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The Effect of Persistent Organic Pollutants on Thyroid Hormone Levels in Arctic Breeding Kittiwake (*Rissa tridactyla*)

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Environmental Toxicology and Chemistry
Submission date: July 2011
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Cover photo: Kittiwake, Kongsfjorden, Svalbard. Photo by Silje Solheim

Preface

This master thesis was written at Department of Biology, at the Norwegian University of Science and Technology (NTNU). The thesis is a part of the International Polar Year (IPY) project "Contaminants in polar regions" (COPOL). The present project was financed by the Norwegian Polar Institute (NPI). The field work was carried out in Kongsfjorden, Svalbard. The field work 2008 was done by Tore Nordstad, while the field work in 2009 was done by the writer of the present study. The thyroid hormone analyses were performed at Chizé Centre for Biological Studies (CEBC), France, and the persistent organic pollutants analyses at the Norwegian Institute for Air Research (NILU), Tromsø. The thesis was written under the supervision of Prof. Geir Wing Gabrielsen, NPI and Prof. Bjørn Munro Jenssen, NTNU.

There have been several unforeseen challenges during the work with this master thesis, which have caused changes of the thesis throughout the process. This includes non-comparable blood samples from the chick-rearing period, non-comparable thyroid hormone samples collected and analysed in 2007, and contamination of all the whole blood samples collected in 2009.

Several persons have helped me along the way. I am so thankful for their help, making this thesis possible. First I would like to thank my supervisor Geir Wing Gabrielsen who gave me the opportunity to work with kittiwakes at Svalbard. Thank you for always being in a good mood and your positive attitude. Next, I would like to thank Bjørn Munro Jenssen for your guidance with the written material, and your encouragement and support during this process.

I am grateful for Claus Bech and Børge Moe, sharing their knowledge about kittiwake biology and Svalbard with me. Elin Noreen, I am so thankful for all your help during this process. You have been so wonderful to me. Dorte Hertze, Charline Parenteau and Oliver Chastel have been very helpful with the laboratory work. Thank you Olivier, for letting me stay in Chize. Also, thank you Aurleie Goutte and Olivier Chastel, for letting me use your field data. Thanks to Oddveig Øien Ørvoll, for letting me use the map from Kongsfjorden.

Silje, you have been a fantastic field assistant, it had never been the same without you. I will never forget our adventures at Krykkjefjellet. Thank you Tore, for advice, your happy face, and a place to stay when visiting NPI and NILU in Tromsø. Furthermore, I would like to thank fellow students and roommates for making Trondheim an inspiring place to study, and my family for their support. Last, but not least, thank you Håvard for believing in me, and for your optimistic thoughts.

Trondheim, June 2011

Ida Egge Johnsen

Abstract

Black-legged kittiwakes (*Rissa tridactyla*) breeding in the Arctic, are exposed to annual fluctuating environmental conditions. The impact of inter-year variations in environmental conditions on the blood concentrations of persistent organic pollutants (POPs) was examined in black-legged kittiwakes breeding in Kongsfjorden, Svalbard. In addition, it was examined if inter-year variations in concentration of POPs were affecting circulating thyroid hormone (TH) levels.

Blood samples were collected from breeding kittiwakes during the incubation period in 2008 (n=46) and 2009 (n=31), two different years in respect of environmental conditions. The whole blood samples were analyzed for POPs: polychlorinated biphenyls (PCBs; PCB-28, -52, -99, -118, -101, -138, -153, 180, -183, and -187), hexachlorobezene (HCB), hexachlorocyclohexan (HCH), dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE) oxychlordane, cis-chlordane, trans-chlordane, cis-nonachlor, trans-nonachlor, heptachlor, heptachloroepoxid, and mirex, using gas chromatography - mass spectrometry. Plasma was analysed for concentrations of total triiodothyronine (T3), using radioimmunoassay. Associations between variables of POPs and T3 were analysed using univariate statistics. Biometric measurements of the kittiwakes; body mass, skull length, and wing length where included in the statistical analysis, together with calculated body condition (BC; body mass controlled for body size). Associations between the variables were analysed using multivariate statistics.

The PCBs were the dominant contaminants in both 2008 and 2009. The three major constituents in the kittiwakes were PCB-153, followed by PCB-138 and PCB-180. Even though the BC of the kittiwakes was significantly poorer in 2009 compared to 2008, the concentrations of PCBs and DDE did not differ between 2008 and 2009. The concentration (ng/g wet weight [w.w.]) of HCB and oxychlordane were significantly higher in 2009 compared to 2008. There was a strong negative association between HCB and BC of the kittiwakes. Also the concentration of oxychlordane was inversely correlated to BC. Thus, kittiwakes with poor BC had higher levels of HCB and oxychlordane. Additionally, HCB, oxychlordane and PCB-183 were positively associated with egg-laying date, while BC was negatively correlated to egg-laying date. The results showed that kittiwakes with a poor BC and thus, high levels of HCB and oxychlordane, were associated with late breeding. The low

BC in 2009 indicated poor food availability and unfavourable conditions for the kittiwakes that year.

There were no associations between whole blood concentrations of POPs and plasma T3 levels in the kittiwakes, in neither 2008 nor 2009. This is probably due to that the concentrations of POPs were below the threshold for POP-induced alterations on the circulating T3 levels in kittiwakes from Kongsfjorden, Svalbard.

List of abbreviations

∑PCBs	Sum of individual polychlorinated biphenyl congeners (PCBs)
BC	Body condition
BM	Body mass
BMR	Basal metabolic rate
CECB	Chizé Centre for Biological Studies
Cl	Chlorine
Cpm	Counts per minute
CV	Coefficient of variation
CYP3A	Cytochrome 3A
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
EDCs	Endocrine disrupting chemicals
EI	Electron ionization
GC-MS	Gas chromatograph - mass spectrometry
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HPT	Hypothalamus-pituitary-thyroid
id	Inner diameter
K_{ow}	Partitioning coefficient between octanol and water
LOD	Limit of detection
NCI	Negative chemical ionization
NILU	Norwegian Institute of Air Research
NIST	National Institute of Standards and Technology
NTNU	Norwegian University of Science and Technology
PBDEs	Polybrominated diphenyl ethers
PC	Principal component
PCA	Principal component analysis
PCBs	Polychlorinated biphenyls
PCR	Polymerase chain reaction
PFAS	Perfluoroalkyl substance
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
POPs	Persistent organic pollutants
Q2	Goodness of prediction
Q-std	Quantification standard
R2x	Goodness of fit

RIA	Radioimmunoassay
RRF	Relative response factor
SE	Standard error
SD	Standard deviation
SIM	Selective ion monitoring
SRM	Standard reference material
T3	Triiodothyronine
T4	Thyroxin
THs	Thyroid hormones
TSH	Thyroid – stimulating hormone
TTR	Transthyretin
UV	Unit variance
w.w.	Wet weight
ZW	Heterogametic
ZZ	Homogametic

Table of contest

1. Introduction

The Arctic is characterized by long food chains, confined species diversity, as well as temporally and spatially variations in the external environment (AMAP 1998, Borga et al. 2004). The fluctuations in the external factors (i.e. food availability, temperature and sea-ice conditions) expose species breeding in the Arctic, to varying levels of natural stress (Bustnes et al. 2008a, Gaston et al. 2005, Moe et al. 2009). In addition, the wildlife is affected by man-made stress, such as persistent organic pollutants (POPs) (AMAP 1998). Studies conducted during the last decade have reported a decreasing trend in the levels of POPs in Arctic wildlife (Barrett et al. 1996, Bustnes et al. 2010b, Murvoll et al. 2006). The decreasing POP levels are a result of restrictions in the use of many pesticides and industrial chemicals since the 1970s (Bustnes et al. 2010b). In addition, in 2001, the Stockholm Convention banned the use and release of certain POPs (Stockholm Convention 2001). As a result of the restrictions in usage of POPs, the concentrations of POPs in the Arctic are expected to decrease in the years to come (Bustnes et al. 2010b). However, as the POPs are resistant to they persist in biota biodegradation (AMAP 1998), and associated biological effects are likely to be detected in the future.

1.1 Persistent organic pollutants

Persistent organic pollutants are transported to the Arctic with air and water currents from mid-latitude industrial and agricultural areas (AMAP 2004). POPs are mainly manufactured chemicals (e.g. polychlorinated biphenyls (PCBs), byproducts of industrial processes (e.g. hexachlorobenzene [HCB]) or pesticides (e.g. dichlorodiphenyltrichloroethanes [DDT], chlordane and heptachlor). These POPs are often termed legacy POPs, because they are included in the Stockholm Convention (Stockholm Convention 2001). It should be noted that there are several novel classes of POPs, such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and perfluoralkyl substances (PFASs; e.g. perfluorooctanoic acid [PFOA] and perfluorooctane sulfonate [PFOS]) (AMAP 2004). Some of these compounds have recently been included in the Stockholm Convention, e.g. octaBDE and PFOS (Stockholm Convention 2009). Although POPs are diverse with respect to their chemical structures, most of them share elements of halogenated aliphatic or aromatic rings. They have also some typical physiochemical properties in common, such as high lipophilicity,

semi-volatility and resistance to biodegradation (AMAP 2004, Borga et al. 2004, Diamanti-Kandarakis et al. 2009). Because of their physiochemical properties they are known to accumulate in the lipid tissue of organisms, and they follow the lipid transfer from prey to consumer, a process called biomagnification (Borga et al. 2004, Hop et al. 2002, Kelly et al. 2007). The increasing concentrations of POPs across trophic levels in the food web, results in high concentrations in apex predators such as glaucous gull (*Larus hyperboreus*) and polar bear (*Ursus maritimus*) (Gabrielsen 2007, Hop et al. 2002, Letcher et al. 2010). However, food web magnification depends on the individual species' ability to biotransform POPs to more water soluble compounds, which are more readily eliminated (Hop et al. 2002). Chemical structures of the compounds are important for biomagnification, as an increasing number of chlorine atoms increases hydrophobicity and reduces biotransformation rates (Borga et al. 2004, Hop et al. 2002). In addition, several biological factors such as trophic position, age, body size, habitat use and seasonality may all affect the bioaccumulation and trophic transfer of POPs in the Arctic marine food web (Borga et al. 2004).

The levels of POPs in Arctic biota are generally lower than in industrialized areas (AMAP 1998, AMAP 2004, Gabrielsen 2007). However the seasonal need of lipids for energy, results in a redistribution of contaminants stored in the lipid tissue to other body compartments (Borga et al. 2004). A range of effects has been reported in Arctic biota after exposure to POPs. In Arctic seabirds, POPs have been linked to impaired reproduction, altered behaviour, reduced efficiency of the immune system, asymmetry of wing feathers, changes in the levels of circulating hormones, reduced body mass (BM) and high stress levels (Bustnes et al. 2001, Bustnes et al. 2002, Bustnes et al. 2004, Bustnes et al. 2008a, Helberg et al. 2005, Nordstad 2009, Sagerup et al. 2009, Verboven et al. 2009, Verreault et al. 2004).

In recent years, the ability of some POPs to interefere with the endogenous hormone system in organisms has received considerable attention. Especially the conflinct between POPs and the sex hormones or the thyroid hormones has recived a lot of interest. The process is called endocrine disruption (Diamanti-Kandarakis et al. 2009). Endocrine disrupting chemicals (EDCs) are chemicals that may bind to, or block, hormone receptors, alter synthesis or metabolism of hormones, or interfere with signalling pathways of the hypothalamus-pituitary-endocrine gland axis (Dawson 2000). However, independent of the mechanisms of actions, the result is changes in the hormonal system that allows the organism to communicate with and respond to its environment (Diamanti-Kandarakis et al. 2009).

1.2 Thyroid hormones and POPs

Thyroid hormones (TH) are necessary for development and maintenance of physiological functions across different taxa. The structure and mechanism by which they are synthesized are the same among vertebrate species. The thyroid gland is a part of the hypothalamic-pituitary-thyroid (HPT) axis. The thyroid gland produces and releases thyroxine (T4), when stimulated by thyroid-stimulating hormone (TSH) released from the pituitary (McNabb 2000, Ucan-Marin et al. 2010, Zoeller and Tan 2007). The synthesis of THs is regulated by a network of feedback mechanisms, and the synthesis is dependent on accessibility to iodine (Zoeller et al. 2007). T4 is transported and distributed by serum binding proteins. In birds, the transport proteins are mainly albumin and transthyretin (TTR). T4 is converted by peripheral deiodination to triiodothyronine (T3), the metabolic active TH, under the control of deiodinase enzymes (McNabb 2000, Ucan-Marin et al. 2010). In birds, THs regulate metabolic heat production for maintenance of a constant body temperature, and stimulate in combination with other hormones growth and cell differentiation (McNabb 2000). Hatching and molt are also processes under TH control, and THs are important for the development and function of the reproductive system (McNabb 2000).

Factors that influence the THs levels are ambient temperature, dietary iodine availability, body condition (BC), age, seasonality, activity and time of day of blood collection. For instance, food intake increase circulating T3 levels, while food restriction decreases T3 concentrations (McNabb 2000). The environmental temperature has a large impact on the TH levels, due to THs importance in controlling thermoregulation. Low temperatures stimulates to an enhancement in the T3 availability to meet the elevated metabolic requirements (McNabb 2000). The presence of specific toxicants may alter the TH levels. All thyroid toxicants are chemicals that reduce circulating levels of THs, either by affecting the biosynthesis, release and transport, or interfere with the metabolism of THs (Diamanti-Kandarakis et al. 2009, Zoeller 2007, Zoeller and Tan 2007). Several interactions have been discovered among toxic chemicals and the thyroid hormone system. The reported effects have been abnormal thyroid gland structure and altered TH concentrations after exposure to POP compounds or their metabolites. The effects have been observed in a number of species: e.g. glaucous gull (Verreault et al. 2004, Verreault et al. 2007), polar bear (Braathen et al. 2004, Skaare et al. 2001), gray seal (*Halichoerus grypus*) pups (Sormo et al. 2005) and rodents

(Kato et al. 2010). The main mechanism of action is interference with the T4 serum binding protein TTR, due to structural similarities between endogenous THs and primarily the metabolites of PCBs; hydroxylated PCBs. PCBs have also the ability to inhibit deoidinase enzyme activity or they can have a direct action on the thyroid hormone receptor (Skaare et al. 2001, Zoeller 2007). Thus, thyroid toxicants have the ability to interfere at different steps of the HPT-axis. The degree of adverse effects on the thyroid hormone system depends on the mechanisms by which different toxicants act (Zoeller et al. 2007).

1.3 The reproduction period of Arctic seabirds

The breeding season in the Arctic is demanding for the seabirds, both energetic and due to environmental factors. The energetic demands of the parent birds are considerable, due to egg-formation, incubation and chick care rather than self-maintenance. BM loss during breeding is a common consequence in many seabird species (Bech et al. 2002, Moe et al. 2002). In addition, food availability, temperature and sea-ice conditions may fluctuate annually and throughout the reproduction season, making breeding more challenging (Kitaysky et al. 1999, Moe et al. 2009). In the Arctic, the timing of breeding to match the chick's energetic requirements with the seasonal peak in food availability, is vital for successful reproduction (Moe et al. 2009, Shultz et al. 2009).Years with extensive sea-ice cover and poor food availability has been associated with delayed egg-laying, small clutches, low BC of the parent kittiwakes, and low reproductive success (Gaston et al. 2005, Moe et al. 2009, Welcker et al. 2010).

1.4 Study species

The black-legged kittiwake (*Rissa tridactyla*), hereafter kittiwake, is a medium sized gull. It has a circumpolar distribution, and is the most numerous gull species in the world (Strøm 2006). The kittiwakes breed in the boreal and arctic zones, including Svalbard. The kittiwakes spend the winter period in the northern part of the Atlantic Ocean, and they arrive at Svalbard in March-April. Kittiwakes breeds in steep cliffs, in colonies. Egg-laying at Svalbard takes place in the middle of June, the clutch size is 1-3 eggs, and the incubation time last for 25-32 days. After hatching the chicks are fed for five to six weeks, until fledgling. Eggs and chicks are cared for by both parents (Strøm 2006).

The kittiwakes are occupying the intermediate third and fourth trophic position in the Arctic marine food web (Hop et al., 2002). The kittiwakes are surface feeders and the main constituents of their diet are polar cod (*Boreogadus saida*), capelin (*Mallotus villosus*) and the pelagic amphipod *Parathemisto libellula*, (Mehlum and Gabrielsen 1993). The diet is lipid rich and the kittiwakes are therefore exposed to persistent organic pollutants, which accumulate in their adipose tissue (Borga et al. 2005, Borga et al. 2007).

At Svalbard, the kittiwake is the most common gull species. However, since 1995 it has been documented a decline in the population and the cause is unknown (Strøm 2006). The kittiwake is listed on the "Norwegian Red List for Species" under the category "Near Threatened" at Svalbard (Artsdatabanken 2010).

1.5 The Objectives

The main purpose of the present study was to compare blood concentrations of POPs in kittiwakes breeding in Kongsfjorden, Svalbard, between two different years, and to assess if POPs have any influence on their plasma levels of T3. The aim was to investigate if a year characterized by extensive sea-ice cover, low temperatures and poor food availability (2009), had any influence on the physiology of the kittiwake, and if this effect was reflected in an altered BM, reduced BC, changed T3 levels and elevated concentrations of POPs.

Hypothesis:

i. A year characterized by food shortage, low temperatures and extensive sea-ice cover causes BM loss and subsequent poor BC of breeding kittiwakes. The mass loss leads to increased blood concentrations of POPs, which results in decreased circulating T3 levels of the kittiwakes.

2. Materials and methods

2.1 Study area and sampling

The field work was conducted during the breeding season in 2008 and 2009. The study location was a colony named Krykkjefjellet (78°54'N, 12°13'E), situated in Kongsfjorden, Svalbard (Figure 2.1.1). The kittiwakes chosen for this study were all breeding. The breeding season was divided in two periods; incubation (June) and chick rearing (July). Blood samples were collected in the incubation period. Every third day throughout the breeding period the nests were checked to determine clutch size, hatching date and chick survival (breeding success).

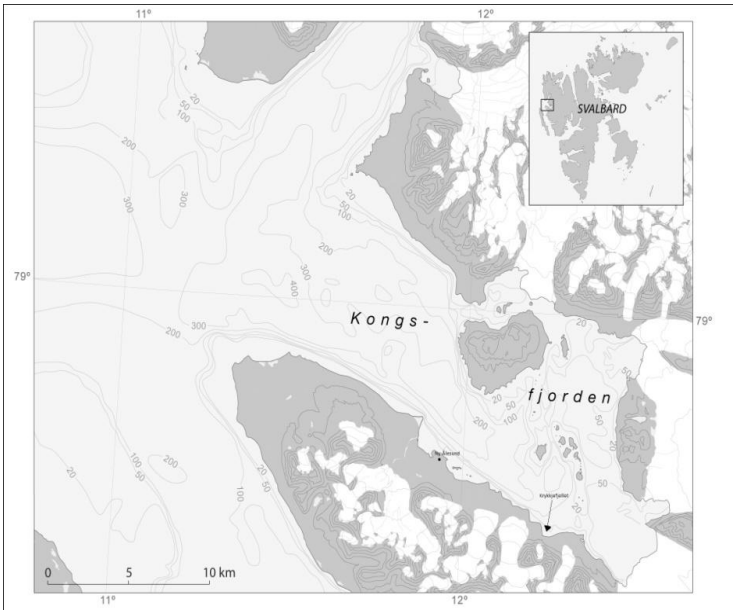

Figure 2.1.1: The study area Kongsfjorden, on the west coast of Svalbard. The study colony Krykkjefjellet is located 6 km east of Ny-Ålesund. Map: Oddveig Øien Ørvoll (NPI).

The kittiwakes were caught on their nest using a fishing rod with a nylon noose attached to one end. Blood (1.5 mL) was sampled from the wing vein using a heparinised (LEO 100 IE/mL) syringe (BD Plastipak) with a 23G needle (BD Microlance). Body mass (g) was determined using a spring balance, skull and bill length (mm) were measured using a sliding calliper, while wing (cm) was measured using a ruler. The identity band was noted**.** If the kittiwake had not previously been caught**,** it was banded with a seven digit metal ring from Stavanger Museum, Norway, and a coloured plastic ring with a unique three-letter code. To avoid recapturing, the kittiwake was marked with dye on the forehead and breast. In the field,

the blood samples were kept on a mixture of salt and ice in a cooling bag. Within five hours after blood sampling, 0.5 mL of the sample was centrifuged at 2,000 rpm (4 min) to separate plasma from red blood cells, and the plasma was stored at -20°C for hormone analyses. The red blood cells were stored for sex determination and the whole blood was stored for chemical analyses, both at -20°C.

2.2 Analyses

2.2.1 Sex determination

The sex determination was carried out at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway.

Principles of sex determination

The method is based on the presence or absence of the female specific W-chromosome. Females are heterogametic (ZW) and the males are homogametic (ZZ). When the polymerase chain reaction (PCR) product is visualized on an agarose gel it appear as one or two bands, showing male and female respectively (Griffiths et al., 1996).

Processing of the sample for sex determination

200 µL 5% chelex-solution (5 g chelex resin in 100 mL water) was added to each eppendorftube and a droplet of blood (2-4 µL) was added. The sample was incubated at 56 °C (20 min), vortexed, incubated at 96°C (8 min), vortexed and centrifuged at 12000 rpm (3 min). In the end the supernatant (20 µL) was transferred to new tubes for PCR.

A stock solution was prepared (0.75 µL Taq, 29.25 µL autoclaved H2O, 6.0 µL Mix, 9.0 µL MgCl, 15.0 µL 10X, 15.0 µL primer 1 (2550), 15.0 µL primer 2 (2718) and 30.0 µL Q) and 8 µL of the solution was transferred to each PCR-well, 2 µL deoxyribonucleic acid (DNA) was added to each well and the samples were run in the PCR (35 cycles, three temperature levels: 94, 46 and 70 °C).

For separation of DNA a 1% agarose gel (1.2 mL 50 x TAE, 59 mL distilled water, 0.6 g agarose, 6 µL ethidium bromid) was made. A running buffer (686 mL water, 14 mL 50 x TAE buffer) was added, the wells were loaded (10 µL sample) and a ladder (10 µL ladder, 80 µL distilled water, 10 µL gel loading buffer) was added. The gel was run at 75 V for 45-50 min. The bands were checked by UV light.

Quality assurance of the method

Vague bands where checked twice to be certain of the sex. Two birds were impossible to sex-determine using the DNA-method (#57 and #79). Sexing was then based on the head plus bill length in relation to the discriminant value of 92.1 mm (Moe et al., 2002). Male kittiwakes were assumed to have skull length above 92.1 mm, while females were assumed to have skull length below the discriminant value.

Chemical analysis of contaminants

The chemical analyses were carried out at the Norwegian Institute of Air Research (NILU), Tromsø, Norway. The following PCB-congeners were analysed PCB-28, -52, -99, -101, -118, -138, -153, -180, -183, and -187 (their structure are reported in Appendix A). Other compounds analyzed included DDE, DDT, α-, β-, and γ-HCH, heptachlor, heptachloroepoxide, HCB, oxychlordane, trans- and cis-chlordane, trans- and cis-nonachlor and mirex.

Principles of gas chromatography - mass spectrometry

The gas chromatography - mass spectrometry (GC-MS) is based on separation of molecules based on their properties as they travel along the column. The time each component use to travel the column is called their retention time. The MS is coupled to the GC and as the components leave the column and enters the MS they are ionized and detected based on their mass to charge (m/z) ratio (Harris 2007).

Processing of the sample for chemical analysis

Disposable glassware was burned or rinsed with cyclohexane prior to use. The sample was made homogenous by mixing, and 1-2 mL of whole blood was transferred to a weighted 15 mL glass tube. 100 µL internal standard solution (POP I 47.09_1:10 in iso-octane (25 pg/µL); individual compound is listed in Appendix B) was added. To denaturate proteins, deionised water saturated with ammonium sulfate and ethanol was added. To allow phase separation 6 mL n-hexane was added, the sample was mixed by shaking and was left in the fume (15 min). After phase separation the organic supernatant was transferred to a weighted 15 mL glass tube. The hexane extraction step was repeated, and the second supernatant was transferred to the same glass tube. The combined extracts were concentrated to 0.2 mL using the vacuum evaporation system RapidVap (Model 7900001, Kansas City, MO, Us) to facilitate evaporation of hexane. For lipid content determination, the sample was left overnight for evaporation and then weighted the following day. In the next step 0.5 mL hexane was added to the sample, before the sample was cleaned with florisil.

To remove fat and other matrix from the extract the sample was run over a florisil column in the RapidTrace SPE workstation (Caliper Life Sciences, Hopkinton, USA) procedure. Florisil (0.15-0.25 mm, Merck, Darmstadt, Germany) was burned (600 °C for 8 hours), and the columns were packed with florisil (1 g (+/- 0.02 g)) between two glass fibre frits in each end of the column. Following the clean up the samples were concentrated using RapidVap to a volume of 0.2 mL. The remaining eluate was transferred to GC-vials and the original glass tube was rinsed with dichloromethane (DCM). The eluate was concentrated down to approximately 50 µL using nitrogen gas, and a recovery standard (octachloronaphtalene [OCN] 10 µL of a 1ng/µL solution in isooctane) was added prior to quantification.

Instrumental setting and quantification

1 µL sample was injected and run in an Aglient 7890 GC connected to an Aglient 5975 MS (Agilent Technologies, Folsom, CA, USA). The GC was equipped with an Aglient DB-5MS column (length 30m, 0.25 µm film thickness (ft), 0.25 mm inner diameter (id); JW Scientific, Folsom, CA, USA). Helium (quality 5.5, Hydrogas, Porsgrunn, Norway) was used as a carrier gas, at a flow rate of 1 mL/min. The following temperature program was used: 70 °C (2min), 15°C/min to 180°C, 5°C/min to 280°C (10 min). The analyses were done using selective ion

monitoring (SIM) with ionization energy of 70 eV. Electron ionization (EI) was used for the analysis of PCBs and DDTs, while negative chemical ionization (NCI) was used for the pesticides. The ion-source temperature was set at 280°C (EI) and 160°C (NCI) with a transferline temperature kept at 280°C.

Quantification was done within the linear range of the detector. To quantify organic compounds, a quantification standard (Q-std) with known concentrations (Conc) of ^{13}C and ^{12}C, was run together with the samples. ^{13}C-labeled quantification standards (purchased from Cambridge Isotope Laboratories, Woburn, MA, USA) represented all groups of PCBs, DDE, HCB and oxychlordane. Reference material were obtained from Promochem (Wesel, Germany) and solvents of pesticide grade were used (Merck, Darmstadt, Germany). A relative response factor (RRF) was calculated from the ratios between ^{13}C and ^{12}C in the quantification standard (Eq. 1)

$$RRF \times \frac{Conc_{13C\_QStd}}{Area_{13C\_QStd}} = \frac{Conc_{12C\_QStd}}{Area_{12C\_QStd}}$$

RRF was used to calculate the concentration of ^{12}C in the samples (Spl) from the known amount of ^{13}C-labeled quantification standard added to the samples (Eq. 2)

$$Conc_{12C\_Spl} = \frac{Area_{12C\_Spl} \times Conc_{13C\_Spl}}{RRF \times \grave{A}rea_{13C\_Spl}}$$

Recoveries were estimated from the calculated (calc) amount of ^{13}C in the samples divided by the amount of ^{13}C added to the samples (Eq. 3).

$$Recovery(\%) = \frac{Conc_{13C\_calc}}{Conc_{13C\_added}} \times 100$$

Quality assurance of the method

As a quality assurance, for every tenth sample it was extracted one blank and one standard reference material (SRM, 1958 human serum from National Institute of Standards and Technology, NIST). There were not observed any contamination of the blanks. The standard reference material met the laboratory's criteria for the limit of accuracy (20%). Limit of detection (LOD) was defined as three times the background noise, and ranged from 0.4 to 269 pg/g w.w. in 2008 and 2009, depending on the specific compound (Appendix C). DDT and lower PCBs (PCB-28, PCB-52) could not be analysed in 2009 due to a switch from EI to NCI on the MS (due to the contamination of the samples in 2009).

2.2.2 Thyroid hormone analysis

Total plasma concentrations of T3 were determined at Chizé Centre for Biological Studies, Chize, France, using radioimmunoassay (RIA).

Principles of radioimmunoassay

RIA is a competitive reaction between labelled antigen (tracer) and unlabelled antigen (analyte) for binding sites on the antibody, when it is limited binding sites available. RIA is a specific and sensitive method. The technique uses radioactive labelled antigens complementary to the antigen of interest, as well as specific and high affinity antibody molecules (Berson and Yalow 1968). The unlabelled antigen is the compound to be quantified on the basis of the counts of the tracer-antibody complex, which are inversely proportional to the antigen concentration. Based on the counts of the bound complex, a standard curve is made. The standard curve is then used to estimate the concentration of an unknown sample (Berson and Yalow 1968).

Processing of the sample for T3 analysis

The antibody molecules used in this assay are immunoglobulin proteins, produced by the immune reaction in rabbit. The labelled antigen used was Iodine 125, a pure gamma emitter.

Precipitation of the antigen-antibody complex was done by the double antibody method, where a second antibody (Sheep serum against rabbit: SMAL) specific to the first antibody was used. Standards with increasing concentrations of T3 were used to calibrate a standard curve. Iodine 125 tracer solution (H*) was obtained from CIS Biointernational. Iodine antibody (Ab) solution was developed in rabbit and obtained from Sigma Chem. Comp. Standards, Ab and H* were diluted in barbital bovine albumin (BSA) buffer (0.075M bovine γ globulins at 6‰) prior to use.

Total activity (AT), non-specific binding (NSB) and maximum binding (B_0) were prepared in polypropylene tubes in duplicates. The NSB and B_0 were added barbital BSA buffer, 110 µL and 10 µL respectively. 10 µL of each standard, control sample and plasma sample were pipetted into their respective vial. All of the standards, controls, and samples were prepared in duplicates. All vials except AT and ANS were added 100 µL Ab using a multipipette. 100 µL H* was added to all vials, followed by gently mixing the vials and incubation at 4°C overnight. The following day 100 µL SMAL was added to all vials except AT, and the vials were incubated at 4°C (12 h). Next day 1 mL barbital buffer was added to all vials except AT, and the vials were centrifuged at 18°C, 4700-4800 rpm (45 min). The vials were decanted, and left to dry. The dry vials were counted for 2 min in a gamma counter and the emitted radioactivity was given as counts per minute (cpm). The software for the gamma counter generated calibration curves, and calculated the T3 concentration in the samples based on their cmp value.

Quality assurance of the method

As quality assurance parameters, quantity control tubes (AT, NSB, B_0, standards and control samples) were included in each assay. The samples from 2008 and 2009 were tested in five different assays. The AT, NSB and B_0, controlled the quality of the tracer and the antibody. The %NSB limit was set at 5%, and %NSB was below this limit (< 2.9%) for all the assays. Eight standards were run in each assay, and the standard curves for the five assays were parallel and well fit. In addition, in the beginning of each assay controls (plasma from canary, goat, human 1 and 2, nF1F2, and wild boar) and standard reference material (Immunoassay Plus Control 1, 2 and 3) were run along with the samples. The coefficient of variation (CV) was calculated for the controls to check for intra- and inter-assay variability. The intra-assay

variation was below 20%, while the inter-assay variation was below 22%. This was considered acceptable. The samples were run in duplicates, and the limit for accepted variation between the parallels was 10%. All analysed samples were accepted. The recovery was 116%. This was 20% over the theoretical concentration, which was accepted. The specificity of the antibody was approved, the cross reactivity was as follows: triiodo-D-thyroacetic acid 6%, L-thyroxine 0.2%, diiodo-L-tyrosine < 0.01%, monoiodo-L-tyrosine < 0.01%. The sensitivity was 0.2 ng/mL and all the plasma samples from the kittiwakes were above the detection limit.

2.3 Statistical analyses

PCB-28, PCB-52 and DDT could not be analysed in 2009, and were excluded from the statistical analyses. Contaminants detected in less than 60% of the kittiwakes within each year were excluded from the statistical analyses. This account for the following contaminants: PCB-101, heptachlor, heptachloroepoxide, trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor, mirex, α-HCH, β-HCH and γ-HCH. The contaminants detected in more than 60% of the kittiwakes within each year, were included in the statistical analyses. Individual concentrations below the limit of detection (LOD) were given a random value using the formula RAND()*(b-a) in Microsoft Excel 2007 (a=0 and b=detection limit).

Biometric measurements, POPs and T3 levels are presented as mean, standard derivations (SD), median, and ranges.

The biometric measurements of wing, head plus bill (skull) and BM were used to calculate the BC of the kittiwakes (the individual biometric measurements are presented in Appendix D; Table D.1 and D.2). The variables wing and skull were reduced to one variable using principal components analysis, followed by linear regression between BM and the new variable. The linear regression gave each individual a BC value, positive or negative dependent on the individual's position in relation to the regression line. The BC was calculated for male and female separately, making the values comparable independent of sexes.

2.3.1 Multivariate data analysis

Multivariate data analysis was performed using Simca-P+ 12.0 (Umetrics, Umeå, Sweden). Prior to PCA, the data was scaled to unit variance (UV) and mean centred, to achieve equally variation between the variables, and equal weight of the variables in the analysis. A principal component analysis (PCA) was used to visualize the relationships between the biological variables, T3 level and concentration of POPs in the kittiwakes. PCA are validated with respect to the goodness of fit (R^2X) and the goodness of prediction (Q^2X) (Eriksson et al. 2006). The principal components were reduced from three to two, explaining 60.9 % of the variation in the data.

2.3.2 Univariate data analyses

The univariate data analyses were performed using PASW Statistics 18.0 (SPSS inc., Chicago, Illinois, USA). The concentrations of POPs were in pg/g wet weight (w.w.), while the levels of T3 were in ng/mL. The biometric variables were normally distributed. The concentrations of POPs were log-transformed (PCB-153, -118, -180 and oxychlordane) or square root-transformed (PCB-99, -138, -183, -187, HCB, and DDE) to achieve normal distribution. After the transformation all the variables were normally distributed (Shapiro-Wilk test $p>0.05$), except T3 (Shapiro-Wilk test $p=0.043$). Parametric tests (Independent-samples t-test) were used for all variables except T3, where non-parametric tests (Mann-Whitney U Test) were used. To check for correlations between variables Pearson Product-moment Correlation Coefficient (two-tailed) was used for all variables except T3, where Spearman's Rank Order Correlation (two-tailed) was used. Preliminary analyses were done to ensure no violation of assumptions. For all the statistic tests the significance level was set at $p=0.05$.

With respect to the concentrations of POPs, both sexes are included in the statistical analyses presented in results. To illustrate if the correlations observed between variables in the present study were caused by inter-year differences, or were a result of between-sexes variation, the correlation plots are presented as both inter-year and between sexes.

3. Results

3.1 Breeding- and physiological conditions

The BM and BC of male and female kittiwakes in 2008 and 2009 are presented in Table 3.3.1. The BM of both male and female kittiwakes were significantly lower in 2009 compared to the BM in 2008 (t-test, df=[36–37], t=2.91, p=0.006). The differences in BM for males and females were 5.63% and 5.90%, respectively. The BM of the females were significantly lower than the BM of males in both years (2008: 9.24%, t-test, df=44, t=5.960, p<0.001. 2009: 9.51%, t-test, df=29, t=3.897, p=0.001). The BC was also significantly lower in both male and female kittiwake in 2009 compared to in 2008 (t-test, df=[36–37], t=[3.16–3.07], p=0.003). The differences in BC for males and females were 102.47% and 107.70%, respectively.

Table 3.1.1: Body mass (g) and body condition presented as mean, standard deviation (SD), median and range in male and female kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard sampled in 2008 and 2009. The results from the t-test are given as df, t and p.

	2008					**2009**					**t-test**		
parameter	**n**	**mean**	**SD**	**median**	**range**	**n**	**mean**	**SD**	**median**	**range**	**df**	**t**	**p**
Body mass (g) male	21	446.29	26.99	447.00	390.00–490.00	17	421.18	25.67	419.00	443.00–458.00	36.00	2.91	0.006
Body mass (g) female	25	405.04	19.88	401.00	392.00–457.00	14	381.14	31.57	381.00	342.00–447.00	37.00	2.91	0.006
Body condition male	21	0.81	0.82	0.83	-0.82–2.11	17	-0.02	0.81	-0.08	-1.70–1.13	36.00	3.16	0.003
Body condition female	25	0.65	0.58	0.64	-0.36–1.95	14	-0.05	0.82	-0.07	-1.63–1.47	37.00	3.07	0.003

The egg-laying date and clutch size for 2008 and 2009 are presented in Table 3.3.2. The egg-laying date was significantly later in 2009 compared to in 2008 (t-test, df = 75, t = 9.446, p < 0.001), and the clutches were significantly smaller in 2009 in relation to in 2008 (t-test, df = 75, t = 3.182, p = 0.002).

Table 3.1.2: Egg-laying date (day in June) and clutch size presented as mean, standard deviation (SD), median and range for kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard in 2008 (n=46) and 2009 (n=31). The results from the t-test are presented as df, t and p.

	2008					2009					t-test		
Variable	n	mean	SD	median	Range	n	mean	SD	median	range	df	t	p
Egg-laying date	46	11.15	2.93	12	9.6 – 27.6	31	18.87	4.25	19	5.6 – 16.6	75	-9.446	< 0.001
Clutch size	46	1.89	0.43	2	1.0 – 2.0	31	1.55	0.51	2	1.0 – 3.0	75	3.182	0.002

3.2 Concentrations of the contaminants

The relative contribution of each compound to the ∑POPs is presented in Figure 3.2.1. The $\sum_7$PCBs was the major contaminant in the kittiwakes in both 2008 and 2009, and constituted 44.7% and 42.12% respectively, of the contaminant burden in the whole blood of the kittiwakes. The individual concentrations of POPs within each year are presented in Appendix E; Table E.1 and E.2).

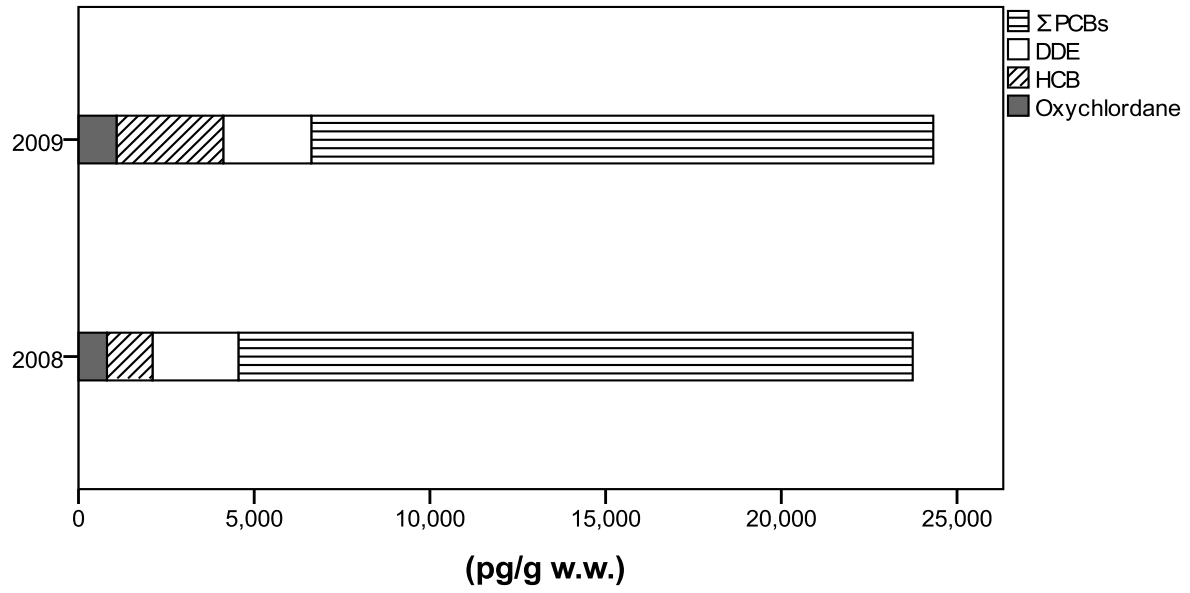

Figure 3.2.1: Relative contribution of each compound to the $\sum_7$POPs (pg/g w.w.) in 2008 and 2009 in whole blood from 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, sampled in 2008 (n=46) and 2009 (n=31).

The whole blood concentrations (pg/g w.w.) of the PCBs in 2008 and 2009 are presented in Figure 3.2.2. There was no significant difference in the PCB levels between 2008 and 2009 (t-test df = 75, t = [-1.484 – 0.762], p > 0.05). The dominant PCBs in the blood of the kittiwakes were the same in both 2008 and 2009. The major PCB was PCB-153 (2008: 6498.33 pg/g w.w, 2009: 5974.74 pg/g w.w) followed by PCB-138 (2008: 5133.07 pg/g w.w, 2009: 4472.45 pg/g w.w) and PCB-180 (2008: 3228.13 pg/g w.w, 2009: 2937.52 pg/g w.w). These three major PCBs constituted 76.57% of the Σ_7PCB[1] burden in the blood of the kittiwakes.

[1]Σ_7PCB: PCB-99, PCB-118, PCB-138, PCB-153, PCB-180, PCB-183, PCB-187.

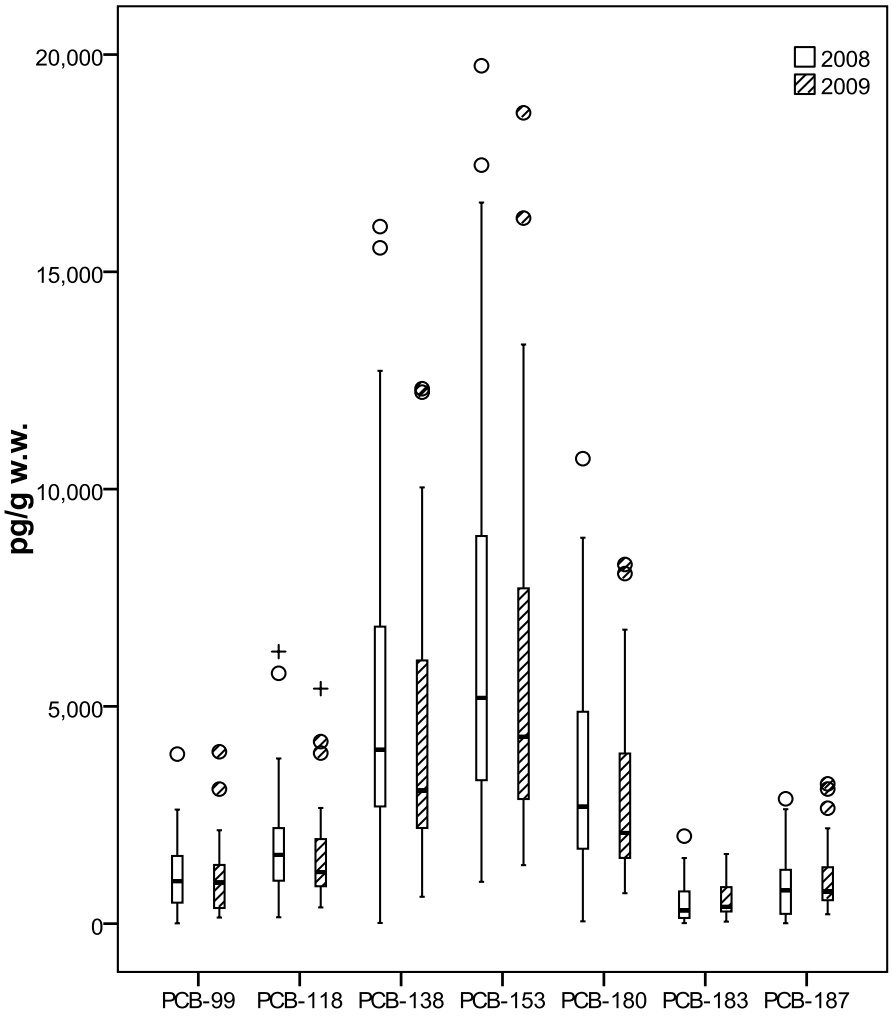

Figure 3.2.2: Concentrations (pg/g w.w.) of PCBs in whole blood from 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, sampled in 2008 (n=46) and 2009 (n=31). The concentrations are given as the mean value for male and female kittiwake in each respective year. The levels are presented as box plots where the rectangle represents 50 per cent of the cases, the whiskers goes out to the variable's top and bottom value and the line inside the box represents the median value. The symbol ○ and + represents outliers. ○: > 1.5 box-lengths from the edge of the box and +: > 3.0 box-lengths from the edge of the box.

The whole blood concentrations (pg/g w.w.) of the pesticides in 2008 and in 2009 are presented in Figure 3.2.3. There was a significant difference between 2008 and 2009, in the concentrations of HCB (134.22%, t-test, df = 75, t = -10.582, $p < 0.001$) and oxychlordane (33.66%, t-test, df = 75, t = -2.268, p = 0.026). The concentrations were higher in 2009, 134.22% and 33.66% respectively, compared to 2008. The level of DDE did not differ between the two years (t-test, df = 75, t = 0.182, p = 0.624). The pattern of pesticides in the kittiwakes differed between 2008 and 2009. Thus, in 2008, the order of the prevalence of the pesticides was: DDE > HCB > oxychlordane, whereas, in 2009 the pattern was: HCB > DDE > oxychlordane.

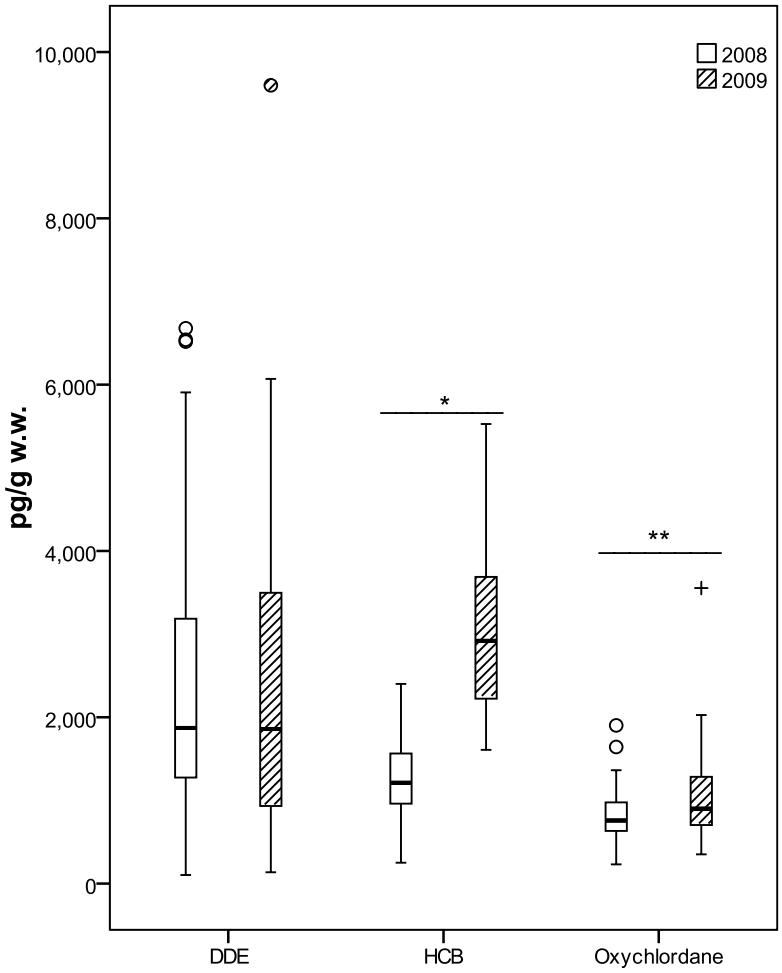

Figure 3.2.3: Concentrations (pg/g w.w.) of pesticides in whole blood from 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, sampled in 2008 (n=46) and 2009 (n=31). The concentrations are given as the mean value for male and female kittiwake in each respective year. The levels are presented as box plots where the rectangle represents 50 per cent of the cases, the whiskers goes out to the variable's top and bottom value and the line inside the box represents the median value. The symbol ○ and + represents outliers. ○: > 1.5 box-lengths from the edge of the box and +: > 3.0 box-lengths from the edge. *: means are significant different at $p < 0.001$. **: means are significant different at $p < 0.05$.

In male kittiwakes, there was only a significant inter-year difference for HCB (t-test, df=36, t=-7.854 p<0.001,), with a higher concentration (93.88%) in 2009. Also in female kittiwakes, the concentration of HCB was significantly higher (183.53%) in 2009 as compared to 2008 (t-test, df = 37, t = -7.770, $p < 0.001$). In addition, the concentration of oxychlordane in females was higher (56.40%) in 2009 compared to the concentration in 2008 (t-test, df = 37, t= -2.317, $p = 0.026$).

With respect to sex-differences in the concentrations of POPs, in 2008 male kittiwakes had significant higher levels of HCB, PCB-138, PCB-153, PCB-180 and PCB-183 (t-test, df = 44, $t > 2.180$, $t = [2.180 – 2.523]$ $p < 0.035$) than females. The concentrations of DDE,

oxychlordane, PCB-99, PCB-118, and PCB-187 did not differ between the sexes in 2008 (t-test, df = 44, t = [0.405 – 1.355], p > 0.05). As opposed to 2008, in 2009 there were no significant differences in the contaminant levels between male and female kittiwake for any of the contaminants (t-test, df = 29, t = [-0.954 – 1.241], p > 0.05).

3.3 Thyroid hormone levels

The plasma concentrations of T3 (ng/mL) for both sexes in 2008 and 2009 are presented in Figure 3.3.1. The individual levels of T3 are presented in Appendix F; Table F.1 and F.2.

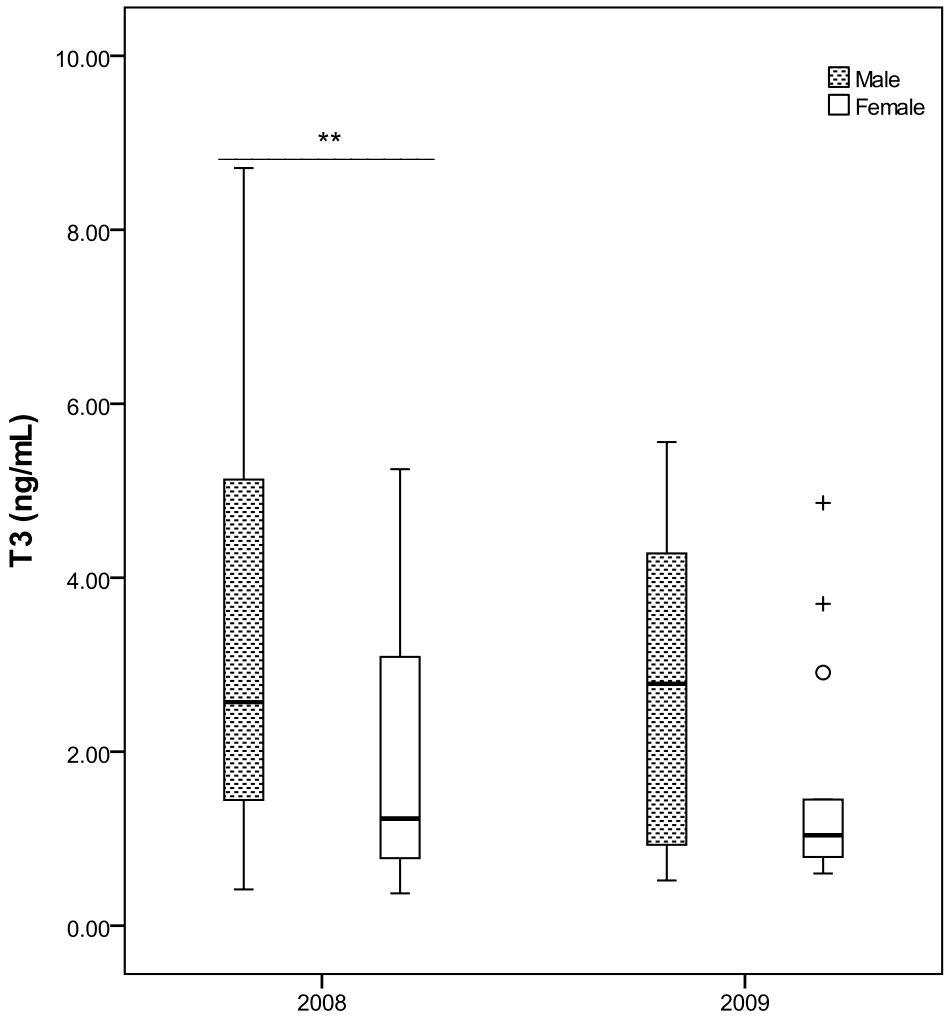

Figure 3.3.1: T3 levels in 38 male and 39 female kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, sampled in 2008 (n=46) and 2009 (n=31). The concentrations are presented as box plots where the rectangle represents 50 per cent of the cases, the whiskers goes out to the variable's top and bottom value and the line inside the box represents the median value. The symbol ○ and + represents outliers. ○: > 1.5 box-lengths from the edge of the box and +: > 3.0 box-lengths from the edge. **: means are significant different at p < 0.05

There was no significant difference in the T3 levels in males or female kittiwakes between 2008 and 2009 (Males: Mann-Whitney U test, U=160, z=-0.543, p=0.587. Females: Mann-Whitney U test, U = 151, z = -0.703, p = 0.482). In 2008, the T3 levels differed significantly between male and female, where female kittiwakes have lower T3 levels (42.58%) than males (Mann-Whitney U test, U = 168, z = -2.084, p = 0.037). In 2009, the T3 levels were also higher in males compared to females. However, the difference was not significant (Mann-Whitney U test, U = 76, z = -1.707, p = 0.088).

3.4 Associations between contaminants, T3 and biological variables

The score plot and loading plot from the analysis are presented in Figure 3.4.1 and 3.4.2, respectively. The analysis resulted in three principal components (PC), explaining 74.5% of the variation in the data (R^2X (cum) = 0.745, Q^2X (cum) = 0.530). PC1 ($R^2X[1]$) and PC2 ($R^2X[2]$) explained 43.0% and 17.9% respectively, of the variation and were used for further analysis.

The score plot illustrates how the observations were related to each other and the plot shows possible outliers, groupings and patterns in the data. As shown in the score plot (Figure 3.4.1), there was a mixed distribution of the individuals from 2008 and 2009, and more individuals were located in the left side of the plot. Males were positioned at the top of the plot, while females were located at the bottom of the plot. There were detected three outliers (9F, 9M and 8M) according to Hotelling T2 ellipse. However, due to individual variations in the levels of contaminants, these individuals represented the span of POPs in kittiwakes and were therefore, not excluded from the analysis.

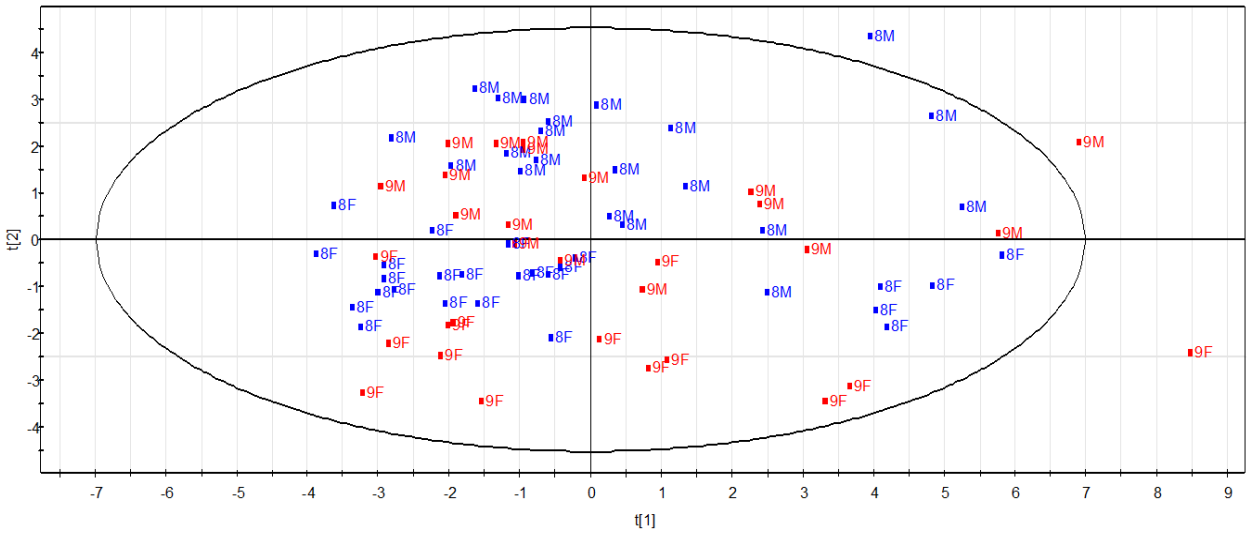

Figure 3.4.1: PCA score plot of 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, sampled in 2008 (n=36) and 2009 (n=31). R^2X [1] = 40.9% and R^2X [2] = 17.9%. The PCA include the variables: BM, BC, sex, skull, wing, T3, DDE, HCB, oxychlordane, PCB-99, PCB-118, PCB-138, PCB-153, PCB-180, PCB-183, and PCB-187. Samples from 2008 are coloured in blue, while samples from 2009 are coloured in red. The first number refers to the year of collection 2008 = 8 and 2009 = 9, while the following letter displays the sex; male = M and female = F.

The loading plot (Figure 3.4.2) visualizes the relationship between the x- variables in the two dimensional coordinate system made up by PC1 (range: 0.0 – 0.3) and PC2 (range: 0.0 – 0.5). The variables located far from the origin of the plot, have the strongest impact on the model. Thus, POPs, BC and BM have the strongest influence on the model, while T3, biometric variables, and clutch have lesser impact ability. The plot illustrates that there were high positive inter-correlations between the PCBs, as they were located close to each other along the PC1 axis. These correlations were confirmed by bivariate correlation analysis (Appendix G; Table G.1, Pearson, n = 77, r > 0.5, p < 0.001). There were also positive associations among the pesticides, and these were also confirmed by bivariate correlation analysis (Appendix G; Table G.1, Pearson, n = 77, r > 0.4, p < 0.002). Furthermore, the pesticide concentrations were positively correlated to $\sum_7$PCBs (Pearson, n = 77, r > 0.4, p < 0.001).

The location of the POPs in relation to T3, illustrates that are no associations between the POP variables and T3, this was confirmed by bivariate correlation analyses (Appendix G; Table G.4, Spearman, n=77, rho = [-0.088 – 0.22], p > 0.054).

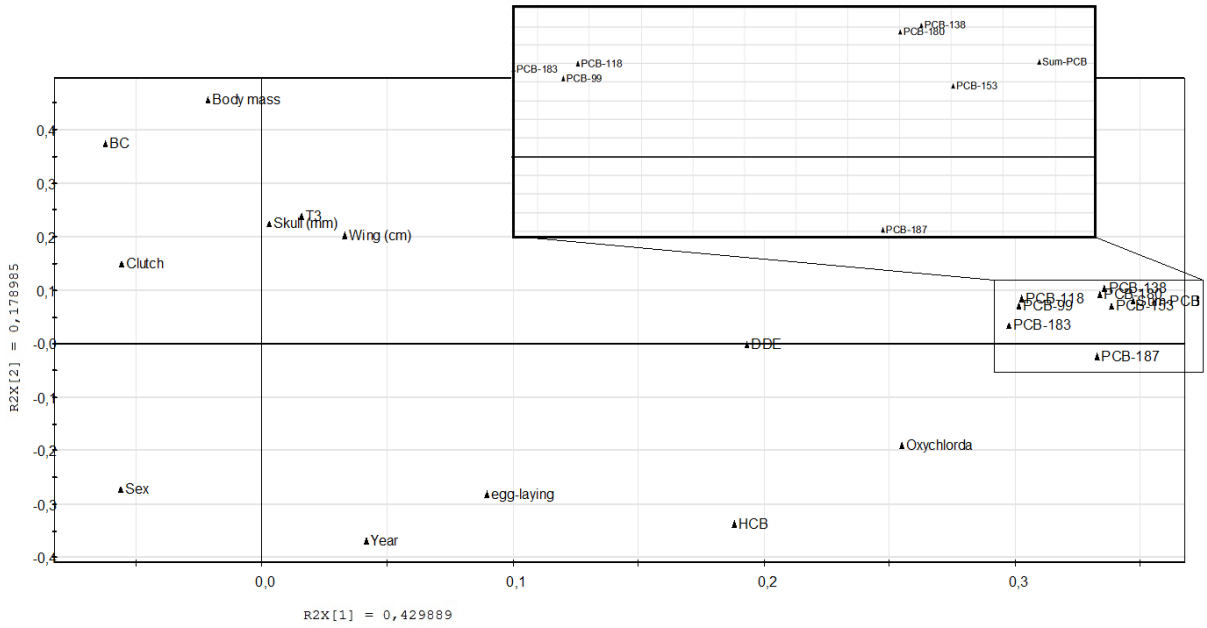

Figure 3.4.2: PCA loading plot of 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, sampled in 2008 (n=46) and 2009 (n=31). R^2X [1] = 43.0% and R^2X [2] = 17.9%. The PCA include the variables: BC, BM, sex, skull, wing, clutch, egg-laying date, T3, DDE, HCB, oxychlordane, PCB-99, PCB-118, PCB-138, PCB-153, PCB-180, PCB-183, and PCB-187.

The variables BM, wing, skull and T3 were located close to PC2. Thus, these variables were important for dispersing the observations along the vertical plane of the plot. Nevertheless not apparent in the PCA loading plot, BM, skull and wing were positively correlated (Appendix G; Table G.2, Pearson, n = 77, r > 0.5, p < 0.001). Whereas, T3 located close to the biometric variables skull and wing, was only significantly correlated to wing length (Appendix G; Table G.3, Figure 3.4.3; Spearman, n = 77, rho = 0.252, p = 0.027). The biometric variables wing, skull, and BM were located in the opposite direction to the variable sex, and were significant negatively correlated to sex (Pearson, n = 77, r = [-0.573 – -0.748], p < 0.001). Also T3 was inverse associated with sex (Spearman, n = 77, rho = -0.323, p = 0.004).

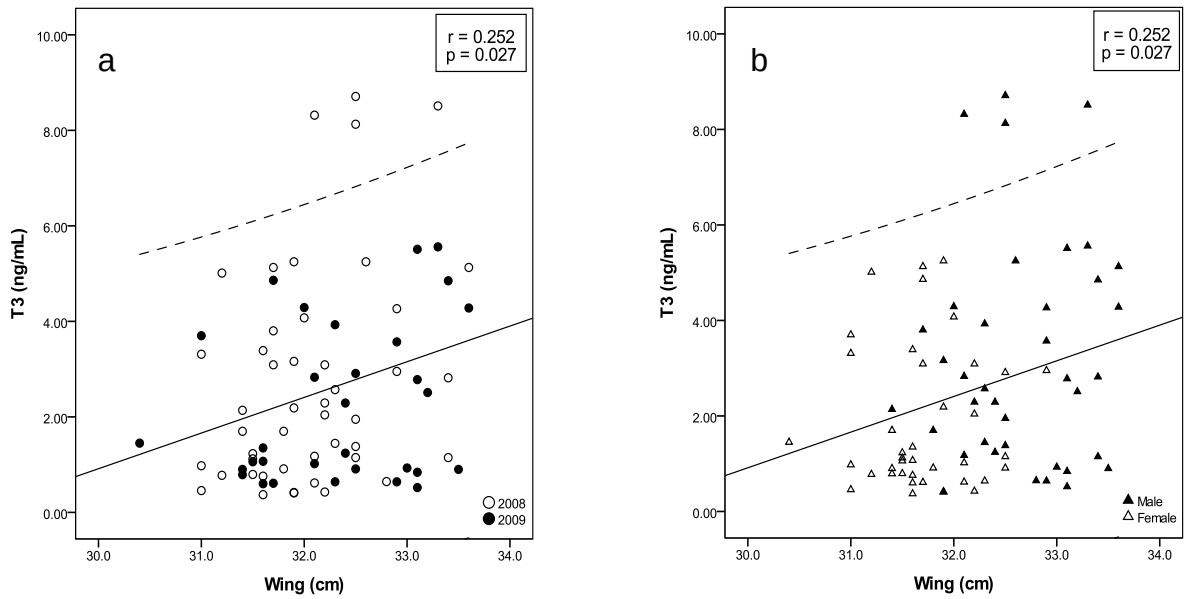

Figure 3.4.3: The wing (cm) length in relation to the T3 level (ng/mL) presented as (a) inter-year and (b) between-sexes in 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden; Svalbard, sampled in 2008 (n=46) and (n=31). The stippled line represents 95% confidence intervals and the r- and p-values are displayed in the plot.

The variable year was located in the opposite direction to the variables BM, BC and clutch, which illustrates a negative relationship between year and the three variables. The negative relationships were further confirmed by bivariate correlation analyses (Pearson, n = 77, r = [-0.303 – -0.453], $p < 0.01$). Thus, kittiwakes in 2009 had lower BM, BC and smaller clutches compared to the kittiwakes in 2008. Furthermore, the variable year was significant positively correlated to the variables HCB (Pearson, n = 77, r = 0.774, $p < 0.001$) and oxychlordane (Person, n = 77, r = 0.253, p = 0.026). Thus, the kittiwakes in 2009 had higher levels of HCB and oxychlordane.

The contaminants were located opposite to BC along PC1, indicating that the contaminants were inversely associated to the BC of the kittiwakes. However, bivariate correlation analysis verified that BC was only inversely correlated to HCB (Figure 3.4.4A; Pearson, n = 77, r = -0.424, $p < 0.001$) and oxychlordane (Figure 3.4.4B; Pearson, n = 77, r = -0.257, p = 0.024). Thus, the concentrations of HCB and oxychlordane increased with a decreasing BC.

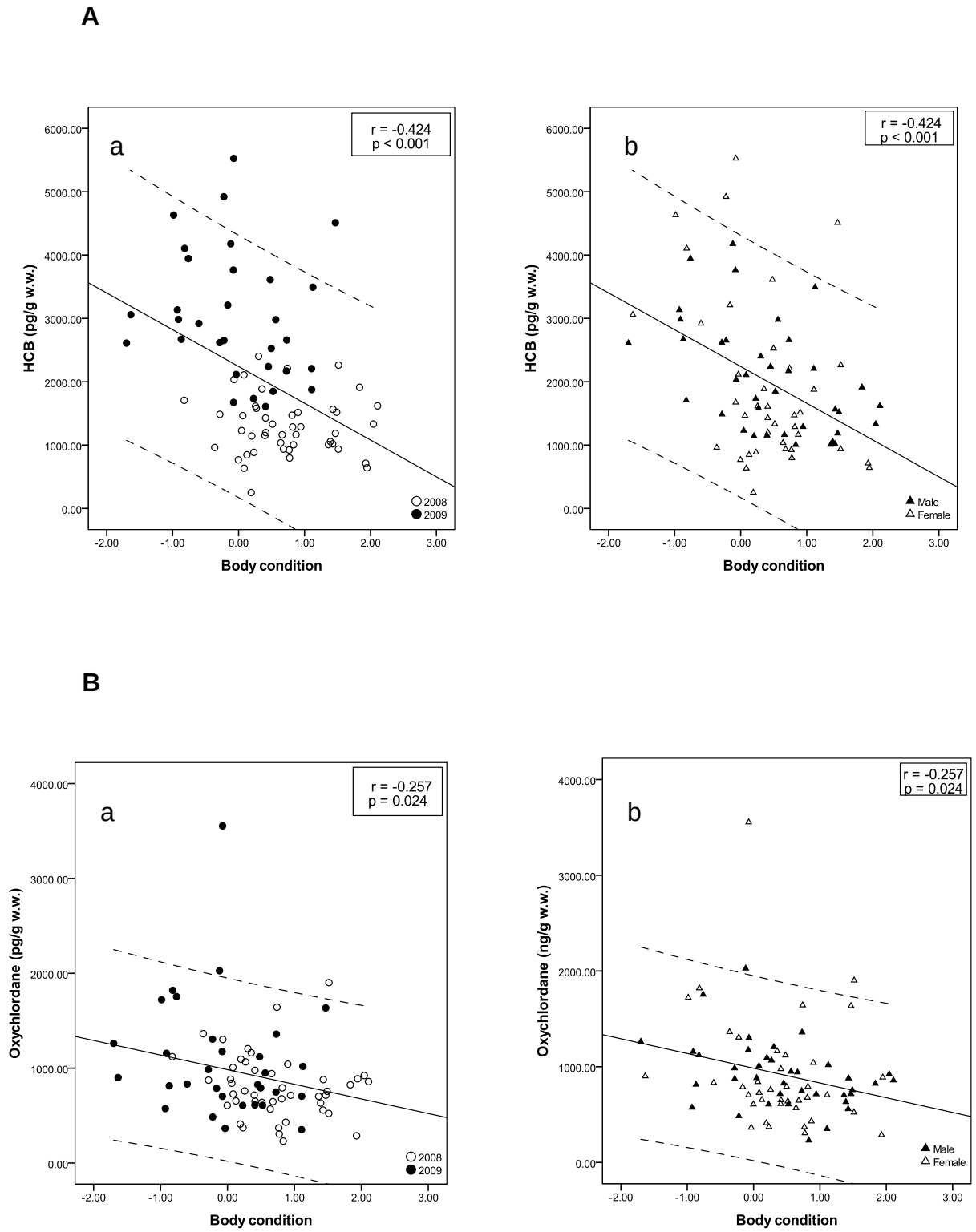

Figure 3.4.4: Correlation plot between (A) body condition and HCB (pg/g w.w.) and (B) body condition and oxychlordane (pg/g w.w.) presented as (a) inter-year and (b)between-sexes in 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, 2008 (n=46) and 2009 (n=31). The stippled lines represent 95% confidence intervals and the r- and p-values are displayed in the plot.

Body condition of the kittiwake was calculated based on the biometric variables skull, wing and BM. However, the correlation analysis confirmed only a significant positive correlation between BC and BM (Appendix G; Table G.2, Pearson, n = 77, r = 0.803, p < 0.001). Body mass was, as BC, inversely correlated to HCB (Figure 3.4.5, Pearson, n = 77, r = -0.299, p = 0.008). Hence, kittiwakes with low BM and a poor BC have higher levels of HCB in the blood.

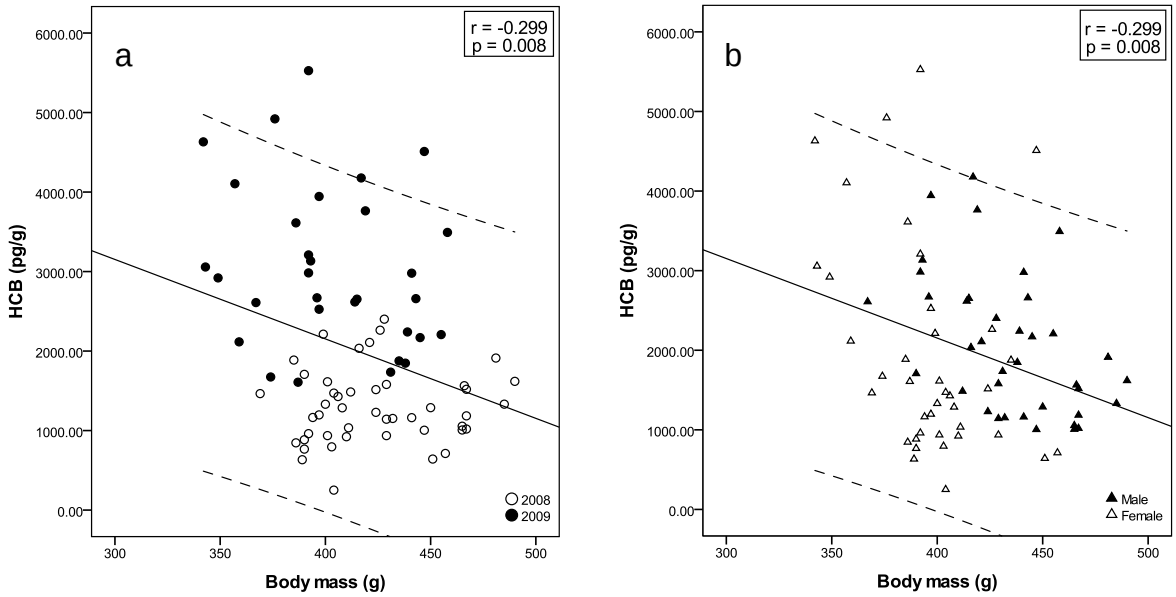

Figure 3.4.5: Correlation plot between body mass and HCB (pg/g w.w.) presented as (a) inter-year and (b) between-sexes in 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, sampled in 2008 (n=46) and 2009 (n=31). The stippled lines represent 95% confidence intervals and the r- and p-values are displayed in the plot.

In the PCA loading plot the variable egg-laying date was located close to the variable year, and the two variables were positively correlated (Pearson, n = 77, r = 0.737, p < 0.001). Thus, kittiwakes in 2009 had later egg-laying, compared to the kittiwakes in 2008. Egg-laying was located at the same side of the coordinate system in the loading plot as HCB, oxychlordane and PCB-183. This indicates positive correlations between egg-laying date and the three contaminants. This positive relationship was further confirmed by bivariate correlation analysis (Figure 3.4.6, HCB: r = 0.642, p < 0.001, oxychlordane: r = 0.406, p < 0.001, PCB-187: r = 0.243, p = 0.33). Egg-laying was not associated to differences between sexes (Figure 3.4.6.b, Pearson, n = 77, r = -0.127, p = 0.271).

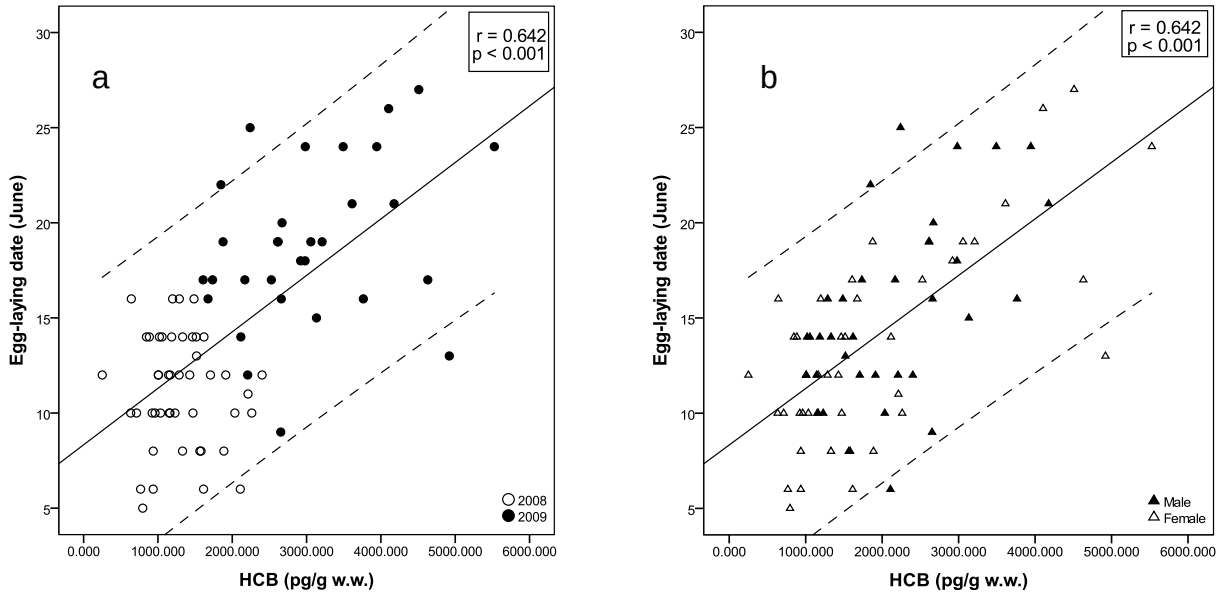

Figure 3.4.6: Correlation plot between egg-laying date (June) and HCB (pg/g w.w.) presented as (a) inter-year and (b) between-sexes of 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, sampled in 2008 (n=46) and 2009 (n=31). The stippled lines represent 95% confidence intervals and the r- and p-values are displayed in the plot.

Egg-laying was located opposite to BC and clutch in the loading plot. The position of egg-laying date indicates a negative association with the physical condition of the kittiwake, as well as the clutch size. The negative relationship was confirmed by bivariate correlation analysis (BC: Figure 3.4.7, r = -0.268, p = 0.018, clutch: r = -0,457). Kittiwakes with poor BC were associated with late breeding and small clutches.

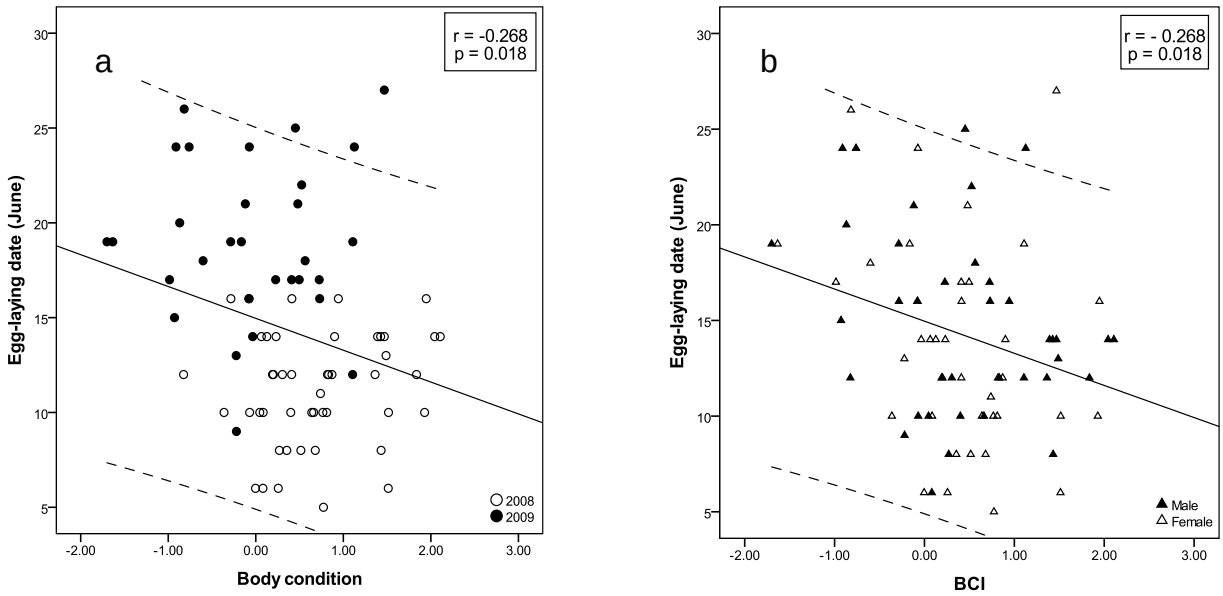

Figure 3.4.7: Correlation plot between body condition and egg-laying date (June) presented as (a) inter-year and (b) between-sexes of 77 kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard, samples in 2008 (n= 46) and 2009 (n= 31). The stippled lines represent 95% confidence intervals and the r- and p-values are displayed in the plot.

4. Discussion

The present study illustrates that annual fluctuations in environmental conditions and food availability in Arctic areas can affect bioaccumulation of POPs and physiological variables, such as BC. The present study demonstrates between-year variations in burden of POPs, which were linked to BM loss most likely caused by annual variations in prey availability. The plasma T3 level was not linked to the POP burden in the kittiwakes.

4.1 Physiology of the kittiwakes and environmental conditions

In 2008 the BM of breeding kittiwakes (Table 3.1.1) was similar to that previously reported in breeding kittiwakes in Kongsfjorden (Langset 2008, Nordstad 2009). However, in 2009, the BM of the breeding kittiwakes was lower compared to these previous studies. In the present study the kittiwakes had significantly lower (~6%) BM in 2009 compared to the kittiwakes in 2008 (Table 3.1.1). Kittiwakes breeding at high northern latitudes are believed to have less flexibility in their energy expenditure compared to kittiwakes breeding in temperate regions (Welcker et al., 2010). Years with unfavourable environmental conditions have been reported to cause BM loss and reduced reproductive success of kittiwakes breeding at Svalbard (Moe et al. 2009, Welcker et al. 2010) and in Alaska (Kitaysky et al. 1999). In the present study both male and female kittiwakes had a significantly poorer BC (Table 3.1.1) in 2009 compared to the kittiwakes in 2008. The difference in physical condition of the kittiwakes between years could be due to the strict energy budget of kittiwakes breeding at Svalbard, and inter-year variations in environmental conditions.

The weather conditions in the European Arctic in April 2008, was characterized by a rapid decline in the sea-ice cover. This was caused by higher than normal surface air temperature (1-5°C) over the Arctic Ocean, and the warm temperatures lasted throughout May (National Snow and Ice Data Center 2008, The Norwegian Meteorological Institute 2008a). On the contrary, the weather conditions in April 2009, was characterized by temperatures lower than average in the Arctic area (The Norwegian Meteorological Institute 2009a), and the sea-ice cover declined much slower compared to 2008 (National Snow and Ice Data Center 2009). In 2009, Kongsfjorden was entirely frozen until mid-May (Chastel 2011, personal comments). During the pre-laying period in 2009, the foraging trips of the kittiwakes were of longer duration, compared to the foraging trips in 2008 (Goutte et al., 2011). The extended duration

indicated poor and / or distant food availability at sea during the pre-laying period in 2009 (Goutte et al. 2011). The presence of sea-ice in the fjord and the food shortage in May 2009, may have forced the kittiwakes to forage far from the colony, which increase the energetic costs of foraging. Thus, the food shortage in 2009 was most likely the cause for the low BM of both sexes that year. This emphasizes how environmental factors have the ability to affect the physical condition of the kittiwakes. Furthermore, the spring of 2009 was dominated by low ambient temperatures, which may have increased the energetic costs to maintain thermoregulation. In summary, the low temperatures, extensive sea-ice cover and food shortage in 2009 did likely make the pre-laying period energetically demanding for the kittiwakes, resulting in low BM and a subsequent low BC.

The females laid eggs significant later (~8 days) in 2009 than in 2008. Also, the clutches were smaller in 2009 compared to 2008 (Table 3.1.2, Chastel 2011, field data). This indicates that early breeding was not feasible for the kittiwakes in 2009, probably due to their poor physical condition. Previous studies have reported that the BC of female kittiwakes from Svalbard, in the pre-laying period is decisive for the timing of breeding. The reason could be that female kittiwakes that are in better BC have the energy resources to lay eggs earlier (Gill et al. 2002, Goutte et al. 2011). In the present study, there was a negative association between BC and the egg-laying date (Figure 3.4.7). Thus, females in a poor BC laid eggs later. This is in accordance with previous studies of breeding kittiwakes at Svalbard (Goutte et al. 2010) and in Alaska (Gill et al. 2002). The BC of the females during the egg-laying period is partly dependent of her mate, as the kittiwakes have courtship feeding (Helfenstein et al. 2003, Kempenaers et al. 2007). During the egg-formation, females stay almost exclusively by the nest and their energetic needs are high. Thus, their partner provides them with food (Helfenstein et al. 2003). Because of the scarcity of suitable prey in 2009, the female kittiwakes may not have been able to build up sufficient energy reserves in 2009, in order to accomplish early egg-laying.

Based on the findings in the present study, it is suggested that the choices to breed in 2009 was at the expense of the kittiwakes' own BC. The breeding season is characterized by a continuous balance between energy allocation to breeding and self-maintenance (Angelier et al. 2009, Kitaysky et al. 1999). Kittiwakes are able to compensate for temporarily unfavourable survival conditions by expanding their foraging range and trip duration, and therefore, invest more energy in reproduction (Kitaysky et al. 2000, Suryan et al. 2002). The extended foraging trips of the kittiwakes in 2009, reported by Goutte et al. (2011), were likely

an attempt to cover their energetic needs. However, in cases of prolonged poor conditions, the kittiwakes are forced to switch to self-preservation (Angelier et al. 2009). This is because the Arctic environment probably causes them to operate close to an intrinsic metabolic ceiling (Welcker et al., 2010). The breeding success for the kittiwakes in 2009 was poorer in comparison to the breeding success for 2008 (Appendix H). The poor survival rate for the chicks in 2009 was an indication that the kittiwakes had less energy to invest in reproduction, and that they prioritized self-maintenance. As the kittiwake is a long-lived seabird, post-breeding survival and increasing the probability of future reproduction, is more evolutionary advantageous than the success of the current reproduction (Kitaysky et al. 1999). The severe environmental conditions which were prevailing during the spring 2009, made the kittiwakes show signs of abandonment of reproduction in 2009 by investing less in egg-laying, and rather focus on own survival.

4.2 Concentration of POPs

The concentrations of POPs in whole blood from kittiwakes from Kongsfjorden have been analysed and reported in one previous study (Nordstad 2009). The levels of POPs found in the present study correspond to the levels reported in those kittiwakes from 2007 (Table 4.2.1). The concentrations of POPs in blood from kittiwakes are much lower than the concentrations reported in polar bear and glaucous gulls from Svalbard (Braathen et al. 2004, Letcher et al. 2010, Verreault et al. 2004), the top predators in the Arctic. The kittiwakes occupy a lower trophic position, than polar bears and glaucous gulls (Borga et al. 2004, Hop et al. 2002). POPs are biomagnified in the Arctic marine food web (Fisk et al. 2001, Hop et al. 2002), it is therefore, not surprising that the concentrations of POPs in kittiwakes were lower compared to species occupying higher tropic positions (Table 4.2.1).

Table 4.2.1: A selection of recently reported exposure levels of some selected persistent organic pollutants in adult free-ranging seabird species. The blood concentrations (pg/g w.w) are presented as mean ± SD.

Species	Sex	N	Location, year	PCB-153	DDE	HCB	Oxychlordane	Reference
Common eider (*Somateria mollissima*)	F	45	Kongsfjorden, Svalbard, 2007[a]	187.3 ± 23.8[b]	183.5 ± 32.4[b]	322.8 ± 31.8[b]	-	Bustnes et al. (2010)
Common eider	F	53	Kongsfjorden, Svalbard, 2008[a]	110.9 ± 21.08[b]	152.8 ± 21.19[b]	129.0 ± 10.05[b]	51.55 ± 4.34[b]	Fenstad et al. (2010)
Kittiwake (*Rissa tridactyla*)	F+M	46	Kongsfjorden, Svalbard, 2008	6498.33 ± 4728.28	2443.46 ± 1774.07	1297.01 ± 468.09	809.99 ± 337.77	Present study
Kittiwake	F+M	31	Kongsfjorden, Svalbard, 2009	5974.74±44 20.07	2504.92 ± 2209.58	3037.81 ± 1019.35	1082.60 ± 633.83	Present study
Kittiwake	F+M	26	Kongsfjorden, Svalbard, 2007	7766.2[c]	2629 ± 668.7[b]	1600 ± 93.5[b]	1566 ± 172.1[b]	Nordstad (2009)
Lesser Black-backed gull (*Larus Fuscus*)	F+M	80	Helgeland, Norway, 2005m	7690 ± 8980	15760 ± 28500	2800 ± 1060	1460 ± 1170	Bustnes et al. (2008b)
Great black-backed gulls (*Larus marinus*)	F	14	Finnmark,Norway 2001[d]	-	32560 ± 7610[b]	6370 ± 1580[b]	4240 ± 1510[b]	Bustnes et al.(2008a)
Great black-backed gulls	M	10	Finnmark,Norway 2001[d]	-	26100 ± 2450[b]	4340 ± 390[b]	3760 ± 730[b]	Bustnes et al. (2008a)
Glaucous gulls (*Larus hyperboreus*)	F	49	Bear Island, 1997	106610 ± 22280	61500 ± 8640	11270 ± 1300	16700 ± 2460	Bustnes et al. (2004)
Glaucous gulls	M	51	Bear Island, 1997	237740 ± 33800	113280 ± 10270	23640 ± 2600	31940 ± 3730	Bustnes et al. (2004)
Glaucous gulls	F	34	Bear Island, 2001	118000 ± 112000	69100 ± 47400	18000 ± 11200	12100 ± 9580	Verreault et al. (2004)
Glaucous gulls	M	32	Bear Island, 2001	179000 ± 166000	122000 ± 92600	22300 ± 12000	14200 ± 7510	Verreault et al. (2004)

-: not reported

[a]day five of incubation

[b]Standard error

[c]Standard derivation not available

[d]Location Loppa,

In the present study the PCB congener patterns in the kittiwakes were dominated by the persistent PCB congeners (PCB-153, -138 and -180) in both 2008 and 2009 (Figure 3.2.1). The PCB congener patterns in kittiwakes are a result of a combination of diet and

biotransformation (Borga et al., 2005). Their ability to eliminate PCBs is linked to the position of the chlorine (Cl) atoms (Borga et al. 2005). Different seabird species are suggested to have a higher ability to eliminate congeners with Cl-unsubstituted meta-para positions than congeners with Cl-unsubstituted ortho-meta positions (Borlakoglu et al. 1988). Because of this congener-selective biotransformation, kittiwakes have higher concentrations of persistent (congeners with Cl-substituted meta-para positions) PCBs (Borga et al. 2005). The fact that the concentrations of the low-chlorinated PCBs were below the detection limit in the present study, suggests that these compounds most likely were biotransformed by the kittiwakes. Kittiwakes also have the capacity to biotransform the chlorinated pesticides DDT, nonachlor and chlordane (Borga et al. 2007). However, kittiwakes are less able to eliminate the persistent metabolites oxychlordane and DDE, and they accumulate in the tissue (Borga et al. 2007). This is confirmed in the present study. The levels of parent compounds (DDT and chlordane) were below the detection limits, whereas the biotransformation products (DDE and oxychlordane) were above the detection limits.

Despite the lower BM of the kittiwakes in 2009 as compared to in 2008, the concentration of PCBs in kittiwakes did not differ between the two years (Figure 3.2.2). The findings are inconsistent with previous studies on common eiders (*Somateria mollissima*) from Svalbard (Bustnes et al. 2010a, Fenstad 2010), kittiwakes from Finnmark (Henriksen et al. 1996) and great black-baked gulls (*Larus marinus*) from Finnmark (Bustnes et al. 2008a, Helberg et al. 2005), showing that blood concentrations of POPs increases when the BM decreases. This is due to as lipids are metabolised, lipid-soluble POPs are released to the blood (Bustnes et al. 2008a, Bustnes et al. 2010a, Helberg et al. 2005). However, Fenstad (2010) reported a critical lower BM limit for release of POPs into the blood of female common eiders. In the eiders, the blood concentrations of POPs did not change as a function of BM when the BM was above1800 g. As the females starved during the incubation period and the BM was reduced to below 1800 g, the further mass loss was associated with increasing blood concentrations of POPs (Fenstad 2010). According to this, a possible explanation for the stable PCB level between years, as observed in the present study, is that the BM in 2009 did not reach the critical lower BM limit for release of PCBs into the blood of breeding kittiwakes.

The levels of HCB and oxychlordane were significant higher in kittiwakes in 2009 compared to the levels detected in 2008 (Figure 3.2.3). Henriksen et al. (1998) suggested that a mass loss of 100 g in glaucous gulls (4-7% of their BM) could cause a doubling of the POP concentrations in the blood. In the present study, the concentration of HCB in the blood of the

kittiwakes was more than two times higher (134%) in 2009 than in 2008. It should also be mentioned that the concentration of HCB in air (pg/m^3), measured at the Zeppelin station, Ny-Ålesund, was slightly higher (~4%) in 2009 than in 2008 (Climate and Pollution Agency 2010). This was mainly due to air currents coming from Russia and Canada (Climate and pollution agency 2010). HCB is a volatile compound and has generally a higher transport potential as compared to the PCBs (Bustnes et al., 2010b). It is possible that the increased concentrations of HCB in the air may have resulted in increased levels in the aquatic environment, and thus a higher bioaccumulation of HCB in kittiwakes in 2009 compared to in 2008. Elevated concentrations of POPs have previously been reported in breeding glaucous gulls from Bear Island, in following years after a high air transport of POPs toward the Arctic (Bustnes et al. 2010b). Nevertheless, the POP concentrations in wildlife are influenced by other confounding factors, such as the diet. Year-to-year variability in diet composition of breeding kittiwakes from Kongsfjorden, in 2008 and 2009, was reported by Gasbjerg (2010). The diet in 2008 was dominated by capelin, crustaceans and polar cod, in equal parts. Contrary to 2008, the diet in 2009 was nearly solely dominated by polar cod (Gasbjerg 2010). The dissimilarity of the diet composition could also be the reason for the different accumulation pattern of HCB and oxychlordane in 2008 and 2009, as observed in the present study.

In the present study, the concentrations of HCB were inversely correlated to BC and BM of the kittiwakes (Figure 3.4.4A, 3.4.5). Also the concentration of oxychlordane increased with decreasing BC (Figure 3.4.4B). However, blood concentrations of oxychlordane were not associated with BM. The relationships demonstrate that kittiwakes in a poor BC had higher levels of contaminants, and the concentrations of HCB and oxychlordane were affected by alterations in BC. This is in accordance with previous studies on common eiders at Svalbard (Bustnes et al. 2010a, Fenstad 2010), glaucous gulls at Bear Island (Bustnes et al. 2003) and black-backed gulls in Finnmark (Bustnes et al. 2008a, Helberg et al. 2005). The inverse relationship between these two contaminants and BC was related to inter-year differences in concentrations, and not associated to differences between sexes (Figure 3.4.4b). However, HCB and oxychlordane were not correlated with BC within each year. This may suggest that it was the choice of prey of the kittiwakes which influenced the kittiwakes' blood concentrations of HCB and oxychlordane.

The levels of PCBs and DDE were not correlated to BC, or BM of the kittiwakes. The compound specific association to BC was unexpected, as several previous studies have

reported concentrations of PCBs and DDE to increase as a function of decreasing BC (Bustnes et al. 2010a, Fenstad 2010, Henriksen et al. 1998). However, PCBs and DDE have also been reported to not be associated to the BC of breeding glaucous gulls at Bear Island (Bustnes et al. 2003, Bustnes et al. 2010b). The findings in the present study could indicate that kittiwakes have compound-specific critical lower BM limits for the release of POPs from the adipose tissue into the blood. The compound-specific critical lower BM limits could be caused by the different compounds' lipophilicity and size. The octanol-water partition coefficient (K_{ow}) gives an indication of a certain compounds' affinity for lipid rich tissue (Noble 1993). A high K_{ow} value is analogous to a high preference for lipids (Simpson et al. 1995). HCB has a log $K_{ow}\approx 6$, which is similar to the log K_{ow} value of DDT, however, HCB is a smaller molecule (Noble 1993). Sormo et al. (2003) reported that HCB was transferred more readily compared to DDT, to the milk from the blubber in maternal gray seals. This suggests that the physiochemical properties of POPs, such as molecular size, influence the transfer between compartments in an individual (Sormo et al. 2005). Thus, the high increase of HCB in the kittiwake blood, compared to the other compounds, could be because HCB's size makes it easier for HCB to pass membranes and travel into the blood compared to the PCBs and DDE.

In the present study, there were positive associations between egg-laying date of the kittiwakes and the concentration of HCB (Figure 3.4.6), oxychlordane and PCB-187. These findings suggest that kittiwakes with high levels of HCB, oxychlordane and PCB-187 breed later. An association between POPs and egg-laying has also been reported in great black-backed gulls at Finnmark (Bustnes et al. 2008a). In a study comparing PCBs, HCB, oxychlordane and DDE, HCB and oxychlordane were reported as the compounds with highest adverse effects potential in glaucous gulls from Bear Island (Bustnes 2006). Even though the concentrations of HCB and oxychlordane in the kittiwakes were lower than those reported in glaucous gulls and great black-backed gulls (Table 4.2.1), is it possible that HCB and oxychlordane in the kittiwakes were involved in the delayed breeding. However, the role of these two compounds in relation to breeding remains to be proven.

Some of the POPs (HCB, PCB-138, PCB-153, PCB-180 and PCB-183) differed between male and female kittiwakes in 2008, where males had higher concentrations of these compounds (Appendix E; Table E1 and E.2). This could be due to the ability of female birds to transfer these POPs to their eggs (Borga et al. 2004, Verboven et al. 2009). The variations in the levels of POPs between sexes could also be a result of different foraging areas for the

two sexes in 2008. Dissimilar foraging areas could be associated with feeding at different trophic levels, hence, unequal exposure to POPs. The mass loss of both sexes in 2009 may be the reason why the concentrations of POPs did not differ between male and female that year. It should also be mentioned that the different foraging areas in the pre-laying period in 2008 and 2009, could be the explanation for the elevated levels of HCB and oxychlordane in 2009.

The large individual variations in the concentrations of POPs, found both in 2008 and in 2009, could be a result of individual variability in migration behaviour and location specific exposure. Contaminant burden in herring gulls (*Larus argentatus*), have been reported to be dependent of their winter migration patterns, as southerly overwintering locations of the herring gulls are more polluted (Hebert 1998). Also, individual-specific biotransformation potential may result in higher concentrations of biotransformation products in some individuals. For instance, Lamba et al. (2002) documented individual differences in the expression of the enzyme CYP3A in humans, due to individual variations in gene-environment causes. Thus, the individual differences observed in the kittiwakes in 2008 and in 2009, may arise from diverse exposure in different overwintering areas and possible individual variations in the ability to biotransform POPs.

4.3 Thyroid hormones

The T3 levels reported in the current study were slightly lower than previous reported levels from studies on kittiwakes in Kongsfjorden, Svalbard. Earlier studies have reported T3 levels in the range 3.10-4.16 ng/mL (Langset 2008, Ronning et al. 2008), while the mean levels in the present study were 2.7 and 2.4 ng/mL for 2008 and 2009 respectively. A possible explanation for the slightly lower T3 levels in the present study is the analytical procedures used in the different studies. Even though the T3 analyses in all these three studies were conducted using the same laboratory, the fact that they were analysed in different assays could result in inter-assay variability.

There was no significant difference in the T3 level between 2008 and 2009. Possible confounding factors, such as age and time of day of capture in the incubation period, were assumed to be similar in the two years. Seawater is abundant in iodine (Whitehead 1984), therefore, iodine deficiency in the diet and possible influences on the THs levels were not likely in the present study. Sex, BM and BC were controlled for in the statistical analyses,

and did not affect the T3 levels. In addition, the environmental conditions were approximately the same during the sampling period in respect of mean temperature for June 2008: 2.6 °C, 2009: 1.6 °C (Norwegian Metrological Institute 2008b, Norwegian Metrological Institute 2009b), and absence of sea-ice in the fjord (personal comments).

Langset (2008) reported that plasma T3 levels decreased with BM reduction, within one breeding season of kittiwakes breeding in Kongsfjorden. According to this, the T3 levels in 2009 should have been lower than in 2008, because of the lower BM of the kittiwakes in 2009 as compared to in 2008. The reason for not observing this relationship could be due to the extent of BM difference between 2008 and 2009. The mass loss of kittiwakes throughout the breeding season (~12%) is considerable greater compared to the BM difference observed between the two years of the present study. Furthermore, it is not expected to find the same relationships between different years as has been found within a season, due to physiological traits may change as they are dependent on environmental context and individual variations (Piersma and Lindstrom 1997).

In 2008 male kittiwakes had significantly higher T3 values compared to females. Previous studies have not reported sex-differences in the T3 levels, but sex-differences were detected in a recent study in other kittiwake colonies in Kongsfjorden (Welcker 2011, unpublished material). Sex-differences in hormone levels have been observed within many species in relation to reproductive and parental care (Lormee et al. 2000, Lormee et al. 2003). However, T3 is not directly linked to the parental behaviour during the breeding season, such as luteinizing hormone, steroid hormones, prolactin and corticosterone (Angelier et al. 2007, Angelier et al. 2009, Goutte et al. 2010). The higher T3 level in males may be a result of a higher BMR in males compared to female kittiwakes. However, the research done on BMR in kittiwakes is so far limited to females (Bech et al. 1999, Ronning et al. 2008). Thus, sex-differences in BMR of kittiwakes have never been studied (Bech 2011, personal comments).

In the present study there was a positive relationship between wing length and T3 levels (Figure 3.4.4). Male kittiwakes have naturally longer wings than females (Appendix D; Table D.1 and D.2), and in the present study the males had higher T3 levels. Thus, the correlation between T3 and wing was most likely caused by the sex-differences in the T3 levels (Figure 3.4.3.b) of the kittiwakes. Thus, wing length, as a measure of body size, appeared to be a determinant biometric variable for plasma T3 concentrations, and not BC or BM.

There were no associations between the whole blood concentrations of POPs and the plasma T3 levels in the kittiwakes (Appendix G; Table G.4). Several studies have linked increasing concentrations of POPs to decreasing levels of THs (Braathen et al. 2004, Skaare et al. 2001, Sormo et al. 2005, Verreault et al. 2004). However, the associations between POPs and THs reported in previous studies have primarily been between POPs and T4 (Dawson 2000, Verreault et al. 2004). In the few studies reporting associations between POPs and T3, the reported effects include both positive and inverse associations between POPs and T3 (Braathen et al. 2004, Sormo et al. 2005, Verreault et al. 2004). For instance, Verreault et al. (2004) reported elevated plasma T3 levels in glaucous gulls exposed to high levels of POPs. On the contrary, the relationship between PCBs and T3 was negative in polar bears at Svalbard, and the authors suggested T3 to be more susceptible for PCB-induced alterations than T4 (Braathen et al. 2004). In addition, several of the studies reporting associations between TH and POPs are laboratory based studies on rodents or field studies on mammals (Dawson 2000). Unlike mammals, where TTR is the major transport protein, T4 is mainly associated with the transport protein albumin in avian species (McNabb 2000). Thus, competitive binding between POPs and T4 for binding sites on albumin has not necessarily the same extent in birds, as competitive binding between POPs and THs for TTR in mammals. The less susceptibility for POP-interference with THs transport protein in birds could be the reason for the unaffected T3 levels in the present study. It is, however, possible that if also T4 was analysed in the present study, an effect on the TH homeostasis of the kittiwakes could have been found.

Verreault et al. (2004) reported oxychlordane and HCB to be the most prominent compounds in terms of their effect on the THs levels in glaucous gulls at Bear Island. In contrast to this, there were no associations between these contaminants and T3 in the present study. This was despite the higher concentrations of HCB and oxychlordane in 2009 as compared to in 2008. It is possible that the lack of these hypothesized inverse relationships is due to the relative low concentrations of POPs in kittiwakes compared to in glaucous gulls (Borga et al. 2001, Hop et al. 2002). Several studies have failed to prove strong associations between POPs and THs in species with lower positions in the arctic food web (Nordstad 2009, Nøst 2009). Since there have been reported several effects of POPs on THs in glaucous gulls (Verreault et al. 2004) and herring gulls (McNabb and Fox 2003), it can be assumed that the levels of POPs in seabirds occupying lower trophic levels may not be sufficient enough to exceed critical threshold levels for POPs-induced alterations on the THs homeostasis. In addition, Ucán-

Marin et al. (2010) reported brominated compounds to be more forceful competitive ligands to gull recombinant albumin and TTR relative to both T3 and T4, compared to chlorinated compounds. Considering the low levels of chlorinated compounds in kittiwakes observed in the present study, and the fact that avian species have been reported to be less susceptible to POP-induced changes to TH levels compared to mammals, the present study suggests that the current environmental concentration exposures of POPs in the Arctic are not sufficient to affect the T3 levels of kittiwakes.

5. Conclusion

The present study is the first to compare inter-year variations of concentrations of POPs, and asses their possible affects on the T3 levels in Arctic breeding kittiwakes. The breeding season in 2009 was characterized by severe environmental conditions compared to 2008. In 2009 the kittiwakes had poor BC, elevated concentrations of HCB and oxychlordane and the egg-laying started later than in 2008. These findings suggest that years with poor environmental condition could make the kittiwakes more susceptible for adverse effects induced by HCB and oxychlordane. However, the levels of POPs in the kittiwakes did not have any effect on the circulating T3 levels. The findings may suggest that the levels of POPs in the kittiwakes are not sufficient to induce the levels of circulating T3.

Since environmental conditions fluctuate between years, it is possible that the vulnerability of the kittiwakes to stress related effects also could differ from one breeding season to the next. Further research is, therefore, needed to verify the links between annual environmental conditions, concentrations of POPs and possible effects in kittiwakes. In addition, the high increase of HCB compared to the other compounds from 2008 to 2009 as shown in the present study, suggests that the adverse effect-potential of HCB should be further examined in Arctic breeding kittiwakes.

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Appendix A – Analysed PCB congeners

Table A.1: Structure of individual PCB congeners, analyzed in the present study (Boon et al. 1997, Borga et al. 2005, Borlakoglu et al. 1988).

PCB congener	Cl- substitution (IUPAC nomenclature)	Position of vicinal H atoms		
		ortho-meta	*meta-para*	*ortho*
Group I:	No vicinal H-atoms			
PCB-153	2,2',4,4',5,5'-hexachlorobiphenyl	-	-	2
PCB-180	2,2',3,4,4',5,5'-heptachlorobiphenyl	-	-	2
PCB-183	2,2',3,4,4',5',6-heptachlorobiphenyl	-	-	3
PCB-187	2,2',3,4',5,5',6-heptachlorobiphenyl	-	-	3
Group II:	Vicinal H-atoms only in *ortho-meta* positions, ≥ 2 Cl in *ortho*-position			
PCB-99	2,2',4,4',5-pentachlorobiphenyl	+	-	2
PCB-138	2,2',3,4,4',5'-hexachlorobiphenyl	+	-	2
Group III:	Vicinal H-atoms only in *ortho-meta* positions, < 2 Cl in *ortho*-positions			
PCB-28	2,4,4'-trichlorobiphenyl	+	-	1
PCB-118	2,3',4,4',5-pentachlorobiphenyl	+	-	1
Group IV:	Vicinal H-atoms in *meta-para* positions, ≤ 2 Cl in *ortho*-position			
PCB-52	2,2',5,5'-tetrachlorobiphenyl	-	+	2
PCB-101	2,2',4,5,5'-pentachlorobiphenyl	-	+	2

Appendix B – Internal standards

Table B.1: Internal standard: POP I (47.09: Utilized as internal standard in PCB-, DDT/HCH- and pesticide analyses) added to the samples in the contaminant analyses.

^{13}C compounds	Concentration pg/µL
PCB-28	246.6
PCB-52	234.3
PCB-101	244.1
PCB-105	242.1
PCB-114	242.1
PCB-118	242.3
PCB-123	238.7
PCB-138	244.1
PCB-153	239.9
PCB-156	243.5
PCB-157	242.3
PCB-167	241.8
PCB-180	245
PCB-189	239.8
PCB-209	243.1
HCH	1022.2
HCH	211.4
HCH	997.6
p.p'-DDE	325.7
p.p'-DDT	319
PeCB	101.8
HCB	104.9
Heptachloroepoxid	1019.7
trans-Nonachlor	364.3
trans-chlordane	521.6
Dieldrin	2544.2
Mirex	1019.2
Endosulfan I	2381.8
Endosulfan II	2392.2
Endosulfan Sulfate	2304
Trifluralin	2488
cis-Nonachlor	2500.8
Aldrin	2521
Endrin	2516.8
Oxychlordane	2432
Isodrin	2411.2
cis- Chlordane	2432.2
Delta-BHC	2518.2
Heptachlor	2501.7

Appendix C – Detection limits

Tabell C.1: Detection limits for POPs in 2008 and 2009

Compound	Detection limit (pg/g)	
PCB-28	208.00	*
PCB-52	94.90	*
PCB-99	314.90	
PCB-101	422.00	
PCB-118	443.00	
PCB-138	433.00	
PCB-153	60.10	
PCB-180	246.00	
PCB-183	209.00	
PCB-187	260.00	
DDT	409.00	*
DDE	268.00	
α-HCH	11.10	
β-HCH	19.00	
γ-HCH	7.80	
HCB	0.40	
Heptachlor	34.80	
Heptachlor epoxide	12.30	
cis-chlordane	3.60	
oxychlordane	244.90	
trans-Nonachlor	2.30	
cis-Nonachlor	1.20	
Mirex	18.50	

* DDT and lower PCBs could not be analysed in 2009, due to a switch from EI to NCI on the MS

Appendix D – Individual biometric measurements

Table D 1: Individual biometric measurements of kittiwakes (*Rissa tridactyla*), from Kongsfjorden Svalbard, captured in 2008.

ID	Sex	Body mass (g)	Head (mm)	Wing (cm)	BCI
KW08-1	M	447.00	92.70	32.60	0.83
KW08-2	M	465.00	93.60	32.50	1.39
KW08-3	M	465.00	96.10	32.10	1.36
KW08-4	M	412.00	94.60	31.90	-0.28
KW08-5	M	485.00	92.00	32.80	2.04
KW08-6	M	421.00	89.60	32.20	0.08
KW08-7	M	429.00	92.90	32.30	0.27
KW08-8	M	466.00	94.10	32.10	1.43
KW08-9	M	428.00	90.30	31.90	0.30
KW08-10	M	450.00	92.10	32.50	0.94
KW08-11	M	481.00	94.90	33.40	1.84
KW08-12	M	416.00	90.40	31.70	-0.07
KW08-13	M	429.00	96.60	32.30	0.20
KW08-14	M	467.00	93.50	31.80	1.49
KW08-15	M	390.00	87.30	31.40	-0.82
KW08-16	M	441.00	90.00	33.30	0.66
KW08-17	M	467.00	92.90	32.50	1.47
KW08-18	M	467.00	94.20	32.90	1.43
KW08-19	M	424.00	93.80	33.40	0.05
KW08-20	M	432.00	90.60	32.50	0.40
KW08-21	M	490.00	95.20	33.60	2.11
KW08-22	F	392.00	91.30	32.50	-0.36
KW08-23	F	369.00	87.30	31.00	0.06
KW08-24	F	408.00	88.50	31.70	0.82
KW08-25	F	424.00	90.40	32.10	0.90
KW08-26	F	399.00	89.20	31.00	0.74
KW08-27	F	406.00	89.60	32.20	0.41
KW08-28	F	401.00	91.50	31.60	0.26
KW08-29	F	401.00	89.50	31.20	0.68
KW08-30	F	389.00	90.30	31.50	0.08
KW08-31	F	411.00	90.60	31.70	0.64
KW08-32	F	394.00	87.00	31.00	0.87
KW08-33	F	385.00	86.00	31.90	0.35
KW08-34	F	404.00	88.60	32.90	0.19
KW08-35	F	426.00	87.70	31.60	1.52
KW08-36	F	386.00	88.00	31.90	0.13
KW08-37	F	451.00	88.40	32.20	1.95
KW08-38	F	400.00	88.00	32.00	0.51
KW08-39	F	404.00	87.30	31.80	0.81
KW08-40	F	410.00	89.70	31.60	0.77
KW08-41	F	457.00	92.50	31.40	1.93
KW08-42	F	390.00	89.00	32.20	0.00
KW08-43	F	429.00	89.70	31.20	1.51
KW08-44	F	397.00	88.40	31.90	0.41
KW08-45	F	390.00	89.40	31.50	0.23
KW08-46	F	403.00	88.30	31.50	0.77

Table D.2: Individual biometric measurements of kittiwakes (*Rissa tridactyla*), from Kongsfjorden Svalbard, captured in 2009.

ID	Sex	Body mass	Head (mm)	Wing (cm)	BCI
KW09-1	M	443.00	92.80	32.00	0.73
KW09-2	M	392.00	93.70	32.10	-0.91
KW09-3	M	397.00	91.80	33.10	-0.76
KW09-4	M	367.00	92.70	32.40	-1.70
KW09-5	M	445.00	93.40	33.30	0.72
KW09-6	M	419.00	93.20	32.90	-0.08
KW09-7	M	439.00	98.00	33.20	0.45
KW09-8	M	455.00	92.10	32.40	1.11
KW09-9	M	458.00	94.50	33.10	1.13
KW09-10	M	415.00	93.50	33.10	-0.22
KW09-11	M	438.00	94.60	32.30	0.52
KW09-12	M	393.00	94.40	32.90	-0.93
KW09-13	M	431.00	96.00	33.40	0.23
KW09-14	M	441.00	94.50	33.60	0.56
KW09-15	M	414.00	95.30	33.10	-0.29
KW09-16	M	396.00	96.10	33.00	-0.87
KW09-17	M	417.00	90.50	33.50	-0.12
KW09-18	F	392.00	91.60	31.70	-0.07
KW09-19	F	342.00	89.00	31.00	-0.98
KW09-20	F	374.00	88.30	31.40	-0.08
KW09-21	F	376.00	89.60	31.50	-0.22
KW09-22	F	447.00	90.20	32.50	1.47
KW09-23	F	343.00	89.50	32.50	-1.63
KW09-24	F	359.00	87.60	30.40	-0.04
KW09-25	F	357.00	90.00	31.40	-0.82
KW09-26	F	386.00	85.90	31.70	0.48
KW09-27	F	392.00	90.40	32.30	-0.16
KW09-28	F	397.00	88.70	31.60	0.50
KW09-29	F	349.00	85.80	31.60	-0.60
KW09-30	F	435.00	91.40	32.10	1.11
KW09-31	F	387.00	87.00	31.60	0.41

Appendix E – Individual concentrations of POPs

Table E.1: Individual concentrations (pg/g w.w.) of POPs in whole blood from Kittiwakes (*Rissa tridactyla*), sampled in Kongsfjorden, Svalbard in 2008.

ID	PCB-99	PCB-118	PCB-138	PCB-153	PCB-180	PCB-183	PCB-187	$\sum_7$PCB	DDE	HCB	Oxychlordane
KW08-1	179.00	249.00	2778.00	2774.00	1596.00	137.00	117.00	7830.00	2194.00	1004.30	231.00
KW08-2	1644.00	1444.00	7205.00	6264.00	4626.00	1015.00	1443.00	23641.00	4943.00	1054.80	633.20
KW08-3	470.00	1784.00	4126.00	5061.00	2929.00	565.00	1103.00	16038.00	2842.00	1007.10	703.00
KW08-4	926.00	1575.00	4680.00	7324.00	3838.00	880.00	811.00	20034.00	2928.00	1485.50	873.80
KW08-5	1566.00	869.00	3532.00	5669.00	3112.00	742.00	768.00	16258.00	869.00	1332.00	922.30
KW08-6	1116.00	1983.00	7602.00	8920.00	4876.00	101.00	94.00	24692.00	2497.00	2108.40	1007.90
KW08-7	1161.00	1977.00	6835.00	9725.00	5288.00	730.00	1367.00	27083.00	1275.00	1579.90	1066.50
KW08-8	903.00	2055.00	5293.00	6288.00	3781.00	748.00	949.00	20017.00	1130.00	1564.30	880.20
KW08-9	2581.00	3415.00	16041.00	12517.00	6790.00	1050.00	2091.00	44485.00	6540.00	2401.10	1205.60
KW08-10	1020.00	1131.00	2859.00	3589.00	1118.00	184.00	96.00	9997.00	2227.00	1288.00	714.20
KW08-11	620.00	724.00	2697.00	3301.00	1882.00	336.00	687.00	10247.00	2555.00	1912.20	824.50
KW08-12	2357.00	2659.00	6388.00	9679.00	5204.00	968.00	1550.00	28805.00	1832.00	2034.70	1302.60
KW08-13	1029.00	2013.00	5337.00	8303.00	2826.00	641.00	1158.00	21307.00	1697.00	1142.70	1094.00
KW08-14	1129.00	1411.00	4407.00	4771.00	3007.00	177.00	181.00	15083.00	2098.00	1519.10	756.80
KW08-15	1558.00	1295.00	9768.00	15878.00	6178.00	903.00	1208.00	36788.00	141.00	1706.90	1121.00
KW08-16	1842.00	3284.00	11716.00	17456.00	8881.00	1313.00	2432.00	46924.00	1291.00	1162.20	942.40
KW08-17	1550.00	2199.00	3381.00	3435.00	2129.00	150.00	232.00	13076.00	1012.00	1184.90	716.00
KW08-18	270.00	3003.00	4554.00	4936.00	2434.00	489.00	130.00	15816.00	3414.00	1019.60	558.50
KW08-19	222.00	1393.00	4706.00	5873.00	2653.00	12.00	769.00	15628.00	3358.00	1229.10	883.20
KW08-20	740.00	1689.00	4809.00	5502.00	3251.00	131.00	888.00	17010.00	1285.00	1152.20	716.60
KW08-21	2252.00	3174.00	10358.00	12448.00	6322.00	1510.00	1525.00	37589.00	6678.00	1619.00	858.80
KW08-22	7.00	719.00	1509.00	2218.00	53.00	67.00	225.00	4798.00	1618.00	961.20	1363.20
KW08-23	2625.00	3801.00	9792.00	12405.00	5740.00	1377.00	2192.00	37932.00	5907.00	1464.70	841.20
KW08-24	1351.00	1829.00	3493.00	5669.00	2845.00	461.00	904.00	16552.00	1624.00	1286.10	792.90
KW08-25	1430.00	1665.00	5295.00	5662.00	2877.00	187.00	870.00	17986.00	3777.00	1515.30	1041.50
KW08-26	2098.00	3252.00	8628.00	13805.00	6314.00	1276.00	1811.00	37184.00	4979.00	2212.30	1642.60
KW08-27	874.00	1588.00	3603.00	4390.00	2730.00	413.00	828.00	14426.00	2334.00	1427.90	976.50
KW08-28	1355.00	2318.00	3563.00	4071.00	2531.00	534.00	107.00	14479.00	6518.00	1613.60	760.20
KW08-29	658.00	988.00	2329.00	3553.00	1632.00	245.00	555.00	9960.00	1311.00	936.50	647.30
KW08-30	816.00	910.00	2313.00	3523.00	1850.00	293.00	729.00	10434.00	103.00	633.30	728.20
KW08-31	1263.00	1545.00	4168.00	4849.00	1908.00	319.00	39.00	14091.00	4915.00	1034.80	569.30
KW08-32	165.00	717.00	1692.00	2018.00	1127.00	20.00	101.00	5840.00	1052.00	1164.50	430.40
KW08-33	936.00	1856.00	3878.00	5328.00	2199.00	17.00	1239.00	15453.00	2919.00	1886.80	1163.10
KW08-34	53.00	146.00	15.00	962.00	387.00	163.00	91.00	1817.00	452.00	250.90	410.80
KW08-35	2110.00	6261.00	9623.00	11253.00	5191.00	57.00	2347.00	36842.00	5474.00	2262.50	1902.30
KW08-36	126.00	426.00	853.00	1426.00	922.00	88.00	9.00	3850.00	1025.00	845.70	654.80
KW08-37	645.00	1077.00	2291.00	3064.00	1724.00	46.00	554.00	9401.00	2141.00	641.80	888.70
KW08-38	1216.00	1748.00	3320.00	5636.00	1922.00	185.00	696.00	14723.00	3185.00	1331.20	639.30
KW08-39	3902.00	5761.00	12722.00	16595.00	5641.00	33.00	2873.00	47527.00	1542.00	1471.20	677.60
KW08-40	483.00	883.00	1822.00	1674.00	906.00	261.00	316.00	6345.00	1006.00	922.50	369.80
KW08-41	477.00	652.00	1055.00	1421.00	914.00	16.00	39.00	4574.00	576.00	712.00	287.30
KW08-42	1934.00	2506.00	15552.00	19742.00	10700.00	2014.00	2634.00	55082.00	487.00	766.70	607.40
KW08-43	378.00	816.00	1705.00	1799.00	801.00	256.00	388.00	6143.00	1536.00	936.30	522.90
KW08-44	714.00	1079.00	2796.00	2592.00	1810.00	324.00	766.00	10081.00	1558.00	1197.00	651.60
KW08-45	613.00	1294.00	3371.00	3638.00	1997.00	476.00	964.00	12353.00	1913.00	884.40	372.00
KW08-46	232.00	1147.00	1661.00	1917.00	1052.00	46.00	392.00	6447.00	1641.00	795.30	306.30

Table E.2: Individual concentrations (pg/g w.w.) of POPs in whole blood from Kittiwakes (*Rissa tridactyla*) sampled in Kongsfjorden, Svalbard in 2009

ID	PCB-99	PCB-118	PCB-138	PCB-153	PCB-180	PCB-183	PCB-187	∑$_7$PCB	DDE	HCB	Oxychlordane
KW09-1	1356.60	2254.10	6699.50	9371.20	4263.30	968.90	1995.70	26909.30	3617.00	2659.30	1359.60
KW09-2	463.70	1577.30	4234.80	4359.10	3262.70	386.00	512.10	14795.70	1918.40	2983.00	1156.60
KW09-3	738.60	2662.70	7391.00	7898.10	5288.50	1040.00	1879.70	26898.60	6069.20	3944.40	1754.30
KW09-4	1312.00	1524.40	4392.40	5929.30	1975.70	828.60	960.90	16923.30	5250.40	2609.90	1261.40
KW09-5	917.30	896.90	2968.80	4350.60	2087.10	398.00	556.60	12175.30	483.40	2169.30	748.30
KW09-6	2000.30	2085.70	6632.40	10564.00	4599.00	852.10	1319.70	28053.20	3152.30	3763.60	1174.70
KW09-7	836.80	1031.20	3062.60	3152.50	1643.60	679.80	935.70	11342.20	2265.50	2240.10	826.70
KW09-8	371.00	458.00	1228.80	1697.80	842.80	156.80	282.60	5037.80	540.10	2207.10	351.50
KW09-9	3955.80	5407.90	12310.80	16237.20	6765.80	1586.10	2656.50	48920.10	6000.60	3492.30	1018.30
KW09-10	611.90	896.70	2076.70	2766.00	1369.00	288.60	464.40	8473.30	1421.70	2652.70	485.20
KW09-11	1037.00	739.50	2165.30	2717.60	1212.10	270.10	390.30	8531.90	745.10	1847.80	608.70
KW09-12	1053.80	1133.10	2744.10	4029.60	2025.10	355.70	622.50	11963.90	1219.20	3133.40	574.40
KW09-13	140.00	674.70	2237.00	3110.40	1677.60	368.60	639.00	8847.30	1158.00	1734.70	607.60
KW09-14	959.70	1017.70	2771.40	4299.20	2120.80	437.20	666.70	12272.70	829.90	2979.60	949.70
KW09-15	1106.50	1426.60	5392.50	4995.30	2408.70	367.50	739.90	16437.00	3162.50	2617.30	985.00
KW09-16	945.20	1187.10	2945.80	3985.80	1529.00	260.90	528.70	11382.50	1776.90	2669.90	814.10
KW09-17	991.20	4189.50	10039.80	13328.00	8056.10	1548.90	3097.80	41251.30	3909.60	4177.10	2027.10
KW09-18	3096.40	3925.80	12231.00	18659.20	8265.30	1602.40	3216.80	50996.90	5812.60	5526.50	3553.80
KW09-19	1950.50	2145.00	8328.00	10187.00	5521.70	1155.10	1681.10	30968.40	1885.10	4631.10	1722.30
KW09-20	349.00	824.10	1908.00	2853.70	1803.90	309.30	603.60	8651.60	1036.40	1673.90	703.90
KW09-21	1346.10	1663.20	4689.00	6337.20	2718.10	539.10	1007.20	18299.90	3822.20	4920.20	1306.00
KW09-22	1408.60	1541.70	4622.60	7535.40	2900.50	533.50	1122.90	19665.20	3376.80	4510.20	1635.10
KW09-23	1386.00	1805.90	5481.70	7531.80	3567.80	692.90	1276.00	21742.10	3175.40	3057.30	901.40
KW09-24	295.60	373.00	872.80	1345.50	699.90	142.40	215.30	3944.50	444.30	2115.60	365.30
KW09-25	2150.00	2593.20	7765.30	11880.50	5301.30	1039.40	2194.20	32923.90	1858.50	4104.50	1820.50
KW09-26	226.00	1543.20	3698.30	3848.70	2441.30	356.60	1146.70	13260.80	9600.10	3612.40	1119.50
KW09-27	212.00	767.80	2071.80	2881.20	1933.00	46.00	957.40	8869.20	136.00	3209.00	788.20
KW09-28	187.00	1028.50	2817.80	2401.60	1354.00	247.50	567.30	8603.70	1463.10	2525.80	792.10
KW09-29	756.00	979.10	2529.90	3380.20	1495.90	303.40	549.50	9994.00	1038.10	2919.90	832.50
KW09-30	162.00	469.40	617.90	1659.00	1204.00	145.80	366.30	4624.40	241.00	1876.20	704.60
KW09-31	216.00	742.90	1718.20	1924.10	729.60	183.00	387.20	5901.00	243.00	1608.10	612.10

Appendix F – Individual levels of triiodothyronine (T3)

Table F.1: Individual levels (ng/mL) of trioodothyronine (T3) in kittiwakes (*Rissa tridactyla*), from Kongsfjorden Svalbard, captured in 2009

ID	Sex	T3
KW08-1	M	5.25
KW08-2	M	1.38
KW08-3	M	1.17
KW08-4	M	0.42
KW08-5	M	0.65
KW08-6	M	2.29
KW08-7	M	2.57
KW08-8	M	8.32
KW08-9	M	3.16
KW08-10	M	1.95
KW08-11	M	2.82
KW08-12	M	3.80
KW08-13	M	1.45
KW08-14	M	1.70
KW08-15	M	2.14
KW08-16	M	8.51
KW08-17	M	8.71
KW08-18	M	4.27
KW08-19	M	1.15
KW08-20	M	8.13
KW08-21	M	5.13
KW08-22	F	1.15
KW08-23	F	3.31
KW08-24	F	3.09
KW08-25	F	0.62
KW08-26	F	0.98
KW08-27	F	2.04
KW08-28	F	3.39
KW08-29	F	5.01
KW08-30	F	1.12
KW08-31	F	5.13
KW08-32	F	0.46
KW08-33	F	0.41
KW08-34	F	2.95
KW08-35	F	0.37
KW08-36	F	2.19
KW08-37	F	0.43
KW08-38	F	4.07
KW08-39	F	0.91
KW08-40	F	0.76
KW08-41	F	1.70
KW08-42	F	3.09
KW08-43	F	0.78
KW08-44	F	5.25
KW08-45	F	0.79
KW08-46	F	1.23

TableF.2: Individual levels (ng/mL) of trioodothyronine (T3) in kittiwakes (*Rissa tridactyla*), from Kongsfjorden Svalbard, captured in 2009

ID	Sex	T3
KW09-1	M	4.29
KW09-2	M	2.83
KW09-3	M	2.78
KW09-4	M	2.29
KW09-5	M	5.56
KW09-6	M	3.57
KW09-7	M	2.51
KW09-8	M	1.24
KW09-9	M	0.84
KW09-10	M	0.52
KW09-11	M	3.93
KW09-12	M	0.64
KW09-13	M	4.85
KW09-14	M	4.28
KW09-15	M	5.51
KW09-16	M	0.93
KW09-17	M	0.90
KW09-18	F	0.61
KW09-19	F	3.70
KW09-20	F	0.79
KW09-21	F	1.06
KW09-22	F	0.91
KW09-23	F	2.91
KW09-24	F	1.45
KW09-25	F	0.90
KW09-26	F	4.86
KW09-27	F	0.64
KW09-28	F	1.35
KW09-29	F	1.07
KW09-30	F	1.02
KW09-31	F	0.60

Appendix G– Correlations

Table G.1: Pearson product-moment correlations between the PCB congeners and pesticides in blood from kittiwakes (n=79). Significant correlations are marked with * ($p \leq 0.05$)

Compound	1	2	3	4	5	6	7	8	9	10	11
1. PCB-99	-	0.778*	0.816*	0.833*	0.764*	0.608*	0.690*	0.850*	0.423*	0.353**	0.508*
2. PCB-118		-	0.868*	0.862*	0.767*	0.540*	0.733*	0.860*	0.603*	0.357**	0.633*
3. PCB-138			-	0.956*	0.859*	0.686*	0.810*	0.904*	0.518*	0.357**	0.594*
4. PCB-153				-	0.873*	0.703*	0.819*	0.928*	0.482*	0.400*	0.679*
5. PCB-180					-	0.668*	0.740*	0.908*	0.419*	0.379**	0.511*
6. PCB-183						-	0.678*	0.782*	0.386**	0.384**	0.495*
7. PCB-187							-	0.843*	0.384**	0.463*	0.647*
8. Sum PCB								-	0.489*	0.470*	0.629*
9. DDE									-	0.347**	0.472*
10. HCB										-	0.672*
11. Oxychlordane											-

* $p < 0.001$ (2-tailed). ** $p < 0.01$ (2-tailed).

Table G.2: Pearson product-moment correlation between biometric measures in kittiwakes (n=79). Significant correlations are marked with * ($p \leq 0.05$)

Biometric measures	1	2	3	4
1. Body mass	-	0.803*	0.583*	0.514*
2. Body condition		-	0.107	0.065
3. Skull			-	0.645*
4. Wing				-

* $p < 0.001$ (2-tailed)

Table G.3: Spearman product-moment correlations between triiodothyronine (T3) and biometric measures of the kittiwakes (n=79). Significant correlations are marked with* ($p \leq 0.05$).

Variables		Correlation coefficient (rho)	p-value
T3	Body mass	0.223	0.051
T3	Body condition	0.042	0.715
T3	Skull	0.214	0.062
T3	Wing	0.252	0.027***

*** $p < 0.05$ (2-tailed)

Table G.4: Spearman product-moment correlations between triiodothyronine (T3) and persistent organic pollutants in blood from kittiwakes (n=79). Significant correlations are marked with * ($p \leq 0{,}05$).

Variables		Correlation coefficient (rho)	p-value
T3	PCB-99	0.096	0.409
T3	PCB-118	0.136	0.238
T3	PCB-138	0.162	0.160
T3	PCB-153	0.099	0.393
T3	PCB-180	0.134	0.245
T3	PCB-183	0.22	0.054
T3	PCB-187	-0.041	0.725
T3	Sum PCB	0.135	0.242
T3	DDE	0.043	0.710
T3	HCB	-0.088	0.447
T3	Oxychlordane	-0.053	0.647

Appendix H – breeding success

Table H.1: Calculated breeding success (chicks per active nest > 12 days) of kittiwakes (*Rissa tridactyla*) breeding in Krykkjefjellet, Svalbard in 2008 (n=46) and 2009 (n=22).

	2008					2009					t-test		
Variable	n	mean	SD	median	Range	n	mean	SD	median	range	df	t	p
Breeding success	46	1.65	0.526	2	0.0-2.0	22[a]	0.91	0.81	1	0.0-2.0	66	4.545	< 0.001

[a]nine of the nests in 2009 were not checked for breeding success