deprivation and refeeding on the liver, thyroid hormones and transthyretin in sea bream. Journal of fish biology 56, 374-387.

Powley, C.R., George, S.W., Ryan, T.W., Buck, R.C., 2005. Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. Analytical chemistry 77, 6353-6358.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. Environmental Science & Technology 40, 32-44.

Rolland, R.M., 2000. A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. Journal of wildlife diseases 36, 615-635.

Sagerup, K., Henriksen, E.O., Skorping, A., Skaare, J.U., Gabrielsen, G.W., 2000. Intensity of parasitic nematodes increases with organochlorine levels in the glaucous gull. Journal of Applied Ecology 37, 532-539.

Sagerup, K., Larsen, H.J.r.S., Skaare, J.U., Johansen, G.M., Gabrielsen, G.W., 2009a. The toxic effects of multiple persistent organic pollutant exposures on the post-hatch immunity maturation of glaucous gulls. Journal of Toxicology and Environmental Health, Part A 72, 870-883.

Sagerup, K., Savinov, V., Savinova, T., Kuklin, V., Muir, D.C., Gabrielsen, G.W., 2009b. Persistent organic pollutants, heavy metals and parasites in the glaucous gull (*Larus hyperboreus*) on Spitsbergen. Environmental pollution 157, 2282-2290.

Scanes, C.G., McNabb, F., 2003. Avian models for research in toxicology and endocrine disruption. Avian and Poultry Biology Reviews 14, 21-52.

Shi, X., Liu, C., Wu, G., Zhou, B., 2009. Waterborne exposure to PFOS causes disruption of the hypothalamus–pituitary–thyroid axis in zebrafish larvae. Chemosphere 77, 1010-1018.

Simon, E., Bytingsvik, J., Jonker, W., Leonards, P.E., de Boer, J., Jenssen, B.M., Lie, E., Aars, J., Hamers, T., Lamoree, M.H., 2011. Blood plasma sample preparation method for the assessment of thyroid hormone-disrupting potency in effect-directed analysis. Environmental science & technology 45, 7936-7944.

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

Skaare, J.U., Larsen, H.J., Lie, E., Bernhoft, A., Derocher, A.E., Norstrom, R., Ropstad, E., Lunn, N.F., Wiig, Ø., 2002. Ecological risk assessment of persistent organic pollutants in the Arctic. Toxicology 181, 193-197.

Smits, J., Fernie, K., Bortolotti, G., Marchant, T., 2002. Thyroid hormone suppression and cell-mediated immunomodulation in American kestrels (*Falco sparverius*) exposed to PCBs. Archives of environmental contamination and toxicology 43, 0338-0344.

StockholmConvention,2010. http://chm.pops.int/Convention/ThePOPs/tabid/673/language/en-US/Default.aspx.Accessed 30.04.14.

Sturkie, P.D., 1965. Avian physiology. Avian physiology. 1965 pp. xxvii+766 pp.

Sørmo, E.G., Ciesielski, T.M., Øverjordet, I.B., Lierhagen, S., Eggen, G.S., Berg, T., Jenssen, B.M., 2011. Selenium moderates mercury toxicity in free-ranging freshwater fish. Environmental science & technology 45, 6561-6566.

Sørmo, E.G., Jussi, I., Jussi, M., Braathen, M., Skaare, J.U., Jenssen, B.M., 2005. Thyroid hormone status in gray seal (*Halichoerus grypus*) pups from the Baltic Sea and the Atlantic Ocean in relation to organochlorine pollutants. Environmental toxicology and chemistry 24, 610-616.

Sørmo, E.G., Salmer, M.P., Jenssen, B.M., Hop, H., Bæk, K., Kovacs, K.M., Lydersen, C., Falk-Petersen, S., Gabrielsen, G.W., Lie, E., 2006. Biomagnification of polybrominated diphenyl ether and hexabromocyclododecane flame retardants in the polar bear food chain in Svalbard, Norway. Environmental toxicology and chemistry 25, 2502-2511.

Thomson, D.L., Monaghan, P., Furness, R.W., 1998. The demands of incubation and avian clutch size. Biological Reviews 73, 293-304.

Trygg, J., Wold, S., 2002. Orthogonal projections to latent structures (O-PLS). Journal of chemometrics 16, 119-128.

Ucán-Marín, F., Arukwe, A., Mortensen, A., Gabrielsen, G.W., Fox, G.A., Letcher, R.J., 2009. Recombinant transthyretin purification and competitive binding with organohalogen compounds in two gull species (*Larus argentatus* and *Larus hyperboreus*). Toxicological sciences 107, 440-450.

UNECE, 1979. The 1979 Geneva Convention on Long-range Transboundary Air Pollution,. http://www.unece.org/env/lrtap. Accessed 12.05.14.

UNEP, 2001. Final Act of the Conference of Plenipotentaries on the Stockholm Convention on Persistent Organic Pollutants. United Nations Environment Program: Stockholm, Sweden,.

UNEP, U.N.E.P., 2008. Preliminary information on riskmanagement evaluation of PFOS's in China. POPRC-SUB F08-PFOS-ADIN-CHI. Stockholm Convention on Persistent Organic Pollutants. Geneva, CH: United Nations Environment Programme;. 2008, p. 6.

Van Birgelen, A.P., Smit, E.A., Kampen, I.M., Groeneveld, C.N., Fase, K.M., Van der Kolk, J., Poiger, H., Van den Berg, M., Koeman, J.H., Brouwer, A., 1995. Subchronic effects of 2, 3, 7, 8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. European Journal of Pharmacology: Environmental Toxicology and Pharmacology 293, 77-85.

Van den Berg, K., Van Raaij, J., Bragt, P., Notten, W., 1991. Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels in vivo. Archives of toxicology 65, 15-19.

Verhoelst, C., Geyten, S., Roelens, S., Darras, V., 2005. Regulation of Thyroid Hormone Availability by Iodothyronine Deiodinases at the Blood-Brain Barrier in Birds. Annals of the New York Academy of Sciences 1040, 501-503.

Verreault, J., Bech, C., Letcher, R.J., Ropstad, E., Dahl, E., Gabrielsen, G.W., 2007. Organohalogen contamination in breeding glaucous gulls from the Norwegian Arctic: Associations with basal metabolism and circulating thyroid hormones. Environmental Pollution 145, 138-145.

Verreault, J., Gabrielsen, G., Bustnes, J., 2010. The Svalbard glaucous gull as bioindicator species in the European Arctic: Insight from 35 years of contaminants research, Reviews of Environmental Contamination and Toxicology Volume 205, Springer, pp. 77-116.

Verreault, J., Gabrielsen, G.W., Chu, S., Muir, D.C., Andersen, M., Hamaed, A., Letcher, R.J., 2005a. Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian Arctic top predators: glaucous gulls and polar bears. Environmental science & technology 39, 6021-6028.

Verreault, J., Houde, M., Gabrielsen, G.W., Berger, U., Haukås, M., Letcher, R.J., Muir, D.C., 2005b. Perfluorinated alkyl substances in plasma, liver, brain, and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. Environmental science & technology 39, 7439-7445.

Verreault, J., Letcher, R.J., Muir, D.C., Chu, S., Gebbink, W.A., Gabrielsen, G.W., 2005c. New organochlorine contaminants and metabolites in plasma and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. Environmental Toxicology and Chemistry 24, 2486-2499.

Verreault, J., Skaare, J.U., Jenssen, B.M., Gabrielsen, G.W., 2004. Effects of organochlorine contaminants on thyroid hormone levels in Arctic breeding glaucous gulls, *Larus hyperboreus*. Environmental health perspectives 112, 532.

Verreault, J., Villa, R.A., Gabrielsen, G.W., Skaare, J.U., Letcher, R.J., 2006. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. Environmental pollution 144, 1053-1060.

Villanger, G.D., Lydersen, C., Kovacs, K.M., Lie, E., Skaare, J.U., Jenssen, B.M., 2011. Disruptive effects of persistent organohalogen contaminants on thyroid function in white whales *(Delphinapterus leucas*) from Svalbard. Science of the Total Environment 409, 2511-2524.

Wade, M.G., Parent, S., Finnson, K.W., Foster, W., Younglai, E., McMahon, A., Cyr, D.G., Hughes, C., 2002. Thyroid toxicity due to subchronic exposure to a complex mixture of 16 organochlorines, lead, and cadmium. Toxicological Sciences 67, 207-218.

Weiss, J.M., Andersson, P.L., Lamoree, M.H., Leonards, P.E., van Leeuwen, S.P., Hamers, T., 2009. Competitive binding of poly-and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicological Sciences 109, 206-216.

Wentworth, B., Ringer, R., 1986. Thyroids, Avian physiology, Springer, pp. 452-465.

Wiklund, S., Johansson, E., Sjöström, L., Mellerowicz, E.J., Edlund, U., Shockcor, J.P., Gottfries, J., Moritz, T., Trygg, J., 2008. Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. Analytical Chemistry 80, 115-122.

Wold, A., Jæger, I., Hop, H., Gabrielsen, G.W., Falk-Petersen, S., 2011. Arctic seabird food chains explored by fatty acid composition and stable isotopes in Kongsfjorden, Svalbard. Polar biology 34, 1147-1155.

Wolkers, H., Van Bavel, B., Derocher, A.E., Wiig, Ø., Kovacs, K.M., Lydersen, C., Lindström, G., 2004. Congener-specific accumulation and food chain transfer of

polybrominated diphenyl ethers in two Arctic food chains. Environmental science & technology 38, 1667-1674.

Xu, L., Krenitsky, D.M., Seacat, A.M., Butenhoff, J.L., Anders, M., 2004. Biotransformation of N-ethyl-N-(2-hydroxyethyl) perfluorooctanesulfonamide by rat liver microsomes, cytosol, and slices and by expressed rat and human cytochromes P450. Chemical research in toxicology 17, 767-775.

Zoeller, R.T., 2005. Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemicals? Molecular and cellular endocrinology 242, 10-15.

Zoeller, R.T., Miller, M., Crofton, K., Rice, D., 2009. Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. Environmental Health Perspective 117, 1033-1041.

Zoeller, R.T., Tan, S.W., Tyl, R.W., 2007. General background on the hypothalamic-pituitary-thyroid (HPT) axis. CRC Critical Reviews in Toxicology 37, 11-53.

Zoeller, T.R., Dowling, A.L., Herzig, C.T., Iannacone, E.A., Gauger, K.J., Bansal, R., 2002. Thyroid hormone, brain development, and the environment. Environmental Health Perspectives 110, 355.

Örn, U., 1997. Synthesis of Polybrominated Diphenyl Ethers and Metabolism of 2, 2', 4, 4'-tetrabromo [14C] diphenyl Ether, Fil. lic. Thesis, Stockholm University, Stockholm, Sweden.

APPENDIX A: Detection limits of OHCs

Table A1. Limits of detection (LOD, ng/g ww) for OHCs analyzed in plasma of glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013.

	LOD		
Compound	2011	2012	2013
α-HCH	0.015	0.009	0.011
β-HCH	0.046	0.027	0.022
γ-HCH	0.008	0.012	0.006
HCB	0.334	0.335	0.236
c-chlordane	0.006	0.007	0.007
t-chlordane	0.004	0.003	0.002
oxy-chlordane	0.376	0.323	0.125
c-nonachlor	0.005	0.005	0.006
t-nonachlor	0.004	0.002	0.002
mirex	0.046	0.024	0.018
o,p'-DDT	0.010	0.007	0.007
p,p'-DDT	0.022	0.011	0.011
o,p'-DDD	0.002	0.001	0.001
p,p'-DDD	0.002	0.001	0.001
o,p'-DDE	0.001	0.001	0.001
p,p'-DDE	0.002	0.001	0.002
PCB-28	0.011	0.003	0.015
PCB-52	0.011	0.007	0.008
PCB-99	0.022	0.015	0.019
PCB-101	0.025	0.017	0.021
PCB-105	0.024	0.016	0.021
PCB-118	0.022	0.015	0.018
PCB-138	0.680	0.439	0.595
PCB-153	0.532	0.345	0.455
PCB-180	0.021	0.019	0.019
PCB-183	0.016	0.015	0.015
PCB-187	0.019	0.018	0.017
PCB-194	0.185	0.129	0.183
PBDE-28	0.100	0.100	0.100
PBDE-47	0.040	0.040	0.040
PBDE-66	0.465	0.465	0.465
PBDE-85	0.199	0.199	0.199
PBDE-99	0.025	0.025	0.025
PBDE-100	0.050	0.050	0.050
PBDE-138	0.417	0.417	0.417
PBDE-153	0.150	0.150	0.150
PBDE-154	0.125	0.125	0.125
PBDE-183	0.250	0.250	0.250
PFHxS	0.030	0.030	0.030
PFBS	0.050	0.050	0.050
PFOS	0.03	0.03	0.03
Iso-PFOS	0.03	0.03	0.03
PFOSA	0.29	0.29	0.29
PFDS	0.085	0.085	0.085
PFHxA	0.02	0.02	0.02
PFHpA	0.01	0.01	0.01
PFOA	0.01	0.01	0.01
PFNA	0.05	0.05	0.05
PFDcA	0.05	0.05	0.05
PFUnA	0.08	0.08	0.08
PFDoA	0.04	0.04	0.04
PFTrA	0.07	0.07	0.07
PFTeA	0.09	0.09	0.09
6:2 FTS	0.03	0.03	0.03

APPENDIX B: Individual biometric measures

Table B1. Biological measurements, year of capture, body condition index (BCI) and plasma lipid % of the individual male (M, n=15) and female (F, n=24) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013.

ID	Sex	Capture year	Body mass (g)	Head (mm)	Bill (mm)	Gonys (mm)	Wing (mm)	BCI	Lipid %
ACA	M	2011	1740	150.0	63.3	22.8	495	0.0469	1.6939
ACF	M	2011	2030	154.0	67.3	23.3	455	2.2556	1.6187
AAZ	M	2011	1760	154.0	67.0	26.0	480	-0.5941	1.1722
ABJ	M	2011	1740	156.0	64.4	25.0	489	-0.6197	1.2109
AAV	M	2011	1740	148.5	65.6	24.6	502	-0.2494	1.4394
ADT	M	2012	1750	153.1	65.2	23.2	493	-0.1975	1.3399
3037402	M	2012	1730	150.5	62.8	23.2	480	-0.0655	1.4965
ACL	M	2012	1760	152.0	64.9	23.3	501	-0.0524	1.6573
AJD	M	2013	1685	148.0	66.2	22.7	473	-0.476	1.4582
ADN	M	2013	1680	152.0	63.5	22.2	454	-0.4816	1.2034
AJH	M	2013	1810	151.0	63.9	23.4	498	0.5184	1.4798
AAJ	M	2013	1820	151.0	65.0	22.9	487	0.6244	1.3961
AJN	M	2013	1900	149.0	61.0	23.9	488	1.5929	1.5048
AJS	M	2013	1535	147.6	64.1	23.4	459	-1.7679	1.5186
AJV	M	2013	1715	152.0	67.2	22.9	487	-0.5341	1.4626
ACC	F	2011	1440	140.0	59.7	21.1	465	-0.5412	1.2985
ABD	F	2011	1400	135.0	57.9	21.0	460	-0.3353	1.7203
ACH	F	2011	1410	133.7	55.7	21.5	469	-0.0256	1.6551
ABV	F	2011	1380	136.0	56.0	20.2	454	-0.2309	1.6784
AAD	F	2011	1440	141.0	60.4	22.7	470	-1.1187	1.4677
ABX	F	2011	1520	136.0	58.7	20.6	478	0.9856	1.4968
ACJ	F	2011	1400	136.0	56.3	21.0	462	-0.2573	0.9727
ADF	F	2012	1700	141.5	59.9	23.0	475	2.2444	1.4299
ACB	F	2012	1475	135.7	57.9	22.4	461	0.2816	1.5853
ADS	F	2012	1420	139.0	58.1	20.5	474	-0.4865	1.3319
ADD	F	2012	1555	135.6	56.6	20.4	485	1.71	1.2359
ADH	F	2012	1385	133.6	55.1	21.2	460	-0.1217	1.401
ADJ	F	2012	1315	134.7	56.1	20.3	473	-1.2055	1.5584
ABC	F	2012	1600	139.3	59.0	21.1	474	1.6471	1.5344
AJB	F	2013	1345	137.2	56.7	20.7	472	-1.1816	1.3063
AJC	F	2013	1490	139.0	58.0	20.6	467	0.5072	1.1999
AAT	F	2013	1455	134.0	57.9	21.3	458	0.4427	1.5747
AFB	F	2013	1440	136.0	55.0	19.5	455	0.8317	1.9797
AJF	F	2013	1440	136.0	57.6	20.7	461	0.1988	1.5776
AJL	F	2013	1390	137.0	57.5	21.0	457	-0.5726	1.2596
ADL	F	2013	1450	137.0	56.2	21.2	458	0.3266	1.6578
ABA	F	2013	1320	134.0	57.0	20.5	452	-1.0134	1.4795
AFC	F	2013	1435	134.9	57.8	20.1	441	0.5431	1.6823
AJX	F	2013	1400	141.3	63.1	20.6	451	-1.3421	1.1051

APPENDIX C: Individual OHC concentrations

Table C1. Individual OHC concentrations (ng/g ww) detected in plasma of male (M, n=15) and female (F, n=24) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013, quantified by GC-MS. Randomly generated concentrations for samples with levels <LOD are included in brackets.

ID	Sex	β-HCH	HCB	*c*-chlordane	*oxy*-chlordane	*t*-nonachlor	*c*-nonachlor	mirex	*p,p'*-DDE	PBDE-47
ACA	M	0.38	9.93	0.01	11.40	0.30	0.49	5.25	5.11	2.98
ACF	M	0.19	5.95	0.01	5.95	0.40	0.24	3.04	2.13	1.26
AAZ	M	0.73	15.90	0.01	41.9	0.87	0.34	19.40	10.80	2.73
ABJ	M	0.90	15.70	0.03	38.50	1.88	0.50	15.90	11.90	1.95
AAV	M	0.87	11.60	0.02	10.50	0.99	1.26	6.89	4.77	2.51
ADT	M	0.17	4.36	0.03	7.44	0.37	0.28	3.17	3.60	1.23
3037402	M	0.54	13.20	0.01	34.60	0.47	0.61	16.10	10.60	2.25
ACL	M	0.93	12.50	0.02	23.20	1.26	1.41	11.00	7.98	3.60
AJD	M	0.45	6.60	0.01	10.00	1.83	1.15	3.63	4.45	3.13
ADN	M	0.28	5.62	0.01	4.74	1.38	0.71	3.59	2.16	1.26
AJH	M	0.89	13.4	0.02	26.50	2.67	0.87	10.40	9.37	3.77
AAJ	M	0.28	6.07	0.01	8.54	0.09	0.14	4.63	2.20	1.43
AJN	M	0.35	8.69	0.02	6.38	0.76	0.48	2.79	2.62	1.45
AJS	M	0.99	12.2	0.02	16.40	0.38	0.54	8.96	8.71	4.47
AJV	M	0.42	8.84	0.02	12.90	1.14	0.86	5.41	3.56	1.97
ACC	F	0.19	6.67	0.02	8.31	0.47	0.64	4.36	2.43	1.13
ABD	F	0.55	15.1	0.02	25.20	4.82	1.98	7.99	5.26	1.94
ACH	F	0.17	8.05	0.01	4.86	0.58	0.52	1.93	1.39	1.01
ABV	F	0.08	4.02	0.01	1.95	0.52	0.34	0.88	0.92	0.47
AAD	F	0.14	7.50	0.02	5.37	0.35	0.55	2.08	1.47	1.25
ABX	F	0.11	5.06	0.01	3.05	0.41	0.42	1.40	0.77	0.78
ACJ	F	0.28	8.51	0.03	7.31	0.70	1.12	2.41	1.49	0.64
ADF	F	0.25	7.82	0.01	4.69	3.26	1.44	2.80	2.76	2.34
ACB	F	0.12	6.08	0.02	5.27	0.47	0.63	1.91	1.29	0.85
ADS	F	0.16	6.64	0.04	4.63	0.86	0.74	1.39	1.26	0.98
ADD	F	0.11	3.97	0.01	2.54	0.48	4.74	0.85	0.74	0.87
ADH	F	0.39	7.20	0.08	11.60	1.15	1.07	3.04	3.54	1.19
ADJ	F	0.20	7.43	0.01	4.63	0.55	0.65	2.17	1.53	1.05
ABC	F	0.31	8.58	0.02	14.50	0.67	1.01	8.64	3.46	1.55
AJB	F	0.18	5.47	0.01	3.59	0.67	1.04	1.49	1.21	2.61
AJC	F	0.24	5.23	0.02	4.80	1.08	1.14	2.04	1.85	1.93
AAT	F	0.51	9.51	0.03	17.00	1.79	2.00	10.20	6.42	2.48
AFB	F	0.69	8.62	0.04	9.21	1.83	1.74	3.31	4.51	3.04
AJF	F	0.26	7.46	0.02	13.10	1.07	0.87	9.32	4.19	1.11
AJL	F	0.34	7.88	0.03	17.20	0.87	0.61	4.18	4.31	2.13
ADL	F	0.12	4.56	(0.006)	3.00	0.26	0.40	1.43	1.21	1.42
ABA	F	0.24	6.93	(0.006)	4.75	0.41	0.98	1.69	1.79	1.92
AFC	F	0.52	9.35	0.06	13.60	2.53	1.46	3.78	4.54	6.24
AJX	F	0.54	10.90	0.02	18.10	1.80	1.72	6.89	9.00	6.68

Table C2. Individual OHC concentrations (ng/g ww) detected in plasma of male (M, n=15) and female (F, n=24) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 andv2013, quantified by GC-MS. Randomly generated concentrations for samples with levels <LOD are included in brackets.

ID	Sex	PCB-28	PCB-52	PCB-99	PCB-105	PCB-118	PCB-138	PCB-153	PCB-180	PCB-183	PCB-187	PCB-194
ACA	M	0.18	0.20	5.80	2.25	10.20	25.60	45.80	19.80	3.18	4.62	2.56
ACF	M	0.09	0.12	2.40	0.94	4.14	9.68	20.10	9.78	1.41	2.24	1.57
AAZ	M	0.39	0.20	13.90	6.15	30.20	63.60	162.00	75.50	9.95	6.75	9.63
ABJ	M	0.30	(0.011)	13.40	4.73	23.60	66.40	143.00	82.40	9.59	9.35	12.20
AAV	M	0.23	0.50	5.53	2.36	9.85	23.80	52.30	21.80	3.69	6.32	2.90
ADT	M	0.09	(0.003)	3.85	1.17	5.25	17.50	35.80	13.50	2.11	2.80	1.60
3037402	M	0.24	0.21	12.60	5.24	24.80	59.40	134.00	66.70	9.29	6.88	8.97
ACL	M	0.29	0.23	8.49	3.92	17.10	33.20	70.60	35.90	5.68	8.68	4.38
AJD	M	0.26	0.04	6.55	0.31	11.00	22.80	36.70	12.90	2.16	4.17	1.28
ADN	M	0.14	0.05	2.93	1.41	5.79	10.50	26.80	14.00	2.05	2.84	2.00
AJH	M	0.42	0.04	13.60	5.43	23.10	43.30	98.40	43.00	6.08	8.58	6.46
AAJ	M	0.15	(0.007)	3.43	1.62	6.66	16.50	28.60	16.10	2.42	2.50	2.63
AJN	M	0.20	0.04	3.93	2.10	7.97	13.80	22.00	9.81	1.62	2.89	1.17
AJS	M	0.40	0.27	11.30	4.58	19.30	45.60	104.00	35.70	6.28	8.62	4.22
AJV	M	0.25	0.05	5.15	2.60	10.60	23.90	41.70	26.20	3.49	3.92	4.49
ACC	F	0.11	0.24	2.77	1.34	5.65	10.10	27.80	15.20	1.95	1.82	2.34
ABD	F	0.27	0.12	7.51	3.67	14.70	26.90	58.10	28.00	3.75	3.68	3.94
ACH	F	0.12	0.25	2.05	0.98	3.79	8.26	15.40	6.33	1.02	1.45	0.80
ABV	F	0.05	(0.007)	0.89	0.35	1.39	2.87	6.53	2.63	0.49	0.99	0.42
AAD	F	0.13	(0.011)	2.04	1.12	3.88	7.74	18.10	7.55	1.10	1.25	1.10
ABX	F	0.08	0.15	1.15	0.58	2.05	5.06	9.37	4.73	0.68	0.82	0.70
ACJ	F	0.14	(0.008)	2.00	1.05	3.65	7.26	17.30	7.47	1.12	1.79	1.15
ADF	F	0.14	(0.004)	3.68	1.61	6.03	18.30	23.90	8.82	1.61	3.33	0.96
ACB	F	0.11	0.09	1.99	1.08	3.78	6.21	13.50	5.48	0.90	1.23	0.73
ADS	F	0.09	0.24	1.72	0.87	3.00	6.08	11.00	5.37	0.83	1.16	0.83
ADD	F	0.07	0.08	1.20	0.60	2.23	3.50	9.05	2.63	0.44	0.85	0.32
ADH	F	0.13	(0.005)	4.29	1.12	4.59	16.70	29.60	10.70	1.91	3.11	1.25
ADJ	F	0.13	0.19	2.18	1.09	4.06	8.07	16.90	7.79	1.10	1.73	1.13
ABC	F	0.14	0.09	4.14	2.20	9.38	19.40	49.70	30.70	3.75	2.60	5.11
AJB	F	0.14	0.30	2.08	1.05	3.49	8.43	13.70	6.89	1.05	1.47	1.08
AJC	F	0.14	0.38	2.48	1.26	4.51	8.59	20.10	9.13	1.44	2.03	1.14
AAT	F	0.30	0.75	7.65	4.03	17.20	31.00	87.00	52.80	6.59	4.91	7.32
AFB	F	0.22	0.74	5.19	2.37	8.69	20.80	31.90	11.60	2.11	4.65	1.42
AJF	F	0.15	0.25	0.51	2.65	12.10	24.90	72.50	48.70	5.54	2.13	7.57
AJL	F	0.17	0.03	6.44	2.31	8.78	23.00	50.10	22.00	3.07	3.45	2.80
ADL	F	0.09	0.09	1.69	0.88	2.92	7.15	8.43	4.17	0.71	1.23	0.71
ABA	F	0.14	(0.004)	2.97	1.43	4.79	11.00	12.70	5.43	1.02	2.18	0.65
AFC	F	0.24	0.71	6.05	2.10	9.89	19.20	28.10	11.80	2.32	5.25	1.21
AJX	F	0.33	0.42	11.10	4.56	15.70	37.10	53.40	27.10	4.79	9.72	2.98

Table C3. Individual OHC concentrations (ng/g ww) detected in plasma of male (M, n=15) and female (F, n=24) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013, quantified by UHPLC-MS/MS. Randomly generated concentrations for samples with levels <LOD are included in brackets.

ID	Sex	PFHxS	PFOS	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFTrA	PFTeA	PFOSA
ACA	M	1.19	26.99	(0.006)	2.47	0.83	3.37	1.16	3.44	0.36	0.88
ACF	M	1.89	21.54	0.34	4.34	1.00	4.54	1.50	4.91	0.85	3.67
AAZ	M	0.58	12.23	(0.005)	2.81	1.27	6.68	1.89	7.73	1.36	0.56
ABJ	M	0.41	6.84	0.10	2.40	0.81	3.25	0.96	3.46	0.52	(0.12)
AAV	M	0.67	18.34	(0.005)	6.25	1.90	5.45	0.97	4.22	0.52	1.43
ADT	M	0.49	10.02	(0.003)	1.15	0.67	4.80	1.05	2.96	0.36	0.50
3037402	M	0.94	33.34	(0.004)	5.49	2.56	7.76	1.69	4.99	0.71	1.49
ACL	M	1.13	18.10	0.13	4.42	1.80	6.74	1.71	4.98	0.74	1.43
AJD	M	0.03	12.29	0.03	1.18	0.96	3.78	0.99	2.81	0.39	0.39
ADN	M	(0.015)	7.47	0.14	2.23	0.57	2.95	0.65	2.52	0.37	0.66
AJH	M	0.50	5.98	0.12	2.15	0.82	2.97	0.82	3.15	0.52	0.78
AAJ	M	1.60	13.42	0.13	1.41	0.56	3.16	1.07	3.70	0.52	0.40
AJN	M	0.57	10.85	0.12	1.35	0.45	2.47	0.72	2.92	0.48	0.89
AJS	M	0.20	10.33	(0.007)	0.95	0.76	4.60	1.19	3.85	0.46	0.35
AJV	M	0.22	6.21	0.06	1.52	0.89	3.81	0.99	3.68	0.42	(0.19)
ACC	F	1.22	10.87	0.68	2.73	1.21	4.09	0.87	3.23	0.38	0.39
ABD	F	0.37	10.49	0.09	2.86	1.22	5.06	1.09	3.38	0.43	0.33
ACH	F	0.63	9.14	0.35	3.35	1.23	4.81	1.06	4.72	0.47	0.72
ABV	F	1.09	11.52	0.31	2.81	0.63	3.36	0.77	3.26	0.32	1.81
AAD	F	6.15	194.57	0.25	2.88	1.14	4.31	0.88	2.82	0.26	(0.08)
ABX	F	1.11	24.82	0.20	3.81	0.97	5.09	0.97	3.30	0.35	1.05
ACJ	F	(0.012)	6.08	0.12	1.41	0.86	4.87	1.00	3.24	0.44	(0.20)
ADF	F	18.73	507.66	0.26	1.03	0.55	2.21	0.69	1.45	(0.08)	0.56
ACB	F	0.27	11.55	(0.007)	4.42	2.09	10.60	2.49	5.88	0.54	0.84
ADS	F	0.50	11.48	(0.004)	1.96	1.34	7.20	1.42	4.67	0.33	0.78
ADD	F	0.57	9.69	0.22	2.47	0.93	3.67	0.58	2.31	0.09	0.89
ADH	F	0.90	4.53	0.25	2.51	0.63	3.37	0.73	3.34	0.62	0.61
ADJ	F	0.37	6.02	0.26	1.81	0.55	3.08	0.78	2.65	0.33	0.60
ABC	F	1.05	15.10	0.75	4.38	1.66	6.10	1.15	3.50	0.27	0.43
AJB	F	0.70	19.02	0.33	1.36	0.53	2.72	0.75	2.04	0.21	0.53
AJC	F	0.37	9.16	0.11	1.33	0.59	2.59	0.44	1.88	0.19	1.09
AAT	F	0.15	4.90	0.42	2.16	0.51	1.94	0.26	1.40	0.16	0.80
AFB	F	(0.008)	6.70	0.34	2.05	0.62	3.19	0.72	2.23	0.39	0.80
AJF	F	0.37	7.70	(0.007)	1.95	0.72	2.85	0.60	2.08	0.32	0.62
AJL	F	0.60	4.93	(0.005)	0.30	0.31	1.28	0.21	1.12	0.07	0.48
ADL	F	4.36	53.95	0.35	2.51	0.73	2.52	0.52	2.16	0.22	0.83
ABA	F	9.36	183.21	0.30	2.25	0.73	2.53	0.65	1.75	(0.04)	(0.10)
AFC	F	0.25	2.89	(0.009)	1.23	0.48	1.53	0.25	1.20	(0.07)	(0.05)
AJX	F	(0.016)	2.56	0.10	1.03	0.36	1.33	0.23	0.98	(0.06)	(0.20)

APPENDIX D: Individual TH concentrations

Table D1. Individual levels of total T3 and T4 (nmol/L) and free T3 and T4 (pmol/L) in male (M, n=15) and female (F, n=24) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013.

ID	Sex	TT3 (nmol/L)	TT4 (nmol/L)	FT3 (pmol/L)	FT4 (pmol/L)
ACA	M	1.848	14.893	0.795	4.936
ACF	M	4.379	13.672	6.119	12.850
AAZ	M	1.709	43.555	1.730	23.600
ABJ	M	1.098	22.814	1.184	14.825
AAV	M	2.115	26.076	3.450	24.988
ADT	M	2.101	32.214	1.478	12.803
3037402	M	2.814	13.645	3.579	8.196
ACL	M	0.715	43.629	5.732	129.009
AJD	M	1.592	13.164	1.585	8.717
ADN	M	2.769	16.276	4.056	12.319
AJH	M	1.856	18.558	2.373	15.522
AAJ	M	3.233	29.397	4.726	24.600
AJN	M	3.810	14.075	5.058	10.573
AJS	M	1.952	43.710	5.093	133.153
AJV	M	1.149	22.180	1.418	18.763
ACC	F	1.616	24.339	2.121	15.403
ABD	F	1.550	10.617	1.540	6.255
ACH	F	3.034	24.534	4.283	23.029
ABV	F	1.903	30.534	3.022	21.248
AAD	F	4.594	27.171	6.569	20.953
ABX	F	1.350	30.004	2.157	28.513
ACJ	F	2.306	47.233	3.267	36.060
ADF	F	3.257	24.915	3.756	16.010
ACB	F	2.699	29.325	3.522	24.386
ADS	F	4.540	37.112	6.480	31.032
ADD	F	1.758	36.237	2.961	32.519
ADH	F	2.368	22.062	3.003	13.110
ADJ	F	2.000	25.523	0.736	9.956
ABC	F	1.558	32.208	1.307	17.438
AJB	F	2.390	47.457	3.316	37.145
AJC	F	2.950	44.592	5.069	39.074
AAT	F	3.017	24.520	3.108	13.535
AFB	F	2.541	37.014	2.327	20.277
AJF	F	1.778	23.360	1.789	17.788
AJL	F	1.671	50.901	2.032	41.473
ADL	F	3.589	24.629	4.614	21.179
ABA	F	2.643	29.401	2.955	19.574
AFC	F	1.286	31.697	0.880	18.508
AJX	F	0.815	21.353	0.549	19.026

APPENDIX E: OPLS-regression model (Y=BCI)

OPLS regression with body condition index (BCI) as Y variable was performed for male glaucous gulls to assess which variables are most related to body condition. This resulted in a significant (p= 0.049) model (R^2X=0.707, R^2Y=0.945, Q^2=0.729) (Figure E1 and E2).

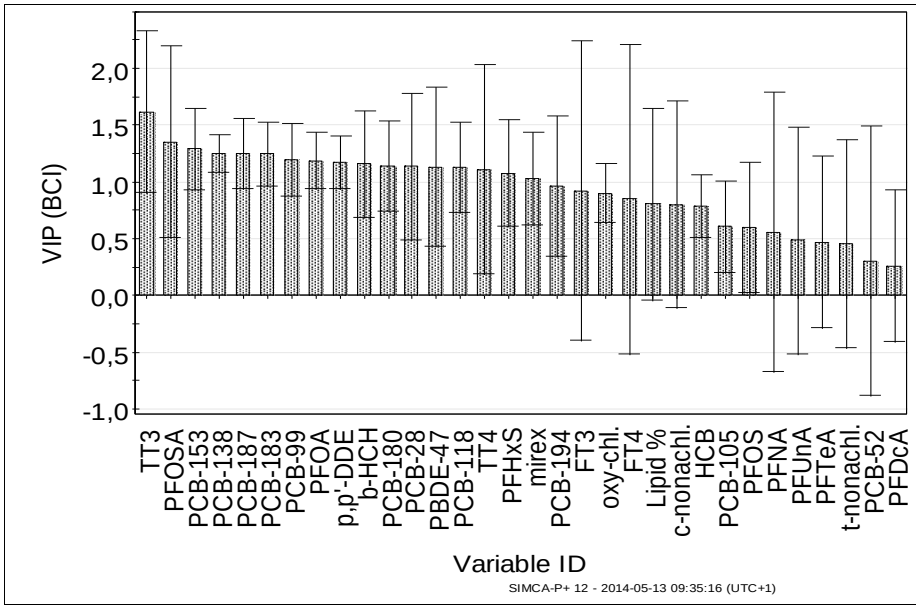

Figure E1. OPLS VIP plot organizing the X-variables according to their explanatory power of BCI in male (n=15) glacous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. Variables with VIP-values over 1.0 are considered most important in explaining BCI. The error bars represent the 95 % confidence interval.

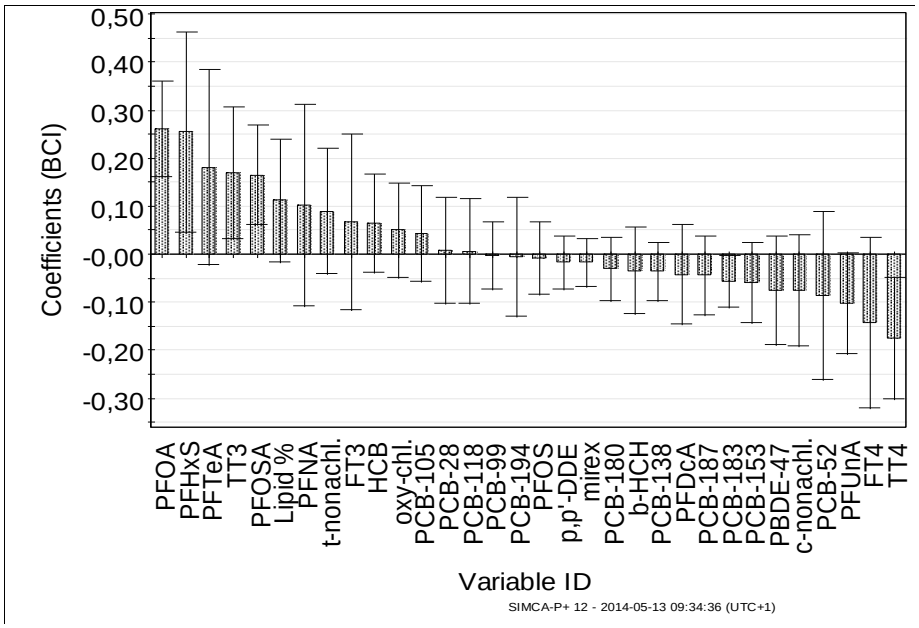

Figure E2. OPLS coefficient plot describing the direction of relationships between X-variables and BCI in male (n=15) glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard during the summer of 2011, 2012 and 2013. The error bars represent the 95% confindece interval.