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Levels and Effects of Organohalogens on Corticosterone Hormones in glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard

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Front page: Glaucous gull (Larus hyperboreus) silhouette. Photo: Mari Engvig Løseth

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"When the bird lands in your lap, you pet it".

Trondheim,
Mari Engvig Løseth

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Abstract

Long-range atmospheric transport, ocean currents, sea ice and rivers are transporting environmental contaminants into the Arctic. Some of these contaminants can reach high concentrations in the upper trophic levels in the Arctic food web due to processes of bioaccumulation and biomagnification. The present study indicates a sex-specific pattern of levels and effects of selected organohalogenated contaminants (OHCs) in the avian top predator, glaucous gull (*Larus hyperboreus*), breeding in Kongsfjorden, Svalbard. The aim of this present study was to report levels of OHCs and investigate whether the high levels detected in glaucous gulls can induce stress and thereby influence the stress response (measured by corticosterone concentration). No statistical differences were recorded for stress-induced or baseline corticosterone concentrations for males and female glaucous gulls. In females, a significant negative association was reported for lipid weight in blood plasma and baseline corticosterone. In male glaucous gulls, positive associations were found between levels of twenty-two OHCs and elevated baseline levels of corticosterone; indicating for the first time a "cocktail" effect of specific OHCs in blood plasma associated with high baseline levels of corticosterone in male glaucous gulls. It is suggested that the high levels of OHCs may act as a chronic stressor. The OHCs may interfere with the Arctic seabirds' ability to respond to environmental stressors, such as climate change and food availability, by disrupting the baseline levels of corticosterone and weakening the feedback mechanisms of the stress axis. Elevated baseline levels may lead to suppression of immune parameters and reduced survival rate. Due to a small sample size assessed in the present study, more research is needed to confirm a possible relationship between the disrupted stress axis and environmental contaminants in the Arctic seabirds.

Sammendrag

Atmosfærisk langdistanse-transport, havstrømmer, sjøis og elver er med på å transportere miljøgifter til Arktis. Noen av disse miljøgiftene kan få høye konsentrasjoner i de øvre trofiske nivåer i det arktiske næringsnettet på grunn av bioakkumulering og biomagnifisering. Denne studien indikerer et kjønnsspesifikt mønster av nivåer og effekter av utvalgte organohalogenerte stoffer (OHCs) i polarmåke (*Larus hyperboreus*) som hekker i Kongsfjorden på Svalbard. Målet med studien var å rapportere nivåer av OHCs og undersøke om de høye nivåene påvist i polarmåke kan forårsake stress og dermed påvirke fuglene stressrespons. Ingen statistisk signifikante forskjeller ble registrert for hverken stressinduserte eller grunnivåer av kortikosteron konsentrasjon for hanner og hunner. En betydelig negativ assosiasjon ble rapportert for lipidvekt i blodplasma og grunnivåer av korticosteron i hunn polarmåker. Positive assosiasjoner ble funnet mellom nivåer av tjueto utvalgte OHCs og forhøyede grunnivåer av kortikosteron hos hanner. Dette indikerer for første gang en "cocktail"-effekt av spesifikke OHCs i blodplasma forbundet med høye grunnivåer av korticosteron i hannlige polarmåker. De høye nivåene av OHCs kan fungere som en kronisk stressfaktor og dermed forstyrre de arktiske sjøfuglenes evne til å svare på miljømessige stressfaktorer, som for eksempel klimaendringer og næringstilgjengelighet. Dette ved å forstyrre grunnivåene av kortikosteron og svekke tilbakemeldingsmekanismer i stressaksen. Forhøyede grunnivåer kan føre til nedsettelse av immunfunksjoner og redusere overlevelsesevne. Ettersom denne studien hadde en liten utvalgsstørrelse er mer forskning nødvendig for å bekrefte en mulig sammenheng mellom en forstyrret stressakse og miljøgifter i arktisk sjøfugl.

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Abbreviations

ACTH	Adrenocorticotropic hormone
AVP	Arginine vasopressin
BCI	Body condition index
B-CORT	Baseline corticosterone
BFR	Brominated flame retardant
CA	Catecholamines
CHL	Chlordane
cm	Centimeters
CORT	Corticosterone
cpm	Counts per minute
CRH	Corticotropic releasing hormone
CV	Coefficient of variation
CV-ANOVA	Cross validated analysis of variance
DDE	Dichlorodiphenyltrichloroethylene
DDT	Dichlorodiphenyltrichloroethane
dT	Handling time, (T2-T1)
EDC	Endocrine disrupting chemicals
EI	Electron impact
GC	Glucocorticoid
GC/MS	Gas chromatograph/Mass spectrometer
I-CORT	Increase corticosterone
LC	Liquid chromatograph
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
min	Minutes
ml	Milliliters
mm	Millimeters
µl	Microliters
n	Number of observations
NCI	Negative chemical ionizaton
NILU	Norwegian Institute for Air Research
ng	Nanogram
nm	Nanometer
NPI	Norwegian Polar Institute

NTNU	Norwegian University of Science and Technology
OC	Organochlorine
OHCs	Organohalogenated contaminants
OCPs	Organochlorinated pesticides
p	Probability of rejecting the hypothesis
PBDE	Polybrominated diphenyl ethers
PC	Principal Component
PCA	Principal Component analysis
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PFCs	Perfluorinated compounds
PFCA	Perfluorocarboxylic acid
PFSA	Perfluorosulfonic acid
pg	Picogram
PLS	Projection to latent structures
POP	Persistent organic pollutant
ppm	Parts per million
PVN	Paraventricular nucleus
Q2	Goodness of prediction coefficient
Qstd	Quantification standard
r	Pearson correlation coefficient
r_s	Spearman's correlation coefficient
R2X	Explained variance
R2Y	Goodness of fit, correlation coefficient
rpm	Rounds per minute
S-CORT	Stress-induced corticosterone
SD	Standard deviation
SRM	Certified reference material
T1	Time of first sample (B-CORT)
T2	Time of second sample (S-CORT)
UHP	Ultra high pressure (gas chromatograph)
UV	Unit variance
VIP	Variable importance plot
w.w	Wet weight

1. Introduction

The Arctic is imaged as clean and relatively untouched, but in reality the seemingly pristine region acts as a sink for anthropogenic contaminants (Oehme, 1991). Even though there has been minimal direct use of organohalogens of anthropogenic origin in the Arctic, these substances are detected in marine food webs in these areas. With long-range atmospheric transport, ocean currents and rivers, transpolar ice drift and migrating biota, contaminants reach the Arctic from southern latitudes where they are produced and used (Macdonald *et al.*, 2000; de Wit *et al.*, 2004; Lohmann *et al.*, 2007). The potential biological effects of these contaminants in exposed Arctic wildlife are of concern. High levels of a range of these contaminants can be found in the Arctic top predators, with documented effects on the health of these organisms (Letcher *et al.*, 2010).

1.1. Organohalogen contaminants

A range of organohalogenated (chlorinated, brominated and fluorinated) compounds (OHCs) have been investigated in the Arctic for decades (Letcher *et al.*, 2010). Organochlorines (OC), such as polychlorinated biphenyls (PCBs) and several organochlorinated pesticides (OCPs) (dichlorodiphenyltrichloroethane (DDTs), chlordanes (CHLs), mirex, hexachloro-cyclohexanes (HCHs), and hexachlorobenzene (HCB)), are (by now) well-known to occur in this area, and were among the initial contaminants listed under the Stockholm Convention in 2001 (Buccini, 2003; Stockholm Convention, 2011). This global treaty, aimed at reducing or eliminating the release of contaminants classified as persistent organic pollutants (POPs) to the environment, has raised an awareness to the production and use of certain POPs (Stockholm Convention, 2011). Recently, new classes of contaminants such as brominated flame retardants (BFRs) and perfluorinated contaminants (PFCs), mainly per- and polyfluorinated alkyl substances (PFASs), have become a global environmental concern (de Wit *et al.*, 2004; Vonderheide *et al.*, 2008; Suja *et al.*, 2009).

The OHCs general physicochemical properties of semi volatility, high lipophilicity and resistance to chemical and biological degradation results in a long half-life in the environment (Mackay *et al.*, 1997; Letcher *et al.*, 2010). These qualities are possessed both by the "legacy" POPs listed in the Stockholm Convention, and the novel contaminants e.g BFRs, such as for instance polybrominated diphenyl ethers (PBDEs) (Rahman *et al.*, 2001). Their chemical qualities and slow degradation leads to high uptake of OHCs in biota, followed by bioaccumulation and biomagnification processes of these compounds, especially in the lipid

rich Arctic marine food web (Borgå *et al.*, 2001; Braune *et al.*, 2005). As lipids are important for energy storage and insulation in a cold climate with fluctuating food availability (Sakshaug *et al.*, 2009), Arctic organisms possess a high capacity for storage of lipophilic OHCs. The lipophilic OHCs are thereby highly influenced by lipid dynamics. As lipophilic OHCs are stored in lipid rich organs and visceral fat, they are released into the blood as lipids are metabolized (Ulfstrand, 1972; Freitas and Norstrom, 1974; Anderson and Hickey, 1976; Henriksen *et al.*, 1996). This seasonal redistribution of lipophilic contaminants may expose the individual and target organs to high concentrations, increasing the probability of interference with several important biological processes (Rozman and Klaassen, 2001).This has raised concerns for the health of exposed organisms.

PFCs are regarded as novel or emerging POPs, due to their recent discovery in environmental matrices. Although PFCs have been manufactured for over 50 years, little attention has been devoted to this OHC group until recently, when new methods of detection become available (Hansen *et al.*, 2001). PFCs are used in the production of stain repellent agents, fluoropolymers, pesticides, lubricants, paints, medicines and fire-fighting foams (Key *et al.*, 1997; Hekster *et al.*, 2003). Despite the fact that PFCs are mostly non-volatile, they have reached a global distribution (Giesy and Kannan, 2001; 2002), possibly due to transport mechanisms similar to those of the legacy POPs (Prevedouros *et al.*, 2006).

PFCs exhibit physicochemical properties of both hydrophobicity and lipophobicity (amphipatic), making the compounds able to repel both water and lipids; they are extremely resistant towards abiotic and biotic degradation (Giesy and Kannan, 2002; Verreault *et al.*, 2005b). This has also lead to bioaccumulation and biomagnification of several PFCs in the Arctic marine food web (Haukås *et al.*, 2007; Kelly *et al.*, 2009). Their chemical properties enable PFCs to bind to biological structures, such as plasma proteins, and thereby interfere with metabolism and production of endogenous compounds (Jones *et al.*, 2003). The degree of bioaccumulation generally increases with perfluorolkyl chain length (Martin *et al.*, 2003) and trophic position of the organism (Van de Vijver *et al.*, 2003).

As contaminants enter an organism they may be converted to more or less toxic metabolites by its biotransformation- and enzyme systems. This process is target specific (mainly occurring in the liver) and expected to result in excretion of the newly formed metabolite(s). The fate and metabolism of OHCs can be influenced by lipid cycling, bioenergetics, seasonal physiological changes of the individual (Anderson and Hickey, 1976; Rozman and Klaassen,

2001; Letcher *et al.*, 2010). The complexity of enzyme and metabolic systems become more developed with increasing trophic levels (Borgå *et al.*, 2004). Studies, in which metabolites have been analyzed, indicates an ability to metabolize several OHCs in e.g. polar bears (*Ursus maritimus*), glaucous gulls (*Larus hyperboreus*) and other arctic seabird species (Letcher *et al.*, 1996; Letcher *et al.*, 1998; Borgå *et al.*, 2005; Verreault *et al.*, 2005a).

The high levels of PCBs, BFRs, DDT, OCPs, PFCs, and their metabolites reported in the upper trophic levels (Verreault *et al.*, 2005a; Letcher *et al.*, 2010; Verreault *et al.*, 2010) indicate the persistency of these chemicals in the Arctic food web. With increasing concentrations, the potential adverse effects become more evident. At the same time, several factors influence the potential of these contaminants to bioaccumulate, such as sex and ability of maternal transfer through egg-yolk or milk, age, feeding ecology, trophic level of feeding and site-specific accumulation of contaminants in specific organs in the animal (Bustnes *et al.*, 2000; Bustnes *et al.*, 2003b; Borgå *et al.*, 2004; Verreault *et al.*, 2010). Alarmingly high concentrations of PCBs in dead and dying glaucous gulls were reported at Bjørnøya in 1989 (Gabrielsen *et al.*, 1995), and effort was focused on investigation of the potential adverse physiological and ecological effects caused by OHCs. Various studies have shown that high concentrations of OHCs may affect behavior, reproduction, development and survival, immune system and bioenergetics in glaucous gulls (Bustnes *et al.*, 2003b; Verreault *et al.*, 2004; Verreault *et al.*, 2006a; Verreault *et al.*, 2007a; Letcher *et al.*, 2010). These important processes are controlled by the endocrine system, and may function as targets for disruption by OHCs.

1.2. Endocrine disruption

By mimicking or blocking the action of endogenous hormones, endocrine disrupting chemicals (EDCs), with similar chemical structure as hormones, may influence or disrupt endocrine systems (Tyler *et al.*, 1998). The mechanisms could be direct interaction with hormone receptors, modulation of transport proteins or interference with biosynthesis, feedback signaling or interference with breakdown of endogenous hormones (Giesy *et al.*, 2003).

Most studies on EDCs have been laboratory studies of potential adverse effects of environmental chemicals on reproductive hormones, but there is also evidence that EDC can affect the hypothalamo-pituitary-adrenal (HPA) axis in birds and mammals (Lorenzen *et al.*, 1999; Boonstra, 2004; Verboven *et al.*, 2010).

1.2.1. The stress-axis

The HPA axis and the limbic system (*denate gyrus* and *hippocampus*) are critical components of the neuroendocrine system that coordinate the organism to successfully adapt to a changing habitat. Together, these two components are referred to as the stress axis (Boonstra, 2004), a term that will be used further in this study. The stress axis is involved in responses from normal daily activities to acute stressors and mediated reactions spanning from short-term adaption to activation the "fight or flight" response (Gabrielsen and Smith, 1995). A stress response is activated by several different environmental stressors such as severe weather changes (Astheimer *et al.*, 1995; Romero *et al.*, 2000), attacks from a rival or a predator or psychological stressors such as fear of an attack (Sapolsky *et al.*, 2000; Boonstra, 2004). In order to handle the stress and reestablish homeostasis from such stressors, rapid cascades of reactions are initiated (Figure 1) (Sapolsky *et al.*, 2000; Boonstra, 2004). Within seconds after the stressed event, activation of the sympathetic system causes the adrenal medulla, a part of the adrenal gland, to release epinephrine and norepinephrine (both catecholamines, CA) into the general circulation. The paraventricular nucleus (PVN) of the hypothalamus releases primary corticotropic releasing hormone (CRH) and arginine vasopressin (AVP) into the portal system. This causes the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the blood stream. In response to the release of ACTH, the adrenal cortex releases glucocorticoids (GCs) into the blood stream within minutes. In avian species the regulation of the stress axis is very similar to mammals and the primary GC is corticosterone (CORT) (Carsia and Harvey, 2000; Boonstra, 2004).

This stress response stimulates the production of glucose in the liver and mobilizes this energy to the muscles, at the expense of tissues not needed for short-term survival. Such activation of the stress axis is, not without costs. In both mammals and birds the heart rate is increased, immune function is altered, inflammatory response is supressed, pain preception is reduced, reproductive physiology and behavior is supressed, feeding behavior and appetite is depressed and the cognitive performance is sharpened (Gabrielsen and Smith, 1995; Boonstra, 2004). When these events happen, GCs exert negative feedback to the brain at three levels; at the anterior pituitary, PVN and the limbic system, in order to reset the body to pre-activation state and retain homeostasis. Under situations where the stress is chronic, the feedback signals are weakened and the stress response remains activated for a longer time. This might affect important body processes and can be potentially harmful for the organism (de Kloet *et al.*, 1999; Boonstra, 2004).

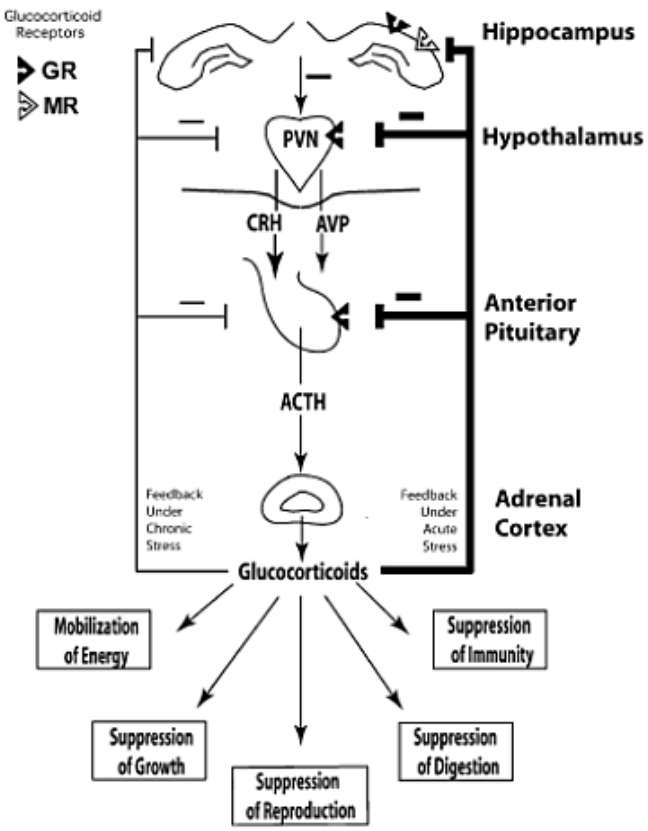


Figure 1: From Boonstra (2004), showing the stress axis in the mammalian brain. The hippocampus regulates the overall functioning of the hypothalamic-pituitary-adrenal (HPA) axis. A stressor causes the hypothalamic paraventricular nucleus (PVN) to release corticotropin releasing hormone (CRH) and vasopressin (AVP). This initiates the release of adrenocorticotrophin (ACTH) by the anterior pituitary (AP). ACTH initiates synthesis and release of glucocorticoids (GCs) from the cortex in the adrenal glands. GCs act at multiple sites within the body to maintain homeostasis, and due to the detrimental effects of pro-longed exposure to GCs, the stress-axis is tightly regulated through negative feedback (inhibition indicated by -) on GCs receptors to inhibit further stress-axis activity. GCs feeds back on hypothalamus and AP to cause a rapid inhibition of CRH release. When the stressor is acute, feedback mechanisms act efficiently and the system is returned to normal, resulting in only short-term body effects. Under situations when the stressor is chronic, feedback signals are weakened and the system remains activated for a longer time, resulting in long termed and detrimental effects on body processes. Glucocorticoid (GR) and mineralocorticoid receptors (MR) occur in the limbic system and GR occur in the PVN and AP. MRs have a higher affinity for GCs than GRs in the brain. MRs are often occupied, whereas GRs remain largely unoccupied.

1.2.2. Investigating stress

When measuring the stress response, the capture-challenge protocol is the most common when studying vertebrates (Boonstra 2004). A simplification of the protocol was applied in the present study.

This protocol uses the capture/trapping of the bird as a stressor and a blood sample is withdrawn within the first 3 minutes, in order to detect the baseline levels of coricosteroid hormones. The baseline levels will reflect the concentration of corticosteroid in the blood when the bird is relatively unstressed. The stress response takes a couple of minutes, and there is a window of approximately 3 minutes before the levels of corticosteroid rise after capture. After the first blood sample is drawn, the bird is placed in a dark place. Blood is then withdrawn preferrably at intervals of 5, 10, 20 and 30 minutes in order to detect the stress-induced levels of corticosteroids (Boonstra, 2004; Romero and Reed, 2005). After a capture-challenge stress examination has been conducted the levels of GCs will rise exponentially due to the stress response within the individual. The levels of corticosteroids after an hour post-capture will reflect the sensitivity of the stress axis to acute stressors in general (Wingfield *et*

al., 1995), as well as increased baseline levels may reflect a chronically stressed situastion. It is important to note that the response to stress is very different when comparing the levels of GCs post-capture beteween different species and taxa (Wingfield *et al.*, 1995; Boonstra, 2004).

1.2.3. Environmental stressors and adaptaion challenges

The Arctic climate is changing. The nature and timing of weather events, changes in temperature, increased precipitation and the spatial and temporal process variability have a profound impact on the Arctic environment (Hinzman *et al.*, 2005b). The changing Arctic climate may result in alterations in the environmental fate of the contaminants, as well as changes in a range of abiotic factors that may affect both uptake and toxicity of contaminants, such as salinity, pH and ultraviolet-radiation (Noyes *et al.*, 2009). This complex interaction between climate and contaminants may be particularly problematic for species living on the edge of their physiological tolerance range, and thereby interfering with the species ability to acclimate and adapt to the environmental stressors by physiological and behavioral processes (Zala and Penn, 2004; Jenssen, 2006; Noyes *et al.*, 2009). These climatic events may also pose an additional stressor to the animals living and breeding in these habitats (Jenssen, 2006). At the same time may an individual's personality contribute to how these challenges are approached and handled (Groothuis and Carere, 2005; Cockrem, 2007). The breeding conditions for seabirds during the Arctic summer is characterized by continuous daylight, an average temperature of 5 ^{o}C and fluctuating external conditions (e.g. temperature, precipitation, food availability, sea-ice conditions) (Hop *et al.*, 2002). The levels of corticosterone in seabirds during the Arctic summer are thereby not influenced by the daily cycles of light that may be observed (Romero and Remage-Healey, 2000). However, the stress axis in birds breeding in these areas might be influenced by variation in food availability (Kitaysky *et al.*, 1999; Reneerkens *et al.*, 2002), which in turn is strongly influenced by ongoing climate changes (Parmesan, 2006). Variation in food availability initiates depletion of body fat reserves during breeding, which may lead to increased levels of contamination in the blood (Anderson and Hickey, 1976), as well as the disruptions in the breeding stages (Hario *et al.*, 1991; Sandberg and Moore, 1996). The fluctuation of external conditions expose seabirds to varying levels of natural stress and – on top of this – the combined effects from OHCs and other anthropogenic stressors (Bustnes *et al.*, 2008; Moe *et al.*, 2009).

1.2.4. OHCs and stress

Although the study of OHCs' ability to influence the stress axis in Arctic seabirds is an emerging research area, several studies have shown that the exposure to high concentrations of contaminants may have effects on the stress axis.

Nordstad *et al.* (2012) conducted a study on black-legged kittiwake (*Rissa tridactyla*) from Kongsfjorden, Svalbard, where stress hormone levels were investigated at three stages during breeding. They found that the concentrations of POPs and CORT increased, whereas the body mass decreased progressively from the pre-egg-laying period to the incubation and the chick rearing period. At the same time the levels of PCB correlated positively with the baseline corticosterone in the pre-laying period, but not during the chick rearing period (Nordstad *et al.*, 2012). In another study of black-legged kittiwakes from the same area, increased levels of corticosterone were found to have a significant effect on the survival of the birds (Goutte *et al.*, 2010). In this study, Goutte and colleagues experimentally increased corticosterone levels in male birds for two days by inserting a corticosterone-implant (CORT-implant). The experimental procedure was followed by a two years survey of the birds' survival rate, indicated by their return rate to the nest sites. The birds with CORT-implants had a 30.9 % lower survival rate than the control birds (Goutte *et al.*, 2010).

Verboven *et al.* (2010) suggested that exposure to POPs may increase the glaucous gulls' (*Larus hyperboreus*) vulnerability to environmental stressors and can potentially compromise their ability to adapt to a changing environment as currently seen in their Arctic habitats. Verboven *et al.* (2010) measured high levels of organochlorines (OCs), BFRs and metabolic-derived PCB products (OH-PCB and MeSO2-PCB) in blood plasma of incubating glaucous gulls collected on Bjørnøya, Svalbard. A significant positive correlation with high baseline levels of the stress hormone corticosterone and the contaminant levels was reported (Verboven *et al.*, 2010). The high baseline levels were detected in both sexes and in addition a negative association between plasma POP levels and corticosterone levels in males was reported.

1.3. Present study species

The glaucous gull is used as an avian bioindicator for levels of contamination in the Arctic due to its potential apex predator position in the marine food web (Verreault *et al.*, 2010). For this reason, and because of the previous indication of impairment of the stress axis due to high OHC levels (Verboven *et al.*, 2010), the glaucous gull was chosen for the present study of stress responses to OHC contamination.

The glaucous gull is one of the largest avian top predators in the Arctic marine food web (Anker-Nilssen *et al.*, 2000; Verreault *et al.*, 2010). As a generalist predator and opportunistic scavenger, the diet varies from pelagic and marine invertebrates, fish, eggs, chicks of waterfowl and seabird species, passerines, small mammals and vegetation (Weiser and Gilchrist, 2012). As the glaucous gull can feed at several or specific trophic levels, some of the intraspecific variation in OHCs levels detected in this species may be explained by the diet (Bustnes *et al.*, 2000; Sagerup *et al.*, 2002; Verreault *et al.*, 2010).

Previous studies have documented relatively high levels of different OHCs in glaucous gulls from the Arctic (Gabrielsen *et al.*, 1995; Borgå *et al.*, 2001; Bustnes *et al.*, 2003b; Buckman *et al.*, 2004; Bustnes *et al.*, 2008), with possible consequences for their endocrine system and fitness (de Wit *et al.*, 2006; Letcher *et al.*, 2010; Verreault *et al.*, 2010).

Some OHCs are shown to influence the health of the birds (Sagerup *et al.*, 2000; Verreault *et al.* 2010), probably through disruption of endocrine function (Jenssen, 2006; Letcher *et al.*, 2010). Hitherto, endocrino-toxicological studies of the glaucous gull have mainly focused on modulation of the thyroid gland and thyroid hormone levels (Verreault *et al.*, 2004; Verreault *et al.*, 2007a; Sonne *et al.*, 2013), and to a lesser extent on reproductive (Verreault *et al.*, 2006a; Verreault *et al.*, 2008) and stress hormones (Verboven *et al.*, 2010).

1.4 Aim of study

The main aim of this study is to 1) investigate the effects of single and combined OHCs on baseline and stress-induced corticosterone and 2) possible sex differences in stress response in relation to OHC burden in glaucous gulls from Kongsfjorden, Svalbard. The study also report concentrations of OHCs.

2. Materials and methods

2.1. Study area

Field work was conducted (between the 7rd and the 17th of June 2013) in Kongsfjorden, located in the vicinity of Ny-Ålesund (79° N, 12-13°E), on the northwest coast of Spitsbergen in the Norwegian Arctic. The sampling location is characterized by the surrounding mountains making Kongsfjorden in leeward position with productive waters, by many bird cliffs and small islets (Hop *et al.*, 2002) – a perfect nesting place for seabirds. The small islets (14 in total) are important breeding areas for common eider (*Somateria mollissima*) (Mehlum, 1991), pink-footed goose (*Anser brachyrhynchus*) (Isaksen and Bakken, 1995) and Barnacle goose (*Branta leucopsis*) (Tombre *et al.*, 1998). As a generalist predator the glaucous gulls feed on eggs and chicks from other birds, resulting in good nutritional conditions for glaucous gulls breeding in Kongsfjorden (Mehlum and Gabrielsen, 1995; Barrett *et al.*, 2002). A survey during fieldwork noted an approximate of 40 breeding glaucous gull pairs in the area in 2013.

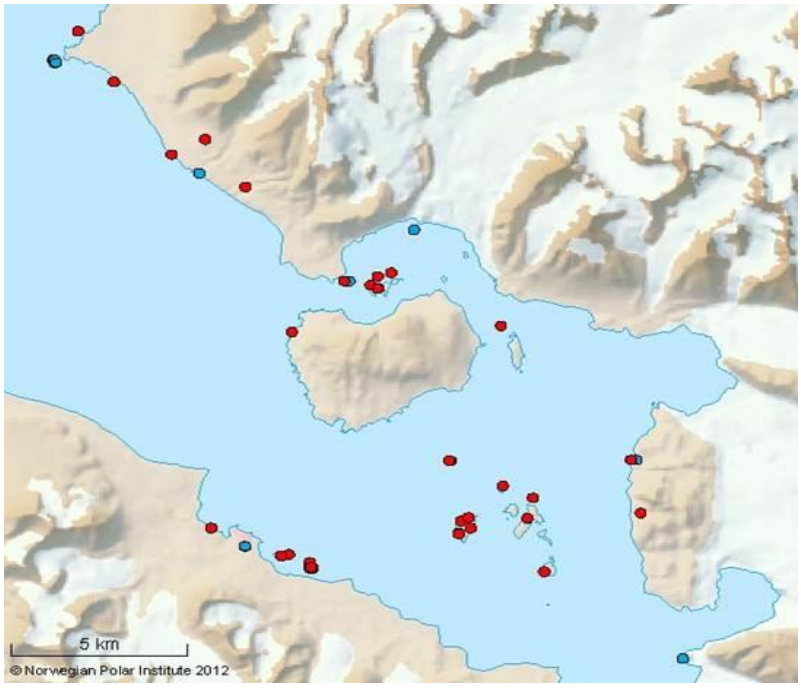


Figure 2: The sampling area in Kongsfjorden, Svalbard. The dots represent located nests of breeding glaucous gull (*Larus hyperboreus*) pairs. Map: Norwegian Polar Institute.

2.2. Field sampling

The preferred and standardized capture challenge protocol (Boonstra, 2004; Romero and Reed, 2005) was not possible to perform in the present study, therefore only two blood samples were withdrawn from each individual, similar to a previous study of stress response in glaucous gulls (Verboven *et al.*, 2010).

Incubating glaucous gulls were caught using an automatic triggered nest trap or a net canon (Super talon). The birds were caught within the second half of their incubation period. The eggs were collected and replaced by wooden "dummy" eggs to prevent damage during trapping. Throughout the procedure the eggs were kept in a heated bag for protection. A nylon snare on the edge of the nest bowl was set by a metal snare attached to cord-retracting mechanism from an old vacuum cleaner. Using a radio transmitted signal the tension was released from afar, once the bird returned to the nest. The snare would then tighten around the bird's feet, trapping it before it was carefully collected. The first of the two blood samples was immediately collected with a heparinized syringe (Terumo syringe 5 and 10mL with 2 drops of Heparin Leo ® 5000 IE/mL, Pharma AS) or heparinized vacutainer blood collection tube (3mL, Venosafe vacuette with Lithium Heparin, Terumo) using 23G fine-jet one-time cannula (0.6x30mm, Neolus, Terumo or BD Microlance) or 22G fine-jet one-time needles (Vacuette®, Greiner Bio-One). These samples represent the baseline corticosterone concentration (B-CORT). Biometric data was recorded for all birds: body mass (± 10g), wing length (±1 cm), head- and bill length, gonys depth and culmen length (± 0,1mm). The time (mm:ss) from when the trigger signal was transmitted until the blood samples had been obtained was measured using a stopwatch. All birds were ringed with numbered steel rings from the Norwegian Ringing Centre, Stavanger museum and a white three letter code plastic ring to simplify later identification with binoculars. Following biometric measuring the second blood sample was taken within approximately 28 minutes after entrapment. These samples represent the stress-induced corticosterone concentration (S-CORT). The birds were released and the eggs returned to the nest. Both blood samples (total of 10 mL) were taken from the brachial veins on the inside of the wings. The blood was stored in a bag at ambient temperatures (~1-5° C) immediately after sampling. After 1-8 hours, when returning from field, the blood was processed at the lab facilities in Ny-Ålesund. Plasma was collected after the blood sample was centrifuged (10min, 10x1000 RPM) and transferred to sterile 1.0 mL cryotubes (Nalgene© cryoware cryogenic vials, USA). Red blood cells were transferred to 1.2 cryotubes (Nalgene© cryoware cryogenic vials, USA) for sex determination. The samples

were immediately frozen down to -20 and kept frozen until further analysis. All birds sampled were breeding, thus sexually mature, so the ages of the birds were therefore assumed to be four years or older (Gaston *et al.*, 2009; Weiser and Gilchrist, 2012).

This project was approved by the Governor of Svalbard (Reference number 2013/00050-28, RIS ID 5267), and all sampling and bird-handling was conducted in accordance to current regulations of the Norwegian Animal Welfare Act.

2.3. Sex determination

Morphological sex determination was based on size, with males being larger than females (Coulson *et al.*, 1983; Cramp *et al.*, 1983; Bustnes *et al.*, 2000; Olsen and Larsson, 2004). Total head length and bill length was measured according to procedure defined by Coulson and colleagues (1983). Individuals with total head length (THL) < 146 mm were classified as females, individuals with while THL > 150 mm were classified as males. Biometric variables for females and males, as well as individuals, are listed in Tables 9 and 10 in Appendix C, respectively.

The sex of individuals with THL between 146 – 149 mm was identified using a molecular sexing method method established by Griffiths *et al.* (1998), analysing DNA from red blood cells. Chelex 100 was used for extraction of DNA from red blood cells (Walsh *et al.*, 1991), polymerase chain reaction for amplification of DNA strands and gel electrophorese for detection of CHD1Z and CHD1W genes. The males are homogametic (ZZ) and the females are hetererogametic (ZW) (Griffiths *et al.*, 1998), and as the lengths of the introns of CHD1Z and CHD1W are different, two bands were visible for females and one band for males after the gel electrophorese was run. None of the detected bands on the gel electrophorese were vague, so the uncertainty was insignificant.The analysis was carried out by Solveig Nilsen at the Norwegian University of Science and Technology (NTNU).

2.3.1. Quality assurance of the method

Method quality was assured by comparing results from the PCR analysis with biometric measurements obtained in the field. The PCR results matched the sex determination based upon total head length for gull species, as recommended by Coulson *et al.* (1983).

2.4. Contaminant analysis

The analyses of organohalogenated contaminants (OHCs) were performed at the Norwegian Institute for Air Research (NILU) in Tromsø, Norway. The subjected organochlorinated and organobrominated compounds selected for analyses were: PCB congeners (-28, -52, -99, -101, -105, -118, -138, -153, -180, -183, -187,-194), PBDE congeners (-28,-47, -66, -85, -99, -100, -138, -153, -154,-183), α-HCH, β-HCH, γ-HCH, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, Hexachlorobenzene (HCB), Heptachlor, Heptachlor Epoxide, *trans*-Nonachlor (t-Nonachlor), *cis*-Nonachlor (c-Nonachlor), *cis*-Chlordane (c-Chlordane), *trans*-Chlordane (t-Chlordane), Mirex and *oxy*-Chlordane.

The perfluorinated contaminants subjected for analyses were: Perfluorobutan sulfonat (PFBS), Perfluorohexansulfonat (PFHxS), Isomer Perfluoroktan sulfonat (PFOS), Perfluordekan sulfonat (PFDS), Perfluorohexanoic acid (PFHxA), Perfluoroheptanoic acid (PFHpA), Perfluoroctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDcA), Perfluorundecanoic acid (PFUnA), Perfluordodecanoic acid (PFDoA), Perfluortridecanoic acid (PFTrA), Perfluortetradecanoic acid (PFTeA), Perfluorooctansulfonamide (PFOSA) and 6:2 Fluortelomersulfonat (6:2 FTS). Details of the contaminants analysed are listed in Table 6, 7 and 8 in Appendix B.

2.5. Analysis of organochlorinated and organobrominated compounds

2.5.1. Sample extraction

The samples were thawed and homogenized before approximately 500 µl of plasma was transferred to 15 ml glass tubes. 20 µl of each internal standard solution (^{13}C-labeled compounds) was added to each sample for quantification purposes (see below). To denaturize proteins and enhance phase separation, 2 ml of deionized water saturated with ammonium sulfate and 2 ml of ethanol was added to the samples. Vortexing was conducted between every addition of new solution. Finally 6 ml of n-hexane was mixed thoroughly into the sample by vortexing and shaking for 45 seconds. The samples were left in upright position for 15 minutes in order to let the organic phase separate. After phase separation, the supernatant was transferred to cleaned 15 ml glass tubes. The procedure of adding 6 ml n-hexane and separating the supernatant form the plasma was repeated and the second supernatant was transferred to the first supernatant. The combined extracts were concentrated to a volume of 0.5 ml using vacuum and temperature to facilitate evaporation (LabConco RapidVap, Model 7900001, Kansas City, MO, US). Sample concentration was carefully conducted stepwise to

prevent shock-boiling and consequently loss of organic material. The samples were capped and kept in a fridge overnight. 0.5 ml n-hexane was added to the samples, which were then vortexed before they were cleaned with florisil.

Florisil (particle size 0.15-0.25 mm; Merck, Darmstadt, Germany) was dried at 600^0C for 8 hours before 1 g (± 0.03 g) was packed in between two glass fiber frits (Isolute SPE Accessories Frits, 10 µm, 3 ml) in plastic columns. Each sample was run over a florisil column to remove lipids and other matrix using Rapidtrace SPE workstation (Caliper Life Sciences, Hopkinton, USA).

After florisil clean-up the samples were concentrated using RapidVap under careful supervision to avoid complete dryness, until 0.2 ml remained. 5-6 drops of isooctane added to the samples before concentration acted as a "keeper", to avoid rapid evaporation. The remaining extracts were transferred to a 1.8 ml glass vial with insert. Original glass tubes were rinsed with 15 drops of isooctane. Nitrogen (N_2 purity of 99.995%, quality 5.0, Hydrogas, Porsgrunn, Norway) was used to concentrate the sample further, until 30 µl remained. 10 µl recovery standard (^{13}C PCB-159, 213 pg/µl, 25.09.12) was added to each vial. The vials were capped and stored in the fridge until GC-MS analysis.

All labeled, internal standards were purchased from Cambridge Isotope Laboratories (Woburn, MA, USA). Solvents applied were provided by Merck (Darmstadt, Germany).

2.5.2. Details of applied GC-MS/MS

The OHCs were analyzed as described in detail by Herzke *et al.* 2009 and Bustnes *et al.* 2008. In brief, the extracts were detected in an Agilent Technology 7890A GC and Agilent Technology 5975C GC/MSD equipped with a DB-5MS column (length 30 m, 0.25 µm film thickness, 0.25 mm inner diameter (i.d.); J&W Scientific, Folsom, CA, USA). Helium (6.0 quality; Hydrogas, Porsgrunn, Norway) was used as a carrier gas at a flow rate of 1 ml/min. The process was performed with the following temperature program: 70 °C for 2 min, then a steady increase of 15°C/min until 180°C, directly followed by an increase of 5 °C/min up to 280 °C, where the temperature was held for 10 min. The detection of pesticides was performed by an Agilent Technologies 5975C GC/MSD operated in negative chemical ionization (NCI) mode. The detection of DDTs, PBDEs and PCBs were performed by an Agilent Technology 7890A GC using a Waters Quattro Micro MS/MS operated in the

electron impact (EI) mode, with ionization energy of 70 eV. The transfer line was kept at 280 °C, while the EI ion-source temperature was 250 °C for EI and 160°C for NCI.

2.5.3. Quantification

Analytes were quantified according to internal standards provided by NILU. POP I in *iso*-octane (250pg/µl), glass 7 of 7, 20.11 was applied to all samples. BROM I (250 pg/µl) (PBDE I), glas 3, 44.09 to samples from 2011 and 2012. BROM I (250 pg/µl) (PBDE I), glass 5, 25.07 to samples from 2013. Native ^{12}C and added ^{13}C-labeled equivalents were analyzed, representing all the groups of PCBs, PBDEs, DDTs, *p,p'*-DDE, HCHs, HCB, chlordanes and nonachlordanes. The native and labeled standards were used to produce a standard curve from which concentrations were calculated using Equation 2.1

$$C_{sample} = Rf * (C_{ISTD} * A_{sample})/A_{ISTD} \qquad (2.1)$$

Where C_{sample} = concentration of unknown sample, C_{ISTD} = concentration of known standard, A_{sample} = known area from the GC/MS chromatogram of the sample, A_{ISTD} = known area on the GC/MS graph of the standard, given on wet weight (w.w) or ml.Rf = response factor accuired from the calibration curve for each compound.

2.5.4. Quality assurance of the method

To validate the method, control analyses were conducted. For every fifteenth sample, one blank sample without plasma that had been treated exactly like a plasma sample, and two samples of 500 µl of standard reference material (SRM, Human Serum (1957, Ø6Ø213), the National Institute of Standards and Technology (NIST)) were. Blank contamination was observed for γ-HCH, HCB, *trans*-Chlordane, *cis*-Chlordane, *oxy*-Chlordane, *trans*-Nonachlor, *cis*-Nonachor, *p,p'*-DDE and PCB-28.

The limits of detection (LOD) for the contaminants above were calculated using three times signal to noise ratio found in the samples, unless a higher signal was recorded in the blank samples (except for BDE-28, -47, -99 and -154, where LOD was set to 2x signal/noise, due to little matrix disturbances in the MS/MS). The results of the reference samples were within the given limits of accuracy. The specific LOD for every compound/congener is listed in Table 15 in the Appendix E.

The recovery standard (^{13}C PCB-159) was added prior to the instrumental analysis both as a quantification standard (volume correction) and as a test of the performance of the analytical method (recovery of internal standard).

2.6. Analysis of perfluorinated compounds

2.6.1. Sample extraction

Perfluorinated compounds were analysed using the Powley method (Powley *et al.*, 2005). The samples were thawed and homogenised before 200 µl of plasma was transferred to an Eppendorf-centrifuge tube. The samples were then spiked with 20 µl of internal standard solution (0.1 ng/µl ^{13}C-labeled PFC mix, 14.06.13) and then 1 ml methanol was added to the samples before the tubes were capped and the solutions were mixed by shaking and vortexing. The samples were put in an ultrasonic bath three times for 10 min, with vortexing in between. In order to enhance phase separation and sedimentation the samples were centrifuged for 10 min (10 000 rpm). The supernatant methanol was then transferred to 1.7 ml Eppendorf-centrifuge tubes containing 25 mg ENVI-Carb graphitized carbon absorbent and 50µl glacial acetic acid for purification. The tubes were capped, vortexed and centrifuged for 10 min (10 000 rpm). An exact amount of 0.5 ml supernatant was then transferred to glass vials, and 20 µl of recovery standard solution (3,7-diMe-PFOA, 0.102 ng/µl, 23.07.2013) was added.The vials were then capped and vortexed and kept in the fridge until analysis.

Prior to UHPLC-MS/MS analysis an aliquote of 100 µl was transferred from the vials into an autosampler vial with insert and the same amount of 2 mM NH4OAc in water was added. The autosampler vials were then capped, vortexed and stored in the fridge until UHPLC-MS analysis.

All labelled and internal standards were provided by NILU (IRMM-427, ID 0119). Solvents applied were provided by Merck (Darmstadt, Germany).

2.6.2. Details of applied UHPLC-MS/MS

Quantification of PFCs was performed by ultra-high pressure liquid chromatography triple-quadropole mass spectrometry (UHPLC-MS/MS). Detailed description of the analysis is described by Hanssen *et al.* (2013), Herzke *et al.* (2009) and Berger and Haukås (2005). Analysis was performed on a Thermo Scientific quaternary Accela 1250 pump (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a PAL Sample Manager (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a Thermo Scientific Vantage MS/MS

((Vantage TSQ) (Thermo Fisher Scientific Inc., Waltham, MA, USA)). An injection volume of 10 µL was used for sample separation on a Waters Acquity UPLC HSS 3T column (2.1 ×100 mm, 1.8 µm) equipped with a Waters Van guard HSS T3 guard column (2.1 × 5 mm, 1.8 µm) (Waters Corporation, Milford, MA, USA). In order to separate the PFCA contamination leaching out from the pump and the degasser, a Waters XBridge C18 column (2.1 × 50 mm, 5 µm) was installed after the pump and before the injector.

Separation was achieved using 2 mM NH_4OAc in 90:10 MeOH:water and 2 mM methanolic NH_4OAc as the mobile phases. Further details of analytical conditions and instrumental performance are given in (Hanssen *et al.*, 2013).

2.6.3. Quantification

Quantification was conducted by NILU using the internal-standard method with isotope-labeled PFCs and the LCQuan software from Thermo Scientific (Version 2.6) (Thermo Fisher Scientific Inc., Waltham, MA, USA). Internal standard and recovery standard were provided by NILU, containing a ^{13}C-labeled PFC mix described in Table 8 in Appendix B. Native ^{12}C and added ^{13}C-labeled equivalents were analyzed, representing all the groups of PFCA and PFSA. The native and labeled standards were used to produce a standard curve from which concentrations were calculated using Equation 2.1. The limits of detection (LOD) of the contaminants above were calculated by using three times signal to noise ratio found in the samples, unless a higher signal was recorded in the blank samples. The results of the reference samples were within the given limits of accuracy. The limit of detection was threefold the signal to noise ratio

2.6.4 Quality assurance of the method

For every fifteenth sample one blank sample without plasma, but treated as a plasma sample, and two samples of 200 µl of standard reference material (SRM, 1957, Ø6Ø213, Human Serum, NIST) were analyzed to validate the method. No blank contamination was observed.

LOD values were calculated by using three times signal to noise. The results of the reference samples were within the given limits of accuracy. The specific LOD for each compound is listed in Table 16 in Appendix E.

The recovery standard (3,7-dime PFOA) was added prior to the instrumental analysis both as a quantification standard (volume correction) and as a test of the performance of the analytical method (recovery ability).

Contaminant concentrations were given in blood wet weight (w.w) (ng/ml) as this measure is considered most relevant when assessing toxic effects (Henriksen *et al.*, 1998).

2.7. Lipid analysis

Analyses of tri-glycerides, phospholipids, free cholesterol, and total cholesterol were determined enzymatically in plasma samples by Unilab Analyse AS, Tromsø, Norway. The analysis was performed using Roche Diagnostics kit and a Cobacs c 11 analyser (F.Hoffmann-La Roche Ltd., Rotkreuz, Switzerland).

The total lipid (TL) content was calculated from the amounts of free cholesterol (FC), total cholesterol (TC), triglycerides (TG) and phospholipids (PL) using Equation 2.2 (Akins *et al.*, 1989). The enzymatic determination is considered more accurate than gravimetric lipid determination (Covaci *et al.*, 2006). The results in mg/dl were converted to lipid percentage (%LW) per ml sample.

$$TL = 1.677 * (TC - FC) + FC + TG + PL \qquad (2.2)$$

2.8. Hormone analysis

Hormone analysis was performed at the Department of Biology at the Norwegian University of Science and Technology (NTNU). Blood plasma levels of corticosterone were quantified using enzyme immunoassay (Corticosterone Enzyme Immunoassay kit, ADI-900-097, Enzo ® Life Sciences, New York, NY, USA), described in detail in the kit manual (EnzoLifeSciences, 2010). The kit uses a polyclonal antibody to corticosterone to bind, in a competitive manner, corticosterone in the standard, sample or in the alkaline-phosohatase molecule which has corticosterone covalently attached to it. After incubation at room temperature the excess reagents were washed away with a wash buffer and substrate was added. The enzyme reaction was stopped after a short incubation, and the yellow colour generated was measured for intensity by assessing optical density on a Bio-Tek Synergy HT microplate reader (Bio-Tek instruments, Winooski, VT, USA) at 405 nm. The intensity of the bound yellow colour is inversely proportional to the concentration of corticosterone in the samples and standards. The measured optical density was used to calculate the concentration

of corticosterone in the standards and generate a standard curve, and a regression line to determine the concentration of corticosterone in the samples.

Each sample was analysed in duplicates and the mean optical density of the two was used to calculate concentration of corticosterone per sample. For each 96-wells plate that was analysed three wells were used for blank samples, without plasma, but treated as samples. Additionally, two wells were used to calculate total activity (TA) of the reagents, three wells for non-specific binding (NSB) and three wells for maximum binding (Bo) activity. The TA wells only contained conjugate, substrate and stop solution. The NSB wells contained assay buffer, conjugate, substrate and stop solution. The Bo wells contained assay buffer, conjugate, antibody, substrate and stop solution. The standard and sample wells contained diluted standard solution or diluted samples, conjugate, antibody, substrate and stop solution.

The specific EIA-kit applied in the present study was highly sensitive (EnzoLifeSciences, 2010). Thus, the samples were diluted 100 times (10µl plasma), both when analysing the first and second plasma sample from each of the birds. The analyses were run both with and without the kit Steroid Displacement Reagent (SDR: 1µl SDR per 10µl plasma) to assess both the bound and free fraction of corticosterone in the plasma.

2.8.1. Assay validation

The EIA kit was carefully validated by testing plasma with different dilutions, quality controls and several replicates, before choosing 100x as the optimal dilution. The EIA kit was tested on both extracted and raw plasma samples to test for variation within the plasma samples. The hormones were extracted using ethyl acetate, 600µl per 100µl plasma sample. No significant variation between the extracted and raw samples was observed, and the final analysis used in the present study was thus conducted on diluted raw plasma.

2.8.2. Quality assurance of the method

The repeatability and inter- and intra assay precision was tested for each kit by running duplicates of blanks and a known diluted standard (~20 000 pg/ml). As there was no reference material available in the kit or in the lab, dilutions of plasma samples were used for quality control.

Readings with coefficient of variation (CV) >15% were not desired and reruns were performed if possible. Due to the high sensitivity of the kit some variation was detected

within the samples; due to limitations of kit availability, those with high CV values had the replicate with the lowest concentration removed. This was applied to five B-CORT samples and three S-CORT samples. One individual (AJH/M09) was analysed on five different kits during assay validation, detecting concentrations of 57, 52, 53, 113 and 62 ng/ml corticosterone in the B-CORT sample. Concentrations of 150, 152, 115, 236 and 200 ng/ml corticosterone were detected for the same individual in the S-CORT sample. This resulted in high inter-assay CV values of 38.4 % for B-CORT samples and 28.0 % for the S-CORT samples. One reason for the high CV values may be the high corticosterone levels detected from one of the assays, 113 and 236 ng/ml for B-CORT and S-CORT samples, respectively. When excluding this plate the inter-assay CV values were 7.9 and 22.8 % respectively for B-CORT and S-CORT samples. These results indicate that there may be differences in sensitivity between the assays.

The CV values of the B-CORT samples ranged from 2-14 % and S-CORT samples ranged from 0-13%, as no CV could be calculated from the samples with only one replicate. The intra-assay CV values of the diluted standard (~20 000 pg/ml) was 3 % and 10 % for each kit, and the inter-assay CV value was 19.6 %. The CV values of the reference plasma sample were only calculated for one of the assays (10 %), and the inter-assay CV value was 21.3 %. To test the precision of dilution two duplicates of the same sample were analysed from different dilution stocks. This was carried out for two plasma samples on the same plate and the intra-assay CV values were 7.3 and 11.9 %.

The levels of corticosterone in glaucous gull plasma were very high in relation to the detection range of the kit, which was 32 – 20 000 pg/ml (EnzoLifeSciences, 2010). Hence the samples were diluted 100 times; sensitivity of the EIA kit was 26.99 pg/ml. One reason for the high CV values detected for inter-assay quality assurance for the diluted standard and the reference plasma sample, may be that the diluted standard was outside the 20-80 % binding range of the hormone, while the plasma samples were in the ~60 % binding range of the hormone. It is also quite common to detect high inter-assay variation when analysing steroids (> 10 %) (Fraser *et al.*, 2010). The high CV values for specific samples can possibly be explained by the natural variation of availability of corticosterone hormones within the samples or the cross-reactivity of related steroid compounds (EnzoLifeSciences, 2010; Fraser *et al.*, 2010).

Even though some of the corticosterone concentrations are not generated from duplicates, all samples were included in statistical analysis.

2.9. Statistical analysis

Simca P12+ (Umetrics, Umeå, Sweden) was used for the multivariate data analysis and regression. R 2.15.1 (R Development Core Team, 2011) was used to generate random numbers. SPSS Statistical software (Version 21.0, IBM, SPSS Inc., Chicago, IL) was used for all other statistical analysis and calculations.

2.9.1. Body condition index

A morphological body condition index (BCI) was calculated for the birds as a condition factor using principal component analysis followed by a linear regression model to obtain a single measure of size (Brown, 1996). Since the sexes are dimorphic, BCI was calculated for each sex separately. For females, one principal component for the total head length, bill length, gonys depth and wing length was created as a single size measure, with an eigenvalue of 2.09 and explaining 69.7 % of the variance. The first principle component for males had an eigenvalue of 1.68 and explained 55.9 % of the variance. Body weight and standardized residuals from a linear regression of the first principal component for the respective sex were used as the individual BCI. BCI for females and males are listed in Table 9 in Appendix C while individual BCIs are listed in Table 10 in Appendix C.

2.9.2. Selection of variables

2.9.2.1. Statistical treatment of values under Limit of detection (LOD) and Limit of qualification (LOQ)

Compounds detected in more than 60 % of all samples (60 % > LOD) were included in the statistical analysis (Verreault *et al.*, 2006b). Limit of detection was set to 3x signal/noise of blanks. Samples under LOD were set to a random value between 0 and LOD generated by the statistical software R 2.15.1 (R Development Core Team, 2011) for further use in analysis. Limit of quantification was set to 3 times LOD. LOD and LOQ values of the individual OHCs analyzed are listed in Table 15 and 16 in Appendix E.

Based on the criteria above, statistical exclusion was applied to the following compounds: PCB-101, *iso*-PFOS, PFHxA, 6:2FTS, *a*-HCH, *g*-HCH, *t*-chlordane, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD, *p,p'*-DDD and *o,p'*-DDE.

The remaining organohalogenated compounds that were detected in >60 % of the individuals were computed as sums (Σ) of closely related compounds, grouped according to their physiochemical properties; ΣPBDE (n = 5 congeners), ΣPCB (n = 12 congeners), ΣCHL (n = 5 compounds), ΣOCPs (n = 3 compounds), ΣPFCAs (n = 7 compounds) and ΣPFSAs (n= 3 compounds). In the statistical treatments, the OHCs were analyzed both as sums and as separate compounds/congeners.

2.9.2.2. Normal distribution

All variables were tested for normality using the Shapiro-Wilcoxon test, ($p > 0.05$). Non-normally distributed variables were (log)transformed, but without achieving normality. These variables were therefore analyzed in their untransformed state using non-parametric tests.. When further investigating selected variables, parametric tests (ANOVA and Pearson correlation test) were applied to normally distributed variables, since these tests were more appropriate for the respective data.

2.9.2.3. Statistical selection of variables

Mann Whitney U tests and Spearman's rank-order correlation tests were applied to all corticosterone variables to test for differences and correlations between corticosterone samples treated with and without SDR. No significant differences were observed ($p > 0.05$) and the corticosterone variables were strongly correlated ($p \leq 0.000$).

Mann Whitney U tests were applied to test for differences in variation between the sexes on multiple variables. Exact significance level (p; two tailed) was set to 0.05.

To determine if the results from both sexes could be pooled in the statistical analysis, or if the two sexes should be examined separately, differences between contaminant levels and response variables in sexes were tested. Significant differences between the sexes were found for several variables: PFDoA ($U = 9.00$, $p = 0.015$), PFTeA ($U = 1.00$, $p = 0.000$) , PFTrA ($U = 0.00$, $p = 0.000$), ΣPFCA ($U = 10.00$, $p = 0.020$) and BDE-153 ($U = 32.00$, $p = 0.037$).

Because of the distinct sex-specific differences in some of the environmental contaminants, statistical analyses were performed separately for male and female glaucous gulls. However, statistical analyses were also applied on pooled sexes to examine responses that could be independent of sex.

2.9.3. Multivariate data analysis

Principal component analysis (PCA) and Partial least square regression (PLS) was performed on the following variables: baseline corticosterone concentration (B-CORT), stress-induced corticosterone concentration (S-CORT), increase in corticosterone concentration between the two samplings (I-CORT), time from trapping to first sampling (T1), time from trapping to the second sampling (T2), time between the first and second sampling (dT), body condition index (BCI), % lipid weight (%LW), PFHxS, PFOS, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTrA, PFTeA, PFOSA, *cis*-chlordane (c-chl), *oxy*-chlordane (oxy-chl), *cis*-nonachlor (c-nonachl), *trans*-nonachlor (t-nonachl), *p,p'*-DDE (pp-DDE), β-HCH (b-HCH), Mirex, PCB-28, PCB-52, PCB-105, PCB-118, PCB-138, PCB-153, PCB-180, PCB-183, PCB-187, PCB-194, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154.

2.9.3.1. Principal component analysis (PCA)

Principal component analysis was applied to explore similarities and differences between samples, and to identify the OHCs levels and biometrical variables explaining the variation in the corticosterone variables. PCA-X&Y models were applied using Simca P12+(Umetrics, 2008), with corticosterone variables as response (*Y*)-variables and OHCs levels, lipid weight (%), time and BCI as predictor (*X*)-variables. The analyses were performed on untransformed variables. Selected variables (PFHxS, PFOS, *cis*-chlordane, PCB (-52, -99, -105, -153, -180, -194) and BDE-154) were auto-Log_{10} transformed by Simca P 12+ to prevent skewness. The analysis was performed on pooled and separate sexes. For the PCA on females only PFHxS, PFOS, PCB-180, PCB -194 and BDE-154 were auto-Log_{10} transformed in Simca P12+.

A PCA is a multivariate technique that analyses a data table in which response variables (corticosterone) are described by multiple inter-correlated quantitative dependent variables (Abdi and Williams, 2010). In a PCA plot, the important information from the variables are extracted and represented as a set of new uncorrelated orthogonal variables, called principal components, projected onto each other. The variables are unit variance (UV) scaled, so that their contribution to the final model is equal, independent of their absolute values. In addition, the variables are mean centred (the mean value is subtracted) to increase the models interpretability (Eriksson *et al.* , 2006). A critical validation of the PCA is essential. This is performed with respect to explained variance of each of the principal components and the models goodness of prediction. Significant PCs have eigenvalues > 1 (Abdi and Williams, 2010). Only the two components with the highest eigenvalues and explaining most of the variation were extracted to make PCA plots.

2.9.3.2. Partial least square/ projection to latent structure

Projections to latent structures by means of partial least squares (PLS) (Wold *et al.*, 2001; Eriksson *et al.*, 2006) was applied to investigate the indications from the PCA analyses. PLS investigates multivariate relationships between the predictor (*X*)-variables (herein: OHCs, lipid weight (%), time and BCI) and their unidirectional influence on the response (*Y*)-variables corticosterone. This multivariate regression method has been applied to several recent wildlife studies investigating numerous and collinear predictor variables (Murvoll *et al.*, 2006; Villanger *et al.*, 2011; Bechshøft *et al.*, 2012).

PLS regression is different from multiple linear regressions (MLR) as it takes into account that *X*-variables can be strongly correlated. Instead of being treated as "independent" *X*-variables as in MLR, PLS treats the *X*-variables as "predictors", due to their close correlation and noisiness (Wold *et al.*, 2001). The PLS method can assess the relationship between *X*-variables and *Y*-variables, and identify possible combined effects by the *X*-variables. PLS regression does not require normality distributions, is less sensitive to outliers and extreme values, and can deal with datasets consisting of a lower number of observations (individuals) than variables, as well as multicolinearity among the variables (Wold *et al.*, 1984; Eriksson *et al.*, 2006; Umetrics, 2008).

As normality is not required for PLS regression, only selected (PFHxS, PFOS, *cis*-chlordane, PCB (-52, -99, -105, -153, -180, -194) and BDE-154) variables were auto-Log_{10} transformed by Simca P12+ to prevent skewness. All variables were unit variance (UV) scaled and significance level was set to 0.05 (Umetrics, 2008). PLS modeling was validated by the explained variation in the *X*-matrix (R^2X), explained variance of the *Y*-variables by the *X*-matrix (goodness of fit, R^2Y), goodness of prediction (Q^2) obtained by cross-validation and permutation analysis (default, 20 permutations) (Umetrics, 2008) and an analysis of variance testing of cross-validated predictive residuals (CV-ANOVA) (Eriksson *et al.*, 2008). $R^2 > 0.7$ and a $Q > 0.4$ denote significant models when analyzing biological data (Lundstedt *et al.*, 1998).

The importance of individual *X*-variables in explaining the *X*- and *Y*-matrices were evaluated using the variable importance for the regression (VIP) values. A VIP value > 1 denotes high importance for the model, and VIP < 0.5 indicate low or no importance (Umetrics, 2008). Optimizing of the PLS model was performed by stepwise removal of *X*-variables with the lowest VIP values. The regression coefficient (CoeffCS) value was used as another evaluation

parameter for individual *X*-variables, showing the correlative relationship (strength and direction) between each *X*-variable and *Y*-variable (Umetrics, 2008).

2.9.4. Spearman's rank-order correlation coefficient test

Spearman's correlation tests were used to further investigate the indications from the PCA plot. A significance level of $p < 0.05$ and a borderline significance of $p = 0.05$-0.07 was set when investigating the relationships between the corticosterone and explanatory variables. Correlations are expressed using the Spearman's correlation coefficient r_s and the *p*-value of significance. Correlations between selected OHCs (*cis*-chlordane, HCB and BDE-99) and lipid weight were detected in male glaucous gull plasma, although when corrected with partial correlations, these correlations with %LW did not affect the variables correlation with corticosterone variables ($-0.106 > r < 0.794$, $p > 0.109$), which was non-significant ($r > 0.252$, $p > 0.11$). As the percentage of extractable lipids in glaucous gull plasma was not correlated with most OHCs ($-0.010 < r_s < 0.456$, $p > 0.06$), the contaminant concentrations were not corrected for lipid weight.

A correlation between BCI and two PFSA variables were detected in glaucous gull plasma, PFOS in males and PFOSA in females, respectively. Although when corrected for this correlation, with partial correlation, these correlations with BCI did not affect the PFSA variables correlation with corticosterone variables ($-0.460 > r > 0.005$, $p > 0.435$), which were non-significant ($-0.657 > r > 0.200$, $p > 0.156$). The OHCs and corticosterone variables were not controlled for body condition, as there was no significant correlation between BCI and these variables.

The aim of the PCA, PLS and Spearman's correlation was to investigate the combined effects from OHCs and biometric variables on the corticosterone variables.

3. Results

3.1. Levels of organohalogen contaminants

Plasma levels of individual and summed organohalogen contaminants (ΣOHC) detected in < 60% of all samples in female and male glaucous gulls are presented in wet weight ng/ml plasma in Table 1.

Of the OHCs, PCBs were found to have the highest concentrations in both male and female glaucous gulls, representing 67.6 % and 53.4 %, respectively, of the total OHC concentrations in the samples. In female glaucous gulls the total OHC load was represented by; ΣPCBs (53.4 %)> ΣPFSA (23.9 %)> ΣCHL (9.0 %)> ΣOCPs (6.3 %)> ΣPFCA (4.8 %) > ΣPBDEs (2.6 %), respectively (Figure 3). In male glaucous gulls the total OHC load was represented by; ΣPCBs (67.6 %)> ΣCHL (11.0 %)> ΣOCPs (7.5 %)> ΣPFCA (6.0 %)> ΣPFSA (5.9 %)> ΣPBDEs (2.0 %), respectively (Figure 3). Only ΣPFCA showed significant differences between sexes (U = 10.00, p = 0.020). Thus, ΣPFSA (U =28.00, p =0.660), ΣCHL (U=23.00, p =0.350), ΣOCPs (U =25.00, p = 0.462), ΣPCB (U =20.00, p = 0.216), ΣPBDE (U = 21.00, p = 0.256) were not significantly different in variation between sexes. As previously mentioned (statistics section), of the single compounds, only PFDoA (U = 9.00, p = 0.015), PFTeA (U = 1.00, p = 0.000), PFTrA (U = 0.00, p = 0.000), ΣPFCA (U = 10.00, p = 0.020) and BDE-153 (U = 32.00, p = 0.037) differed significantly between the sexes.

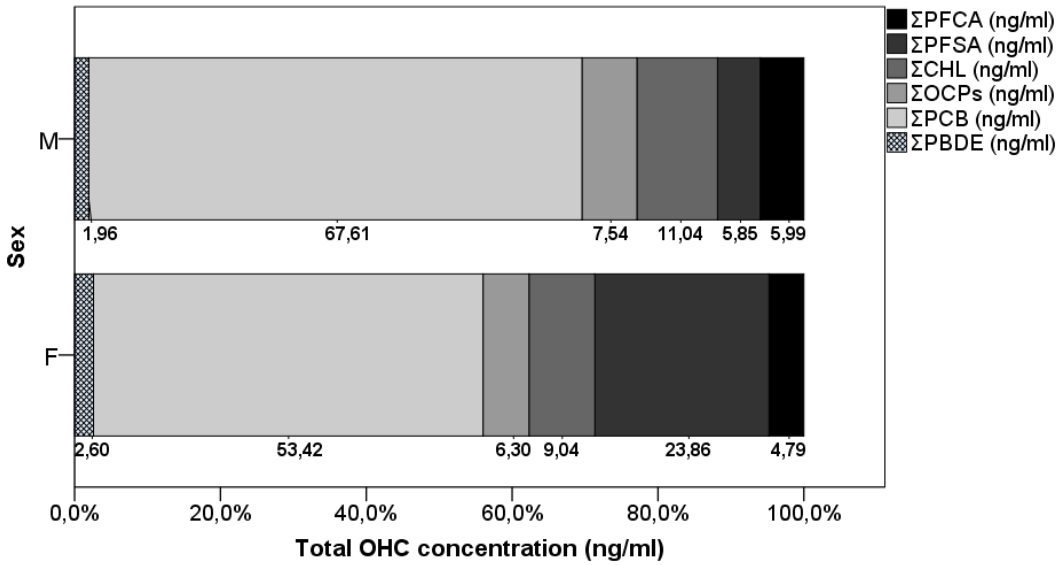


Figure 3: Histogram based on average OHC concentrations showing the sex-specific OHCs pattern in plasma from female and male glaucous gulls breeding in Kongsfjorden, Svalbard.

PCB-153 was found to have the highest concentration of the PCBs in both males and females, followed by PCB-180, -138 and -118. *Oxy*-chlordane and HCB, respectively, were found to have the highest concentrations of the CHLs and OCPs in both males and female glaucous gulls. BDE-47 was found to have the highest concentration of the PBDEs, and PFOS had the highest concentration of the PFCs in both males and females. The range of PFOS in females was very large and the mean value was more than three times the median, indicating a very skewed distribution. This is due the detected level of 183.2 ng/ml PFOS in gull #14 (ABA).

Table 1: Mean ± standard deviation (St.D), median, minimum and maximum concentrations (ng/ml w.w plasma) of individual organohalogen contaminants detected (>60% of the samples) in male and female glaucous gulls from Kongsfjorden, Svalbard 2013. Included are the sums denoted with Σ. For compounds with concentrations <LOD, a random value between 0 – LOD was applied in statistics. The presented concentrations are therefore based on n=6 for the males and n=11 for the females. The "n" presented in the table represents the number of samples with concentrations > LOD. Compounds with significant differences between the sexes (p<0.05) are denoted with *.

	Males				**Females**			
Analyte (ng/ml w.w.)	**n**	Mean ± St.D	Median	Range	**n**	Mean ± St.D	Median	Range
Mirex	6	5.08 ± 2.76	4.13	2.79 – 10.40	11	3.73 ± 3.14	2.31	1.31–10.20
cis-nonachlor	6	0.70 ± 0.35	0.78	0.14 – 1.15	11	1.00 ± 0.56	0.98	0.31 – 2.00
trans-nonachlor	6	1.31 ± 0.89	1.26	0.09 – 2.67	11	1.04 ± 0.71	0.87	0.26 – 2.53
oxy-chlordane	6	11.51 ± 7.87	9.27	4.74 – 26.50	11	8.71 ± 5.53	5.66	3.00 – 17.20
cis-chlordane	6	0.02 ± 0.004	0.01	0.01 – 0.02	9	0.03 ±0.01	0.03	0.009–0.06
ΣCHL[1]	6	18.61 ± 11.36	15.01	10.43 – 40.45	11	14.51 ± 9.03	9.08	5.09 -31.02
HCB	6	8.20 ± 2.88	7.65	5.62 – 13.40	11	6.80 ± 1.91	6.93	4.08 – 9.51
β-HCH	6	0.45 ± 0.23	0.38	0.28 – 0.89	11	0.32 ± 0.18	0.24	0.12 – 0.69
p,p'-DDE	6	4.06 ± 2.74	3.09	2.16 – 9.37	11	3.00 ± 1.82	1.85	1.21 – 6.40
ΣOCPs[2]	6	12.71 ± 5.68	11.58	8.06 – 23.66	11	10.11 ± 3.82	8.96	5.48 -16.44
PCB-28	6	0.24 ± 0.10	0.23	0.14 – 0.42	11	0.16 ± 0.07	0.14	0.08 – 16.44
PCB-52	5	0.04 ± 0.02	0.04	0.008 – 0.05	9	0.37 ± 0.29	0.30	0.03 – 0.75
PCB-99	6	5.93 ± 3.98	4.54	2.93 – 13.60	11	3.57 ± 2.34	2.48	0.51 – 7.65
PCB-105	6	0.01 ± 0.006	0.01	0.003 – 0.02	11	0.62 ± 0.75	1.43	0.002 – 2.11
PCB-118	6	10.85 ± 6.35	9.29	5.79 – 23.10	11	7.27 ± 4.52	4.79	2.92 – 17.2
PCB-138	6	21.80 ± 11.73	19.65	10.50 – 43.30	11	15.38 ± 8.65	11.00	5.43 – 31.00
PCB-153	6	42.37 ± 28.35	32.65	22.00 – 98.40	11	32.37 ± 26.36	20.10	8.43 – 87.00
PCB-180	6	20.34 ± 12.43	15.05	9.81 – 43.00	11	16.85 ± 17.50	9.13	4.17 – 52.80
PCB-183	6	2.97 ± 1.65	2.29	1.62 – 6.08	11	2.36 ± 1.98	1.44	0.71 – 6.59
PCB-187	6	4.15 ± 2.27	3.41	2.50 – 8.58	11	2.74 ± 1.54	2.13	1.23 – 5.25
PCB-194	6	3.00 ± 2.08	2.32	1.117 – 6.46	11	2.32 ± 2.60	1.16	0.51 – 7.57
ΣPCB[3]	6	113.94 ± 69.17	89.40	65.53– 248.43	11	85.79 ± 63.85	51.89	28.14 – 220.87
BDE-47	6	2.17 ± 1.04	1.71	1.26 – 3.78	11	2.37 ± 1.40	1.93	1.11 – 6.24
BDE-99	6	0.21 ± 0.15	0.22	0.0004 – 0.42	11	0.38 ± 0.22	0.38	0.002- 0.85
BDE-100	6	0.42 ± 0.18	0.38	0.27 – 0.68	11	0.42 ± 0.19	0.42	0.18 – 0.86
BDE-153*	6	0.36 ± 0.11	0.35	0.25 – 0.57	11	0.83 ± 0.56	0.68	0.26 – 2.14
BDE-154	6	0.15 ± 0.07	0.14	0.08 – 0.24	11	0.17 ± 0.13	0.13	0.02 – 0.41
ΣPBDE[4]	6	3.31 ± 1.45	2.86	1.88 – 5.68	11	4.17 ± 1.76	3.61	1.81 – 8.40
PFOA	6	0.10 ±0.04	0.12	0.03 – 0.14	7	0.29 ± 0.10	0.32	0.11 – 0.42
PFNA	6	1.64 ± 0.44	1.47	1.18 – 2.23	11	1.80 ± 0.88	1.95	0.30 – 3.59
PFDcA	6	0.71 ± 0.21	0.70	0.44 – 0.96	11	0.62 ± 0.16	0.62	0.31 – 0.92
PFDoA*	6	0.87 ± 0.17	0.91	0.65 – 1.07	11	0.55 ± 0.26	0.60	0.20 – 1.09
PFUnA	6	3.19 ± 0.52	3.07	2.47 – 3.81	11	2.51 ± 0.69	2.59	1.28 – 3.58
PFTrA*	6	3.13 ± 0.48	3.03	2.52 – 3.70	11	1.85 ± 0.44	1.94	1.11 – 2.46
PFTeA*	6	0.45 ± 0.07	0.45	0.37 – 0.52	8	0.22 ± 0.10	0.21	0.07 – 0.39
ΣPFCA[5]*	6	10.09 ± 1.00	10.34	8.50 – 11.38	11	7.69 ± 2.30	7.92	3.28 -11.86
PFHxS	5	0.49 ± 0.59	0.36	0.03 – 1.60	10	2.25 ± 2.95	0.65	0.15 – 9.36
PFOS	6	9.37 ± 3.23	9.16	5.98 – 13.42	11	36.28 ± 54.31	9.16	2.89-183.21
PFOSA	5	0.57 ± 0.22	0.66	0.39 – 0.89	9	0.81 ± 0.30	0.80	0.48 – 1.44
ΣPFSA[6]	6	9.86 ± 3.58	9.46	6.43 – 15.02	11	39.00 ± 57.00	10.62	3.23 – 192.65
ΣPFCs[7]	6	20.52 ± 3.39	19.45	17.59 – 25.99	11	46.69 ± 57.54	17.75	7.97 – 200.93

[1] ΣCHLs: *cis*-Chlordane, *oxy*-Chlordane, *trans*-Nonachlor, *cis*-Nonachlor and Mirex
[2] ΣOCPs: HCB, β-HCH and *p,p'*-DDE
[3] ΣPCBs: PCB;-28, -52, -99, -105, -118, -138, -153, -180, -183, -187 and -194
[4] ΣPBDEs: BDE;-47, -99, -100,-153 and -154
[5] ΣPFCA: PFOA, PFNA, PFDcA, PFDoA, PFUnA, PFTrA and PFTeA
[6] ΣPFSA: PFHxS, PFOS and PFOSA
[6] ΣPFSA: PFHxS, PFOS, PFOSA, PFOA, PFNA, PFDcA, PFDoA, PFUnA, PFTrA and PFTeA
[7] ΣPFCs: PFOA, PFNA, PFDcA, PFDoA, PFUnA, PFTrA and PFTeA

3.2. Levels of corticosterone hormone and sampling time

The levels of corticosterone hormone and sampling time information for female and male samples separately, as well as pooled samples, are listed in Table 2, 3 and 4 in Appendix A. The individual levels of corticosterone hormones and sampling times are listed in Table 5 in Appendix A. The individual increase in corticosterone from the baseline (B-CORT) to the stress-induced (S-CORT) sample is projected in relation to sampling times in Figure 4.

3.2.1. Baseline corticosterone concentration

Baseline corticosterone (B-CORT) concentration averaged 45.78 ± 22.24 ng/ml (range 23.72 – 112.38 ng/ml) and did not differ between males and females (effect of sex on B-CORT concentration: $U = 20.00$, $p = 0.216$). The time from the trapping until the first blood sample was taken (T1) did not differ between the sexes ($U = 30.00$, $p = 0.808$), and averaged at 08:20 ± 02:12 min (range 05:13 – 12:28 min).

3.2.2. Stress-induced corticosterone concentration

Stress-induced corticosterone (S-CORT) averaged 106.65 ± 55.42 ng/ml (range 46.70 – 224.91 ng/ml) and did not differ between males and females (effect of sex on S-CORT concentration: $U = 29.00$, $p = 0.733$). The time from trapping to the second blood sample (T2) was obtained did not differ between the sexes ($U = 33.00$, $p= 1.00$), and averaged at 28:29 ± 06:10 min (range 20:10 – 38:48 min).

3.2.3. Increase corticosterone concentration

The increase in corticosterone (I-CORT) concentration between the first and second sample averaged 60.86 ± 39.62 ng/ml (range 18.44 – 144.86 ng/ml) and did not differ between males and females (effect of I-CORT concentration: $U = 32.00$, $p = 0.961$). The handling time that elapsed between the two samples (dT) did not differ between the sexes ($U = 28.00$, $p = 0.660$), and averaged at 20:08 ± 03:59 (range 14:57 – 26:20).

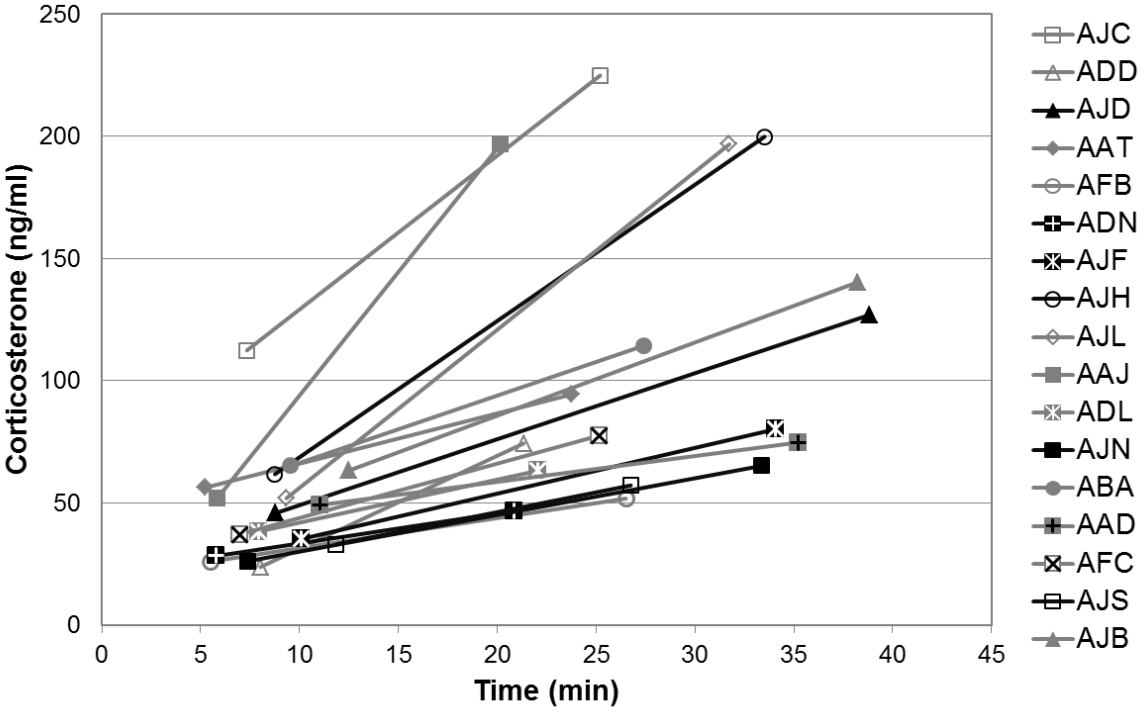


Figure 4: Individual increase in corticosterone concentration as a function of time in glaucous gulls sampled in Kongsfjorden, Svalbard 2013. The graph includes B-CORT and S-CORT samples for each individual and their respective sampling times from trapping; T1 and T2, respectively. Females (AJB, AJC, ADD, AAT, AFB, AJL, AAJ, ADL, ABA, AAD and AFC) are marked with grey markers and lines. Males (AJD, AND, AJF, AJH and AJN) are marked with black markers and lines. There was no difference in stress response between sexes.

3.3. Multivariate data analysis

3.3.1. Principal component analysis (pooled sexes)

The PCA analysis performed on both female and male glaucous gulls resulted in four significant principal components explaining 70.6 % of the total variation of the variables (R^2X=0.706, Q^2 = 0.2). The first component (PC1) explained 38.3 % (eigenvalue 6.51) and the second component (PC2) explained 13.1 % (eigenvalue 2.23). The loadings of the individual OHCs onto the principal components are listed in Table 17 and 18 in Appendix F. These PCs are projected in the biplot of loadings (PC1/PC2) in Figure 5. The remaining third (PC3) and fourth (PC4) components explained 0.098 % and 0.093 %, respectively, and are not included in the loading plot. No outliers were present as all samples were within the Hotellings T2 range (Figure 6). There was an evident clustering of PCBs, CHLs and OCPs, indicating a significant correlation between the variables. The PFCAs and PFSAs are on the opposite side of the PCA plot compared to the PCBs, CHLs and OPCs indicating a negative correlation between these OHCs variables. A clear difference between males and females is shown in the score plot (Figure 6), and separate PCA analyses on each of the sexes were performed.

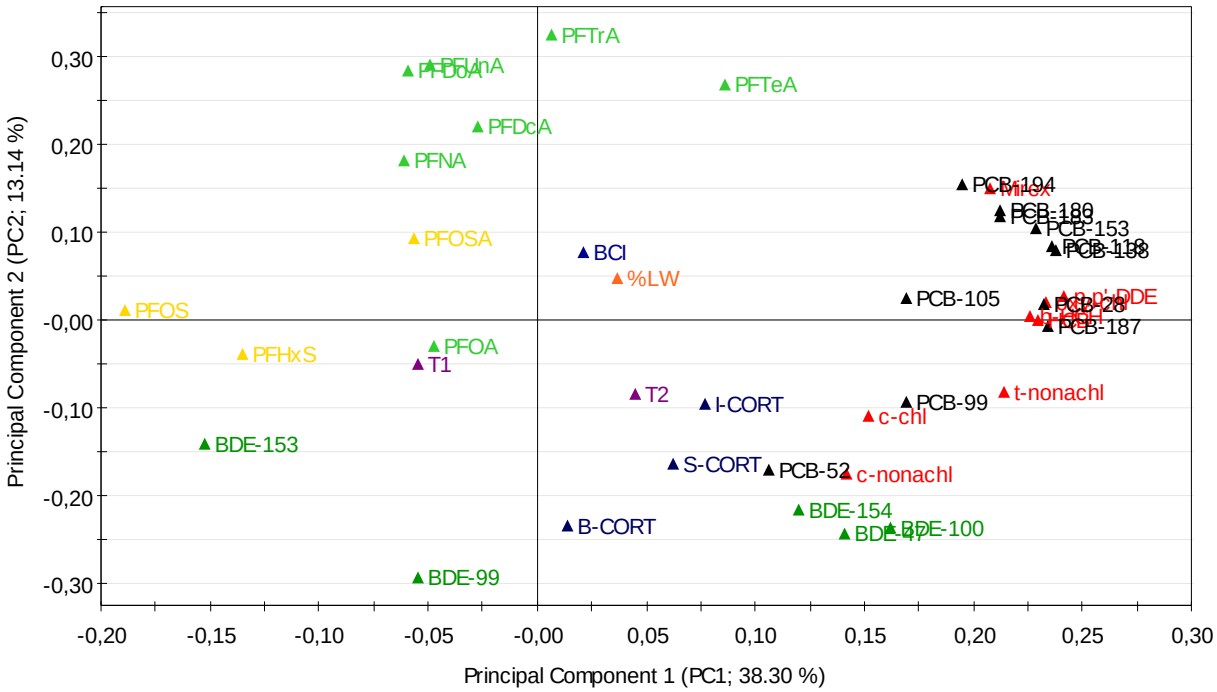


Figure 5: Biplot of loadings in PC1/PC2 dimension based on contaminant and corticosterone hormone concentrations (B-CORT, S-CORT and I-CORT) in plasma of female and male glaucous gulls captured in Kongsfjorden, Svalbard 2013. Individual OHCs, time of first (T1) sampling, second sampling (T2) and the time difference between the two (dT), as well as BCI and lipid weight (LW%) was included.

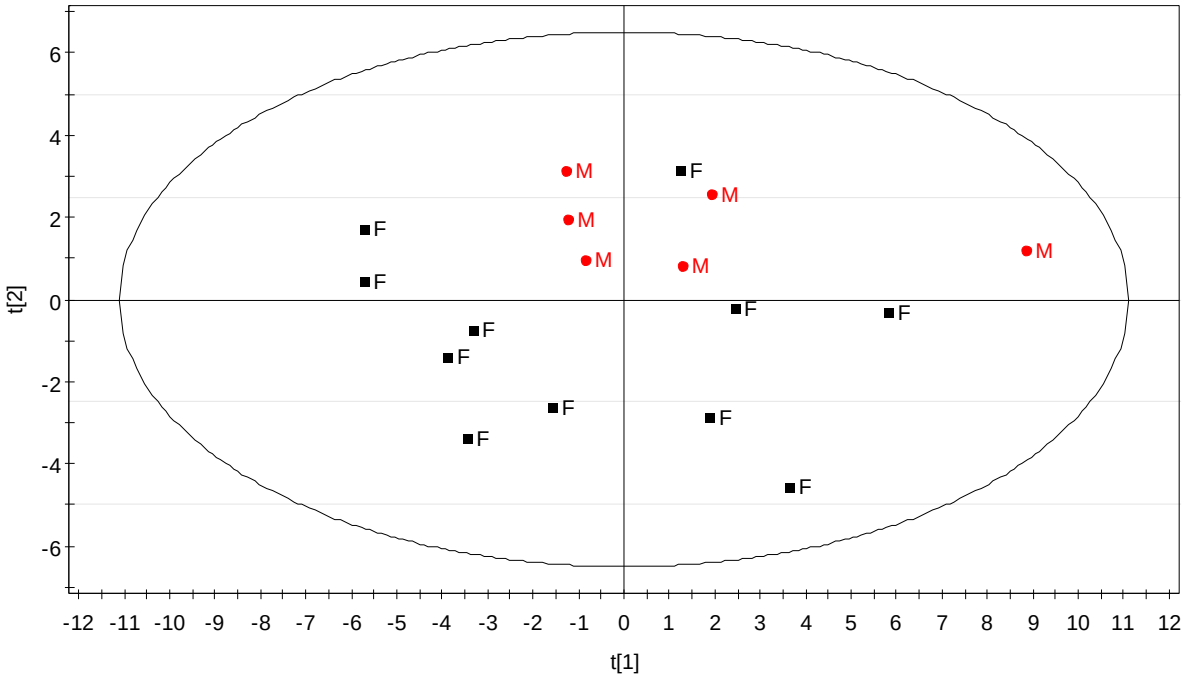


Figure 6: Biplot of scores in PC1/PC2 dimension based on contaminant and corticosterone hormone concentrations (B-CORT, S-CORT and I-CORT) in plasma samples of female (F;black) and male (M;red) glaucous gulls captured in Kongsfjorden, Svalbard 2013. Hotellings T2 range is illustrated by the circle around the scores. All samples are within the Hotellings T2 range.

3.3.2. PCA of female samples

The analysis including female glaucous gull samples resulted in a PCA-model with three significant principal components (R^2X=0.751, Q^2 = 0.355). PC1, PC2 and PC3 explained 45.5 % 15.6 % and 13.9 % of the variation, respectively (Table 19 and 20, Appendix G). The loadings of the individual OHCs onto the principal components are listed in Table 19 and 20 in Appendix G. Only PC1 and PC2 were extracted for a loadings biplot (Figure 11, Appendix G) as these explained most of the variation. No outliers were present as all samples were within the Hotellings T2 range. The PCA analysis including female glaucous gulls indicates negative associations between the corticosterone variables (B-CORT and S-CORT) and some of the PFCs and %LW along PC2. However, correlation analysis only identified a significant inverse relationship between the CORT variables and lipid weight (%) (r > -0.762, p<0.006)(Figure 12, Appendix H). As no relationship with any OHCs variables were identified for the corticosterone variables in female glaucous gulls, the female samples were excluded from further analysis.

3.3.3. PCA of male samples

The analysis of male glaucous gull samples resulted in a PCA-model with only one significant principal component (R^2X=0.514, Q^2 = 0.14). To compute a PCA plot a second non-significant PC was calculated, although this weakens the model (R^2X=0.69, Q^2 = 0.054). PC1 and PC2 explained 51.4 % and 17.6 % of the variation, respectively. The loadings of the individual OHCs onto the principal components are listed in Table 21 and 22 in Appendix I. No outliers were present as all samples were within the Hotelling's T2 range. The biplot of loadings in PC1 and PC2 indicates that several OHCs are clustered together along significant PC1 together with the corticosterone variables (Figure 7). The close clustering of corticosterone variables and specific OHCs variables within the square in Figure 8 is magnified in Figure 13 in Appendix I.

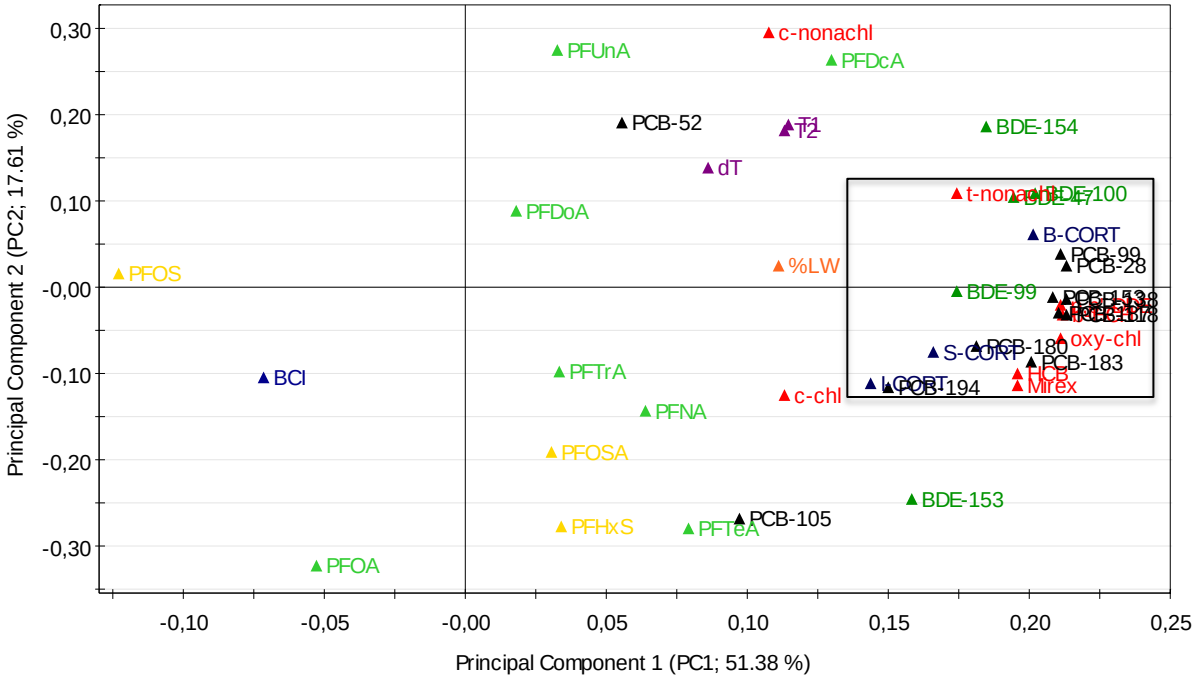


Figure 7: Biplot of loadings in PC1/PC2 dimension based on organohalogen contaminant and corticosterone hormone concentrations (B-CORT, S-CORT and I-CORT) in plasma of male glaucous gulls captured in Kongsfjorden, Svalbard 2013. Individual OHCs, time of first (T1) sampling, second sampling (T2) and the time difference between the two (dT), as well as BCI and lipid weight (%LW) was included. Variables within the marked square is magnified in Figure 13 in Appendix I.

The PCA plot indicates close correlations between several OHCs and the corticosterone variables (B-CORT, S-CORT and I-CORT). The longer distance between the S-CORT and I-CORT variables and their surrounding OHCs, denote less significant positive correlations. As there was a shorter distance between several OHC variables and B-CORT, these positive correlations were more significant. The positive correlation between each of the single compounds; BDE-47, BDE-154, PCB-99, PCB-28, *trans*-nonachlor, *cis*-nonachlor, *p,p'*-DDE, PCB-187, PCB-118, β-HCH, and B-CORT was confirmed with correlation tests (r_s > 0.829, p<0.042). There were no correlations between OHCs variables and the remaining corticosterone variables, S-CORT and I-CORT. PFOS was oriented on the opposite side of the close clustering of corticosterone variables, PCBs, PBDEs, OPCs and CHLs, indicating a negative relationship between these variables. The close orientation of the PFCAs, PFSAs BCI, %LW and the time variables (T1, T2 and dT) towards the center of the plot, indicates that these variables are of less importance to the model.

3.3.4. Partial least square regression (PLS)

To further investigate the relationships observed from the PCA-model, PLS-regressions were applied to investigate the relationship between predictor (*X*)-variables (OHCs, lipid weight (%), time and BCI) and the response (*Y*)-variables (B-CORT, S-CORT and I-CORT). Stepwise removal of the *X*-variables of least importance (Wold *et al.*, 2001) resulted in a significant model. PLS-regressions were applied to all corticosterone (*Y*) variables, separately. Weaker, non-significant models were obtained for S-CORT and I-CORT, these were accordingly not included in the results.

The PLS regression predicted that the variation of B-CORT in male glaucous gulls was significantly explained by twenty-two variables (R^2X = 0.789, R^2Y = 0,905 and Q^2 = 0.869, CV-ANOVA; p = 0.048). The loadings of the individual OHCs onto the principal component are listed in Table 23 in Appendix J. All of these compounds contributed positively to an increase in the B-CORT concentrations. The highest VIP (variable importance in projection) value were detected for BDE-47, *p,p'*-DDE, PCB-99, PCB-187, β-HCH, PCB-28, PCB-118, PCB-138, PCB-153, *trans*-nonachlor, BDE-100, *oxy*-chlordane and BDE-154 (VIP > 1), respectively. Hence, these variables were considered to of most importance, of the remaining variables in the model, in explaining the variation in baseline corticosterone. The information from the VIP plot (Figure 8) is complemented with a coefficient plot (Figure 9). These two plots summarize the overall contribution from each *X* variable, indicating the strength of the

correlation with *Y* (B-CORT) and the direction of the relationship (between *Y* and *X* variables) as positive or negative. The directions of the bars in the coefficient plot denote positive correlations for all OHC variables and B-CORT. The whiskers in both plots (Figure 8 and 9) indicated the 95% confidence interval of each variable, and lengths of the whiskers denote the variables reliability in the model. Shorter whiskers were more significant in the model and variables with whiskers crossing the zero-line indicated lack of significance. Further correlation tests confirmed these positive correlations for BDE-47, *p,p'*-DDE, PCB-99, PCB-187, β-HCH, PCB-28, PCB-118, PCB-138, *trans*-Nonachlor and BDE-154 (r_s > 0.829, p<0.042) with B-CORT.

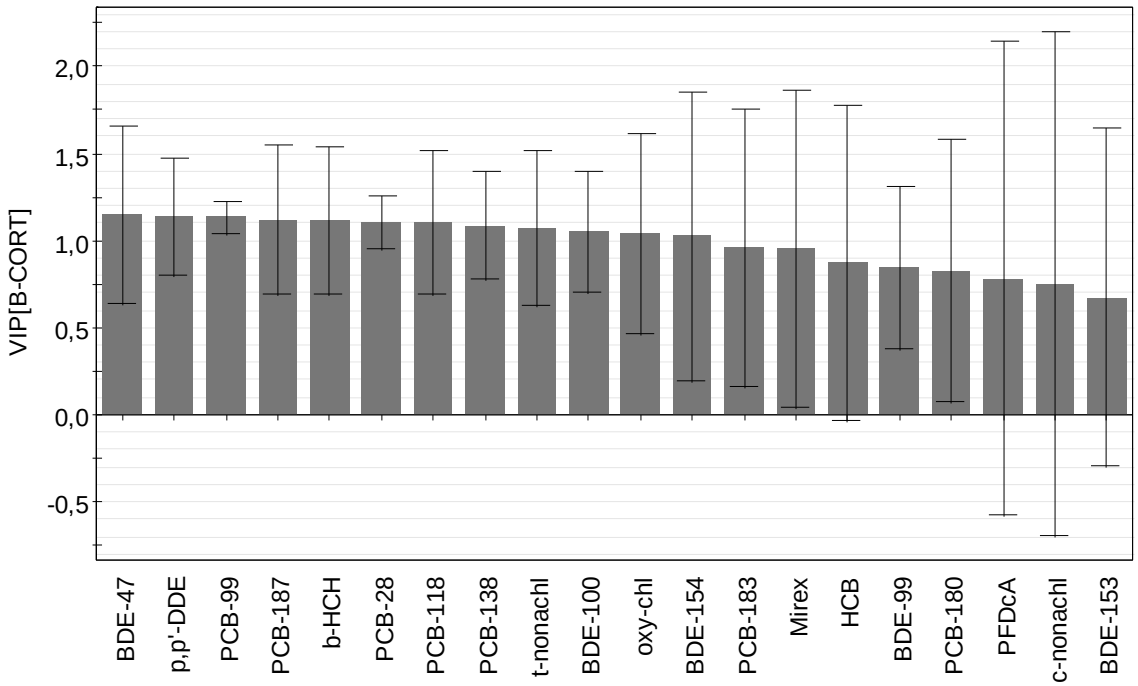


Figure 8: Variable importance in projection (VIP) plot of the final PLS regression, classifying the *X*-variables according to their explanatory power of the *Y*-variable, baseline corticosterone (B-CORT) in male glaucous gulls captured in Kongsfjorden, Svalbard 2013. Variables with a VIP value over 1 are regarded as important. The whiskers represent the 95% confidence interval, and crossing the zero-line indicates lack of significance.

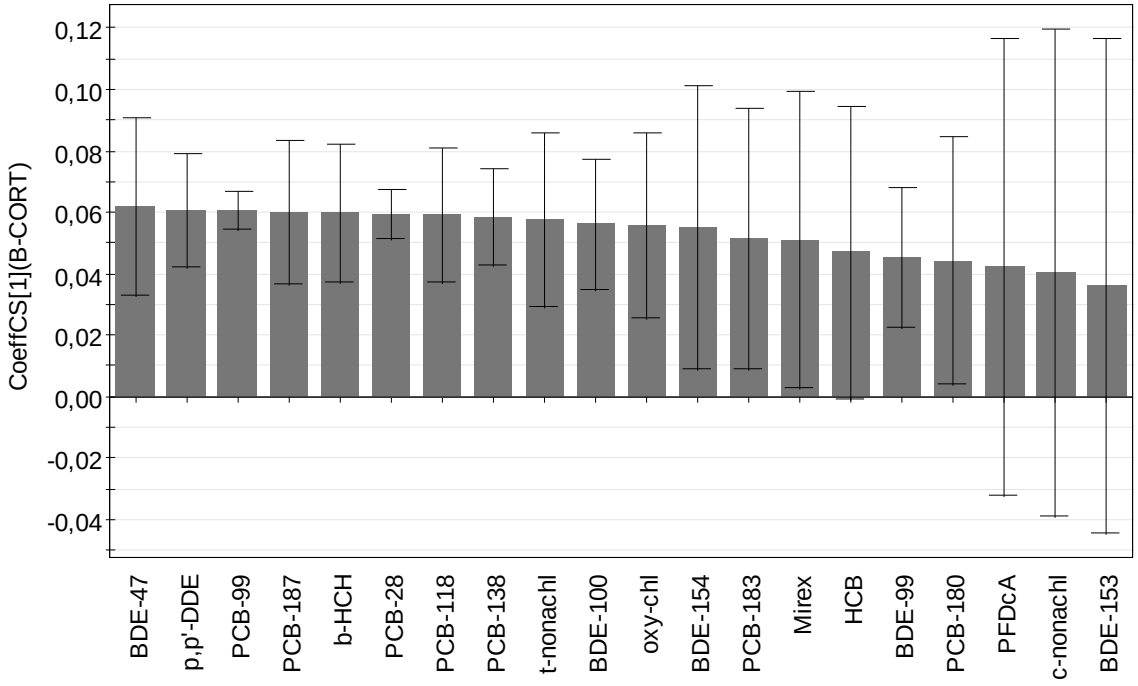


Figure 9: Coefficient plot of the final PLS regression summarizing the relationship between *X*-variables and baseline corticosterone (B-CORT) levels in male glaucous gulls captured in Kongsfjorden, Svalbard 2013. The whiskers represent the 95% confidence interval, and crossing the zero-line indicates lack of significance. The direction of the whiskers denotes significant positive relationships with B-CORT for all variables with VIP values above 1 (Figure 8).

The twenty-two remaining OHCs values, explaining 78.9 % of the variation of B-CORT, were summed (ΣPLS-OHCs) to investigate their combined effects on B-CORT. ΣPLS-OHCs correlated positively with B-CORT (r = 0.907, p = 0.013), and the linear relationship between ΣPLS-OHCs and B-CORT is presented graphically in Figure 10.

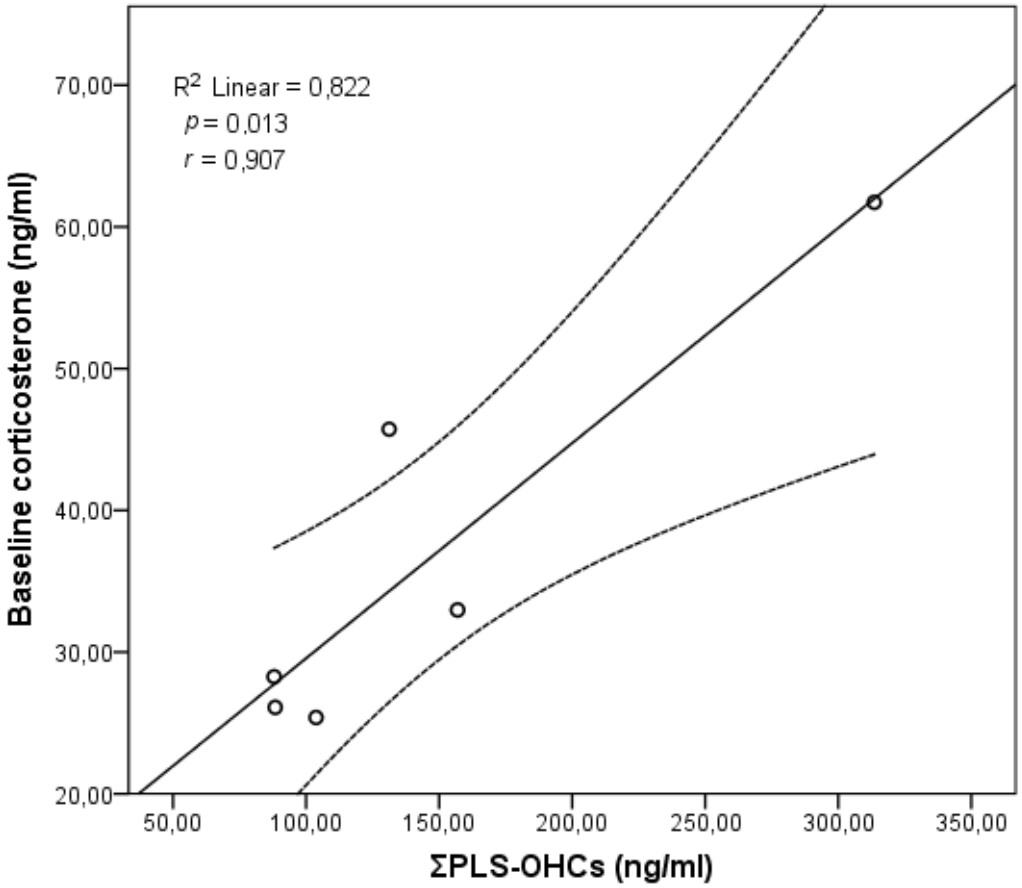


Figure 10: The linear relationship (± 95% confidence interval) between the ΣPLS-OHCs (PBDE; -47,-99, -100, -153, -154, PCB;-28, -99,-118, -138, -153, -180, -183, -187, -194, *p,p'*-DDE, b-HCH, *trans*-nonachlor, *oxy*-chlordane, *cis*-nonachlor, Mirex, HCB and PFDcA), and baseline corticosterone (ng/ml) in male glaucous gulls captured in Kongsfjorden, Svalbard 2013. The positive relationship was confirmed with Pearson's correlation coefficient, r, and significance, p (< 0.05). R^2-values for the linear regression are shown in the plot.

4. Discussion

The present study reports levels of both OHC contamination and corticosterone (CORT) hormone in glaucous gulls breeding in Kongsfjorden, Svalbard. The results indicate for the first time that the combination of specific OHCs might constitute a "cocktail" effect on baseline levels of CORT in male glaucous gulls. The study also indicates sex-differences in CORT responses to OHCs levels, since no significant relationships between CORT responses and OHC levels were identified for females. It is however possible that these findings are due to the identified differences in levels and patterns of OHCs between the sexes.

4.1. Levels of organohalogen contaminants

High levels of PCBs, CHLs and OPCs reflects the general pattern of OHC contamination in Arctic seabirds (Letcher *et al.*, 2010). In the present study, the contamination pattern was dominated by PCBs, CHLs and PFCs in both female and male glaucous gulls. The high levels of PFCs reported in female glaucous gulls were due to high levels of PFOS detected in some females.

It is very challenging to reveal possible effects of OHCs in wildlife. Field studies are often compromised with small sample sizes. In addition, recordings of relative few possible ecological and biological variables hamper the possibility of unveiling confounding effects caused by other factors than OHC levels. The present study is no exemption. A sample size of seventeen individuals is not sufficient to report the levels or effects of OHCs in the whole Arctic population of glaucous gulls. The arctic biota is exposed to a complex mixture of anthropogenic contaminants (Verreault *et al.*, 2005c; Letcher *et al.*, 2010), and the OHCs analysed in the present study reports only a selection of these compounds and contaminant groups. The high degree of covariation between the contaminant variables, in the present study, may complicate the multivariate data analyses when elucidating responses (Wold *et al.*, 1984; Umetrics, 2008). Multivariate data analyses are developed to handle these challenges, shown by inter-correlations. However, there are still uncertainties associated with interpretation of the results. The results presented in the present study may thereby only function as indicators and provide additional basis for further research of OHCs fate and effects in glaucous gulls.

The OHCs levels measured in the present study are overall considerably lower when compared to previously reported levels in glaucous gulls from Svalbard (Bjørnøya) (Bustnes *et al.*, 2003b; Verreault *et al.*, 2010). The levels of ΣPCBs and ΣCHLs reported in the present

study are over five and two times lower, respectively, than the levels previously reported in plasma samples from male and female glaucous gulls (Bjørnøya) (Verreault *et al.*, 2006a). It is possible that this population difference is due to spatial differences in exposure levels, with lower concentrations in Kongsfjorden than Bjørnøya. Although, previous studies show a steady decline in levels of specific OHCs in glaucous gulls (Bjørnøya) (Bustnes *et al.*, 2010). The levels of ΣPBDEs are highly comparable. As the levels of PFCs have not been investigated in the Svalbard glaucous gulls until recent years (Verreault *et al.*, 2005b; Haukås *et al.*, 2007), fewer studies are available for comparison to the present study. Levels of PFCAs and PFSAs were investigated in the Barents Sea food web, including glaucous gulls. This study reported a similar PFC contamination pattern as the present study (Haukås *et al.*, 2007). The major compound detected in liver samples of glaucous gulls from the Barents Sea was PFOS, followed by PFDcA (Haukås *et al.*, 2007). High PFOS levels have been reported in several wildlife studies (Giesy and Kannan, 2001; Butt *et al.*, 2008; Kelly *et al.*, 2009), as sulfonic PFCs are ultimately degraded to PFOS in the environment (Giesy and Kennan 2002).

4.1.2. Organochlorinated and -brominated contaminants

The major compound group detected in the present study was PCBs, with highest levels in measured in male glaucous gulls. The high levels of PCBs may reflect trophic level of feeding (Bustnes *et al.*, 2000; Wold *et al.*, 2011), as PCBs are known to bioaccumulate and biomagnify in the Arctic marine food web (Borgå *et al.*, 2005; Kelly *et al.*, 2007). PCB-153 was the main contributor in both sexes, followed by PCB-138 and PCB-180 respectively, when assessing the median concentrations. This pattern of PCB-congeners has previously been reported in glaucous gull from Svalbard (Bjørnøya) (Verreault *et al.*, 2004). These congeners are also represented in the significant PLS model explaining variation of B-CORT in males, although only PCB-153 and PCB-183 have VIP values above 1. PCB-153 is well known for its persistency in the Arctic marine food web (Wolkers *et al.*, 2004), and high levels of PCB-153 has been reported in several studies of glaucous gulls (Bustnes *et al.*, 2003a; Buckman *et al.*, 2004; Bustnes *et al.*, 2004; Sagerup *et al.*, 2009).

The major organochlorinated pesticide detected in the present study was *oxy*-chlordane, followed by HCB, mirex and *p,p'*-DDE for both sexes. *Oxy*-chlordane is a metabolite from *cis*- and *trans*-chlordane (Polen *et al.*, 1971; Fisk *et al.*, 2001), the major compounds in chlordane, an agricultural insecticide (Sovocool *et al.*, 1977). HCB, mirex and the environmental metabolite of *p,p'*-DDT, *p,p'*-DDE, are persistent OCPs commonly detected in

arctic biota (Borgå *et al.*, 2007; Rigét *et al.*, 2010). These compounds are also important for explaining the variation of B-CORT in males.

The sex-specific OHC pattern detected in the present study is supported by previous studies of glaucous gulls from West-Spitsbergen and the more contaminated Bjørnøya (Bustnes *et al.*, 2000; Verreault *et al.*, 2006a; Verboven *et al.*, 2010; Verreault *et al.*, 2010). Males are generally more contaminated than females, reflecting feeding ecology (Bustnes *et al.*, 2000) and the selective maternal transfer of OHCs into eggs (Verreault *et al.*, 2006b).

Exact ages of the glaucous gulls were not recorded in the present study, as it shown that a steady-state of OCs is reached before the onset of breeding (Anderson and Hickey, 1976). It is rather the trophic levels of feeding that explain the levels of OCs, with highest levels in birds feeding on a higher trophic level (Bustnes *et al.*, 2003a).

The detected level of ΣPBDE in the present study was within the lower range of previous studies from Bjørnøya (Verreault *et al.*, 2007c), with no differences between the sexes. The major congener was BDE-47, in both males and females. This congener is also one of the main PBDEs detected in the Arctic (de Wit *et al.*, 2006), and its levels are shown to increase with higher trophic level in the Arctic marine food web (Kelly *et al.*, 2008). The second and third major congeners were BDE-100 and -153 for males, and BDE-153 and -100 for females, respectively. This is also the general pattern of PBDE contamination previously detected in glaucous gulls from Svalbard (Verreault *et al.*, 2005a). All PBDEs detected in the present study were important in explaining the variation of B-CORT in male glaucous gulls, in spite the low percentage of this group (1.96 % in males, 2.6 % in females) in the total OHC contamination pattern (Figure 3).

The different physiochemical properties of the OHCs contribute to the inter-correlations between the OHC groups in both the PCA analyses, bivariate analyses and the final PLS model. Organochlorinated and brominated OHCs possess similar properties of lipophilicity and may thereby be partitioned similarly into the blood (Henriksen *et al.*, 1998; Yordy *et al.*, 2010) during the lipid cycle. This may account for the correlations between these contaminant groups, relative to PFCs, shown in the PCA plots (Figure 5 and 7, Appendix I: Figure 13). The PFCs possess amphipathic properties and due to their protein affinity will rather be partitioned into protein-rich compartments e.g. blood and liver (Ahrens *et al.*, 2009; Gebbink and Letcher, 2012), compared to the lipophilic OHCs. Thus, the observed intra-

correlation among PFCs and inter-correlations with OCPs, CHLs, PBDEs and PCBs visible in the PCA plots in the present study was expected.

4.1.3. Levels of perfluorinated contaminants

PFOS was the predominant compound of the detected PFCs, in both sexes in the present study. This is consistent with previous reports of PFCs detected in glaucous gulls (Verreault *et al.*, 2005b; Haukås *et al.*, 2007), although the levels previously reported are much higher than in the present study. This might be due to differences in spatial contamination patterns.

The sum concentrations of PFCAs (ΣPFCAs) were lower or equal to the PFOS concentration in the present study. This is consistent with previous studies of arctic biota (Martin *et al.*, 2004; Verreault *et al.*, 2005b; Houde *et al.*, 2011). Of the detected PFCAs the highest levels were reported for PFUnA and PFTrA, in both sexes of glaucous gulls. These contaminants are also the predominant PFCA in eggs of several seabirds from the Canadian Arctic (Braune and Letcher, 2012). The pattern of PFC contamination presented in the present study is similar to previous studies from the Norwegian and Russian Arctic, in eggs from ivory gulls (*Pagophila eburnea*) (Miljeteig *et al.*, 2009) and glaucous gulls (Verreault *et al.*, 2005b).

Interestingly, PFDcA was the only PFC denoted as significant for explaining the B-CORT levels in males, in spite its low concentration. This compound was found to increase between 1993 and 2003 in herring gull (*Larus argentatus*) eggs from two coastal colonies in northern Norway (Verreault *et al.*, 2007b). A similar increase of PFDcA was also detected in livers from northern fulmars (*Fulmaris glacialis*) from 1975 to 2003 was also detected in the Canadian Arctic (Butt *et al.*, 2007).

4.2. Levels of corticosterone

To my knowledge, this is the second study examining circulating levels of corticosterone in glaucous gulls. The previous study by Verboven *et al.* (2010) detected baseline (B-CORT) and stress-induced corticosterone (S-CORT) in glaucous gulls from Bjørnøya. In that study, the average B-CORT concentration was (on pooled sexes) 8.56 ± 1.25 ng/ml (range 1.00 – 35.08 ng/ml) and S-CORT concentration was 35.54 ± 1.78 ng/ml (range 14.90 – 65.83 ng/ml) was detected (Verboven *et al.*, 2010). These results are over five and three times lower than the levels of corticosterone detected in the present study for B-CORT and S-CORT, respectively. Although the mean sampling time for the B-CORT samples was three minutes later and for the S-CORT samples two minutes earlier than the previous study, the differences in detected corticosterone concentrations may not just result from differences in sampling

times. Verboven *et al.* (2010) extracted the plasma samples with diethylether after the adding of tritiated corticosterone for quantitative analyses and diluted the plasma extracts 15 times prior to radioimmunoassay analysis. The evident differences in the method of analysing corticosterone may account for (some of) the differences in detected B-CORT and S-CORT concentration.

The same method of trapping and capture-challenge protocol were applied in both Verboven *et al.*, (2010) and the present study. A considerable amount of time passed by when attempted to trap the adult birds, this is reflected in the individual handling times (dT) (Table 5, Appendix A). Because the eggs had to be replaced into the rest as quick as possible to prevent cooling and delay of the incubation process, a post-capture sample an hour after trapping, to reflect the sensitivity of the stress axis, was therefore not possible to conduct in the present study.

No statistical differences between males and female glaucous gulls were reported for B-CORT, S-CORT or I-CORT in the present study. As the incubation and chick rearing is shared between the male and female glaucous gull when breeding (Weiser and Gilchrist, 2012), it is possible that these stressful situations and the challenge of being trapped are perceived and managed equally by the two sexes. Although, individual differences between B-CORT and S-CORT in correlation to the sampling time are evident in the present study (Figure 4). This may be reflected by differences in personality and how the individual birds handle stressful events. Birds' personalities are usually classified as proactive or reactive. Proactive personalities have relatively active behaviour responses and low corticosterone stress responses, whilst reactive personalities have rather passive behavioural responses and large corticosterone responses (Groothuis and Carere, 2005; Cockrem, 2007). Birds with proactive personalities have been suggested to be more successful in a more stable environment, while reactive personalities may be more cautious and thereby be more successful in a changing environment (Cockrem, 2005; Cockrem, 2007). These personality types were personally observed during field sampling of the glaucous gull in the present study, although not recorded for each bird. Some of the sampled birds were quiet and showed no signs of resistance, whilst others displayed a more active and aggressive behaviour when being trapped.

The individual differences shown in Figure 4 also indicate the complexity and challenges when investigating stress response of animals in field studies. The differences in timing of

the first sample (B-CORT) were due to differences in accessibility of the nest location by boat or on land. While, the differences in in timing of the second sample (S-CORT) reflect the time spend before trapping the bird, when the eggs were kept away from the nest. Short handling time thereby reflects a more time consuming trapping. Although, there are no signs of effect of the time spent attempting to trap the birds, as the B-CORT and S-CORT levels are comparable to each other.

The mean concentrations of B-CORT, S-CORT and I-CORT reported here were generally higher in males than female glaucous gulls. It is important to keep in mind the small sampling size of the present study, weakening the statistical power of the tests applied. The median concentrations may thereby give a more representable insight to the corticosterone concentrations. The median concentration of B-CORT was higher in females than males, and the median S-CORT concentration was higher in males than females. This reflects the higher median I-CORT concentration in males than females. A sample size of six males and eleven females is not sufficient to provide a good estimate of the B-CORT or S-CORT concentrations of the whole glaucous gull population breeding in Kongsfjorden. The present study is therefore providing indications of the possible differences; that may be confirmed by new studies with larger sample sizes and equal sample sizes of the two sexes.

Sex differences in B-CORT levels have previously been reported in free-living Arctic passerine birds (Astheimer *et al.*, 1994; Holberton and Wingfield, 2003), the sub-Arctic willow warbler (*Phylloscopus trochilus*) (Silverin *et al.*, 1997) and the tropical red-footed booby (*Sula sula*) (Lormée *et al.*, 2003), with male birds exhibiting higher levels than females during breeding. Other studies have shown higher levels in females, than in male birds (Rector 2012) (O'Reilly and Wingfield, 2001; Rector *et al.*, 2012) or no differences between the sexes (Verboven *et al.*, 2010). These differences may be due to uniparental or biparental care (O'Reilly and Wingfield, 2001). The levels of B-CORT are important for timing of breeding. By experimentally reducing the release of corticosterone in black-legged kittiwakes with corticosterone inhibiting implants, a study found significant delays in egg laying in females (Goutte *et al.*, 2011; Goutte *et al.*, 2014). Disruption of the breeding process may also be influenced by ecological factors e.g. food availability (Moe *et al.*, 2009).

Differences in levels of B-CORT and S-CORT between species, subspecies or populations have frequently been reported and the mechanism behind is still poorly understood (Astheimer *et al.*, 1994; Boonstra, 2004; Angelier *et al.*, 2011). This is important to keep this

in mind when comparing the levels of stress hormones between species, subspecies or populations.

4.3. Effect of OHCs on corticosterone concentrations

The multivariate data analyses applied in the present study provides the opportunity to investigate associations and pinpoint the most important OHC variables in explaining the variation of corticosterone variables. This method is not widely used in endocrino-toxicological studies, although it has become more applied in recent years (Villanger *et al.*, 2011; Bechshøft *et al.*, 2012; Villanger *et al.*, 2013). The observed positive association between B-CORT and plasma levels of selected OHCs in glaucous gulls in the present study supports the previous results from Verboven *et al.*, (2010). They reported a positive correlation (on pooled sexes) between baseline corticosterone concentration and a PC explaining 77.3 % of the total variation in selected POP classes. Of the selected POP classes, the largest contributions of loadings in PC1 were by PCBs and PBDEs (Verboven *et al.*, 2010). The present study show that the most persistent PBDEs (BDE-47, -100 and -154) and PCBs (-99, -187, -28, -118, -138 and -153), as well as some very persistent OCPs (*p,p'*-DDE β-HCH, *trans*-Nonachlor and *oxy*-Chlordane) were most important in explaining most of the variation in baseline corticosterone in males. This observation also support the previous reports of baseline corticosterone concentrations in herring gull (*Larus argentatus*) and black guillemots (*Cepphus grylle*) experimentally exposed to PCBs (Peakall *et al.*, 1981).

No relationships were found between OHCs and the corticosterone variables (B-CORT, S-CORT and I-CORT) for females, although a significant negative relationship was revealed between B-CORT and lipid weight. Plasma lipids include triglycerides, cholesterol and fatty acids. Changes in these components correspond to changes in food quality (Gavett and Wakeley, 1986). Thus, the lipid weight of plasma is not a good indicator of the body condition in the glaucous gull. The same is true for the visceral and organ lipids. The plasma carries newly absorbed nutrients and nutrients mobilized to tissues, and therefore provides an acute rather than long-term index of nutritional status (Brown, 1996; Gibson, 2005). The lipid content of the plasma may thereby function as an indicator of whether the bird had fed within the last hours. As the females exhibit higher corticosterone concentrations with low lipid content in the plasma, it is possible that the stress of being trapped is magnified, when the bird is already stressed when foraging (Astheimer *et al.*, 1995).

All of the investigated PBDE congeners in the present study were important in explaining the variation of B-CORT in males glaucous gulls- in spite the low levels detected. This sheds light upon the complexity of toxicants and their potency of potential endocrine disruption, even at low concentrations. A study of American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs (BDE-47, -99, -100 and -153) showed immunomodulation and changes in several immune parameters (Fernie *et al.*, 2005), suggesting endocrine disruption by the PBDEs administered. Another study of the same species exposed to environmentally relevant levels of a different PBDE mixture demonstrated modified quality of pair-bond and affected reproductive behaviour of both sexes when exposed for a short period as adult (Fernie *et al.*, 2008). These effects have not been reported in glaucous gulls, although studies indicate that PBDEs may disrupt thyroid system through competitive binding to transthyretin (TTR), an important binding protein (Ucán-Marín *et al.*, 2009).

Oxy-chlordane and HCB have previously been pinpointed as causative agents in POPs mixtures to cause adverse effects in glaucous gulls (Bustnes, 2006). These pesticides are also important compounds explaining the variation in B-CORT in the PLS model for male glaucous gulls in the present study. The altered immune function during stress responses and the chronic exposure to environmental POPs may influence the bird's susceptibility to parasites and disease. Sagerup *et al.* (2000) found a positive correlation between the levels of OCs and parasite intensities in glaucous gulls (Bjørnøya), thereby suggesting chemical alteration of the immune system - making the individuals more susceptible to parasite infections (Sagerup *et al.*, 2000; Bustnes *et al.*, 2004). In glaucous gulls, *oxy*-chlordane has previously been related to decreased adult survival (Bustnes *et al.*, 2003b), and increased time spent away from the nest site during incubation (Bustnes *et al.*, 2005). A study reported that as *oxy*-chlordane concentrations in the blood increased a 10-fold (from 5 to 50 ng/g w.w), survival probability of female and male glaucous gulls was reduced significantly (Bustnes *et al.*, 2003b). This indicates a possible interference with the glaucous gulls' immune system. Strong correlations between the levels of OCs and impairment of immune parameters reported in glaucous gulls (Bjørnøya) strengthen these indications (Bustnes *et al.*, 2004). The authors found strong correlations between several OCs and immune parameters in males, whereas only significant correlations for *oxy*-chlordane and HCB in females. This also reflects the differences in both contaminant levels and toxicological effects between sexes. The immune system is highly influenced by the stress axis, and prolonged activation of the stress axis due

to exintric and insintric factors, such as high contaminant levels, may suppress important immune parameters (Sapolsky *et al.*, 2000; Boonstra, 2004; Martin, 2009).

The results reported by the present study and Verboven *et al.* (2010) are, however, contradictive to an experimental study on captive male American kestrels exposed to a mixture of PCBs. The main congeners of the mixture were PCB-138, -153, -180 and -187 (Love *et al.*, 2003), all which are important in explaining the variation of B-CORT in the present study. Love *et al.* (2003) showed that both B-CORT and S-CORT levels were significantly lower in exposed birds compared with control birds of similar age. Also, the B-CORT levels of corticosterone showed a hormetic response in relation to the relative burden of PCBs in the liver (Love *et al.*, 2003). The hormetic response is characterized by an inverted U-shape. This reflects an increase in baseline corticosterone levels at lower concentrations and a decrease at higher concentrations of PCBs. This response, hormesis, is a well-known toxicological response involved in short-term dosing studies followed by measurement of a biological endpoint, e.g. metabolism, reproduction or survival (Calabrese and Baldwin, 2001). However in the study by Love *et al.* (2003) there was a considerable time period for the contaminants to cause additional effects after exposure and before the sampling (348 days). This made the researchers to suggest that the hormetic response was due to damage of the adrenal cortex by the administered PCB, the same conclusion as Byrne *et al.*, (1988). Corticosterone is also the principal glucorticoid in rats and experimental analysis shows suppression of corticosterone by chronic low-dose (1, 5, 10 or 50 ppm) PCB and polybromobiphenyl (PBB), as well as reductions in adrenal weights, suggesting toxicity to the adrenal gland (Byrne *et al.*, 1988). These variables were not examined in the present study or other studies of glaucous gulls. It is also important to note that the dietary PCBs were administered to the kestrels for 120 days, and then followed by a 348 days period with a relatively clean diet. The study by Love *et al.* (2003) may therefore not reflect the chronic exposure of POPs birds are exposed to in their natural environment, and for that reason the results cannot be fully extrapolated to the wild although the dosage was environmentally relevant.

There might be a difference in the stress response in captive birds and wild birds, due to several factors. A study on several parrot species showed that wild-captured birds showed a prolonged stress response to the stressor of capture and handling, compared to captive-bred birds. After 45 minutes the corticosterone levels of captive-bred birds were reduced, while the levels in wild-captured birds continued to increase (Cabezas *et al.*, 2013). Even though both

the captive-bred and the wild-captured birds had been in captivity for some time, the captive-bred birds would have had considerable more time to acclimatize to the handling by humans. This may be the reason for the rapid homeostasis response in captive-bred birds (Romero, 2004). It may be speculated that since glaucous gulls breeding at Bjørnøya have been studied and trapped for several years (Verreault *et al.*, 2010), they might be more habituated to capture and handling than glaucous gulls breeding in Kongsfjorden. This may explain the high B-CORT and S-CORT levels detected in the present study compared to levels reported by Verboven *et al.* (2010). This is thus, highly unlikely, as the birds are only "exposed" to the researchers once every year during the breeding season, and a considerable amount of time is needed to be fully acclimatized to such situations.

As temperature and other climatic variables were not recorded during the present study, it is not possible to assess the impact of climatic variability on circulating corticosterone concentrations in the glaucous gulls. Thus, it would be interesting to include these factors in further analysis regarding stress in seabirds. As the Arctic is a changing environment (Hinzman *et al.*, 2005a), the interference of OHCs and additional climatic stress can potentially compromise seabird's ability to adapt to a changing habitat. Changes in several climatic variables may disrupt the trophic levels of the Arctic food web (Bluhm and Gradinger, 2008). Disruption of important primary and secondary production events, such as the annual algae blooms in spring, may lead to scarce food availability for the higher trophic levels (Walther *et al.*, 2002; Smetacek and Nicol, 2005; Falk-Petersen *et al.*, 2007). Several trophic levels feed in connection to sea ice, and as the sea ice decreases food availability may decrease (Parmesan, 2006; Post *et al.*, 2009). Decreased food availability may have an impact on body mass and survival (Harding *et al.*, 2011), and may initiate additional mobilization of lipids, thereby increasing the levels of lipophilic OHCs in the blood (Anderson and Hickey, 1976). These events may contribute to additional stress for the Arctic seabirds (Boonstra, 2004). The glaucous gulls sampled in the present study were of generally good condition relative to each other, indicated by BCIs, although it is possible that additional variability in food availability may further influence the stress responses in the birds (Wingfield *et al.*, 1995; Kitaysky *et al.*, 1999; Lanctot *et al.*, 2003).

The stress-axis is very complex and is influenced by both external and internal stressors. It is important to note that OHCs can interfere with numerous stages of the stress-axis. The synthesis, binding and delivery of GCs to the plasma proteins and target cells are critical stages that can be disrupted by OHCs. At the same time regulation of active and inactive GCs

through changes in number and binding affinity of the receptors, as well as the exportation and degradation of GCs (Odermatt *et al.*, 2006). Due to the lack of control samples, B-CORT and S-CORT samples of uncontaminated glaucous gulls, as well as the correlative nature and low sample size of the present and previous studies – the exact relationship between OHCs and corticosterone remain undetermined.

It is also challenging to analyze GCs in blood plasma, as the levels of GCs are fluctuating due to constant changes in external and internal stressor. Thereby, it might be interesting to evaluate a more stable biological matrix when analyzing GCs in birds. A matrix where levels of the GCs are not influenced by the situation of sample collection would be preferred. A situation highly influenced by several extrinsic and logistic factors in Arctic field studies. The levels of GCs in blood will only provide a snapshot of the stress physiology and may thereby provide insight in short-term effects in relation to OHCs. Feathers may be assessed for both OHCs (García-Fernández *et al.*, 2013) and corticosterone, and thereby provide a more long-term insight to the effects of OHCs on stress in birds (Bortolotti *et al.*, 2008; Bortolotti *et al.*, 2009). This is a relatively new method assessed for biomonitoring of OHCs (Jaspers *et al.*, 2006; Jaspers *et al.*, 2011), and further validation of feathers as a sample matrix in endocrino-toxicological research is needed for several birds, including glaucous gulls.

5. Conclusions

In conclusion, the present study indicated sex-specific pattern of levels and effects of selected OHCs in glaucous gulls from Kongsfjorden. A significant negative relationship was reported for lipid weight in blood plasma and baseline corticosterone concentration in female glaucous gulls. This may indicate the stressful situation of being captured "on an empty stomach". No associations were reported between B-CORT and OHCs in females. Although, significant positive associations were reported for B-CORT and twenty-two OHCs in males; indicating for the first time a "cocktail" effect of specific OHCs in blood plasma associated with high baseline levels of corticosterone in male glaucous gulls. No statistical differences in stress response or corticosterone concentrations were recorded for males and female glaucous gulls; although a larger sample size might reveal differences between the sexes. The present study did not find significant associations between S-CORT, I-CORT and the OHCs in either females or males. However, due to the low n available for male individuals, the results must be treated with caution and need further research to validate our findings.

6. Further recommendations

The present study shows indications of a sex-specific pattern of levels and effects of contaminants in glaucous gulls. It also highlights the importance of equal sample size (n) of both sexes to confirm these indications, as well as a larger sample size in general. Larger sample sizes will strengthen the statistical models applied to investigate effects. Further studies investigating stress response and contamination levels in glaucous gulls in Kongsfjorden, should desirably include a sample size of forty individuals. Further studies should also include climatic and ecological variables of average temperature, food availability, breeding conditions and timing of breeding, in order to explain further variation in the stress axis not explained by OHCs. Further studies should also include metabolic derived OHCs, as these are hypothesized to be of greater endocrine disruption potential (Tyler *et al.*, 1998). Due to the close association between several PBDEs and B-CORT levels indicated in the present study, further studies should include other new and emerging BFRs in order to assess these relationships.

Bibliography

Abdi, H., Williams, L.J., 2010. Principal component analysis. Wiley Interdisciplinary Reviews: Computational Statistics 2, 433-459.

Ahrens, L., Siebert, U., Ebinghaus, R., 2009. Total body burden and tissue distribution of polyfluorinated compounds in harbor seals (*Phoca vitulina*) from the German Bight. Marine Pollution Bulletin 58, 520-525.

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

Anderson, D.W., Hickey, J.J., 1976. Dynamics of storage of organochlorine pollutants in herring gulls. Environmental Pollution (1970) 10, 183-200.

Angelier, F., Ballentine, B., Holberton, R., Marra, P., Greenberg, R., 2011. What drives variation in the corticosterone stress response between subspecies? A common garden experiment of swamp sparrows (*Melospiza georgiana*). Journal of evolutionary biology 24, 1274-1283.

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

Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1994. Gender and Seasonal Differences in the Adrenocortical Response to ACTH Challenge in an Arctic Passerine, *Zonotrichia leucophrys gambelii*. General and comparative endocrinology 94, 33-43.

Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1995. Seasonal and acute changes in adrenocortical responsiveness in an arctic-breeding bird. Horm. Behav. 29, 442-457.

Barrett, R.T., Anker-Nilssen, T., Gabrielsen, G.W., Chapdelaine, G., 2002. Food consumption by seabirds in Norwegian waters. ICES Journal of Marine Science: Journal du Conseil 59, 43-57.

Bechshøft, T.Ø., Sonne, C., Dietz, R., Born, E., Muir, D., Letcher, R., Novak, M., Henchey, E., Meyer, J., Jenssen, B., 2012. Associations between complex OHC mixtures and thyroid and cortisol hormone levels in East Greenland polar bears. Environmental research 116, 26-35.

Bluhm, B.A., Gradinger, R., 2008. Regional variability in food availability for Arctic marine mammals. Ecol. Appl. 18, S77-S96.

Boonstra, R., 2004. Coping with changing northern environments: The role of the stress axis in birds and mammals. Integr. Comp. Biol. 44, 95-108.

Borgå, K., Fisk, A.T., Hoekstra, P.F., Muir, D.C., 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. Environ. Toxicol. Chem. 23, 2367-2385.

Borgå, K., Gabrielsen, G., Skaare, J., 2001. Biomagnification of organochlorines along a Barents Sea food chain. Environ. Pollut. 113, 187-198.

Borgå, K., Hop, H., Skaare, J., Wolkers, H., Gabrielsen, G., 2007. Selective bioaccumulation of chlorinated pesticides and metabolites in Arctic seabirds. Environ. Pollut. 145, 545-553.

Borgå, K., Wolkers, H., Skaare, J.U., Hop, H., Muir, D.C., Gabrielsen, G.W., 2005. Bioaccumulation of PCBs in Arctic seabirds: influence of dietary exposure and congener biotransformation. Environ. Pollut. 134, 397-409.

Bortolotti, G.R., Marchant, T., Blas, J., Cabezas, S., 2009. Tracking stress: localisation, deposition and stability of corticosterone in feathers. Journal of Experimental Biology 212, 1477-1482.

Bortolotti, G.R., Marchant, T., Blas, J., German, T., 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Functional Ecology 22, 494-500.

Braune, B., Outridge, P., Fisk, A., Muir, D., Helm, P., Hobbs, K., Hoekstra, P., Kuzyk, Z., Kwan, M., Letcher, R., 2005. Persistent organic pollutants and mercury in marine biota of the Canadian Arctic: an overview of spatial and temporal trends. Science of the Total Environment 351, 4-56.

Braune, B.M., Letcher, R.J., 2012. Perfluorinated sulfonate and carboxylate compounds in eggs of seabirds breeding in the Canadian arctic: temporal trends (1975–2011) and interspecies comparison. Environmental science & technology 47, 616-624.

Brown, M.E., 1996. Assessing body condition in birds, Current ornithology, Springer, pp. 67-135.

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

Buckman, A.H., Norstrom, R.J., Hobson, K.A., Karnovsky, N.J., Duffe, J., Fisk, A.T., 2004. Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. Environ. Pollut. 128, 327-338.

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

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

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

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

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

Bustnes, J.O., Gabrielsen, G.W., Verreault, J., 2010. Climate variability and temporal trends of persistent organic pollutants in the Arctic: A study of glaucous gulls. Environmental science & technology 44, 3155-3161.

Bustnes, J.O., Hanssen, S.A., Folstad, I., Erikstad, K.E., Hasselquist, D., Skaare, J.U., 2004. Immune function and organochlorine pollutants in arctic breeding glaucous gulls. Arch. Environ. Contam. Toxicol. 47, 530-541.

Bustnes, J.O., Miland, Ø., Fjeld, M., Erikstad, K.E., Skaare, J.U., 2005. Relationships between ecological variables and four organochlorine pollutants in an artic glaucous gull (*Larus hyperboreus*) population. Environ. Pollut. 136, 175-185.

Butt, C.M., Mabury, S.A., Kwan, M., Wang, X., Muir, D.C., 2008. Spatial trends of perfluoroalkyl compounds in ringed seals (*Phoca hispida*) from the Canadian Arctic. Environ. Toxicol. Chem. 27, 542-553.

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

Byrne, J.J., Carbone, J.P., Pepe, M.G., 1988. Suppression of serum adrenal cortex hormones by chronic low-dose polychiorobiphenyl or polybromobiphenyl treatments. Arch. Environ. Contam. Toxicol. 17, 47-53.

Cabezas, S., Carrete, M., Tella, J.L., Marchant, T.A., Bortolotti, G.R., 2013. Differences in acute stress responses between wild-caught and captive-bred birds: a physiological mechanism contributing to current avian invasions? Biol. Invasions 15, 521-527.

Calabrese, E.J., Baldwin, L.A., 2001. The frequency of U-shaped dose responses in the toxicological literature. Toxicol. Sci. 62, 330-338.

Carsia, R.V., Harvey, S., 2000. Adrenals. Sturkie's Avian Physiology 5th ed., 489-537.

Cockrem, J., 2005. Conservation and behavioral neuroendocrinology. Horm. Behav. 48, 492-501.

Cockrem, J.F., 2007. Stress, corticosterone responses and avian personalities. Journal of Ornithology 148, 169-178.

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

Covaci, A., Voorspoels, S., Thomsen, C., van Bavel, B., Neels, H., 2006. Evaluation of total lipids using enzymatic methods for the normalization of persistent organic pollutant levels in serum. Sci. Total Environ. 366, 361-366.

Cramp, S., Simmons, K.L., editors, Brooks, D., Collar, N., Dunn, E., Gillmor, R., Hollom, P., Hudson, R., Nicholson, E., Ogilvie, M., 1983. Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic: 3. Waders to gulls.

de Kloet, E.R., Oitzl, M.S., Joels, M., 1999. Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci. 22, 422-426.

de Wit, C., Fisk, A., Hobbs, K., Muir, D., Gabrielsen, G., Kallenborn, R., Krahn, M., Norstrom, R., Skaare, J., 2004. AMAP assessment 2002. Persistent Organic Pollutants in the Arctic. Arctic Monitoring and Assessment Program report. Oslo, Norway.

de Wit, C.A., Alaee, M., Muir, D.C., 2006. Levels and trends of brominated flame retardants in the Arctic. Chemosphere 64, 209-233.

EnzoLifeSciences, 2010. Corticosterone EIA Kit, 96 Wells. Catalog No. ADI-900-097 [Online], http://www.enzolifesciences.com/ADI-900-097/corticosterone-elisa-kit/, [Accessed 20.09.2013].

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Trygg, J., Wikström, C., Wold, S., 2006. In Multi-and Megavariate Data Analysis Part I: Basic Principles and Applications; Umetrics: Umeå, Sweden, 2006. There is no corresponding record for this reference.

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

Falk-Petersen, S., Pavlov, V., Timofeev, S., Sargent, J.R., 2007. Climate variability and possible effects on arctic food chains: the role of Calanus, Arctic alpine ecosystems and people in a changing environment, Springer, pp. 147-166.

Fernie, K.J., Mayne, G., Shutt, J.L., Pekarik, C., Grasman, K.A., Letcher, R.J., Drouillard, K., 2005. Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. Environ. Pollut. 138, 485-493.

Fernie, K.J., Shutt, J.L., Letcher, R.J., Ritchie, J.I., Sullivan, K., Bird, D.M., 2008. Changes in reproductive courtship behaviors of adult American kestrels (*Falco sparverius*) exposed to environmentally relevant levels of the polybrominated diphenyl ether mixture, DE-71. Toxicol. Sci. 102, 171-178.

Fisk, A., Moisey, J., Hobson, K., Karnovsky, N., Norstrom, R., 2001. Chlordane components and metabolites in seven species of Arctic seabirds from the Northwater Polynya: relationships with stable isotopes of nitrogen and enantiomeric fractions of chiral components. Environ. Pollut. 113, 225-238.

Fraser, R., Gower, D., Honour, J.W., Ingram, M.C., Kicman, A.T., Makin, H.L., Stewart, P.M., 2010. Analysis of corticosteroids. Springer.

Freitas, A.d., Norstrom, R., 1974. Turnover and metabolism of polychlorinated biphenyls in relation to their chemical structure and the movement of lipids in the pigeon. Canadian journal of physiology and pharmacology 52, 1080-1094.

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

Gabrielsen, G.W., Smith, E.N., 1995. Physiological responses of wildlife to disturbance. Island Press, Washington.

García-Fernández, A.J., Espín, S., Martínez-López, E., 2013. Feathers as a biomonitoring tool of polyhalogenated compounds: a review. Environmental science & technology 47, 3028-3043.

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

Gavett, A.P., Wakeley, J.S., 1986. Blood constituents and their relation to diet in urban and rural House Sparrows. Condor, 279-284.

Gebbink, W.A., Letcher, R.J., 2012. Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls. Environ. Pollut. 162, 40-47.

Gibson, R.S., 2005. Principles of nutritional assessment. Oxford university press.

Giesy, J.P., Feyk, L.A., Jones, P.D., Kannan, K., Sanderson, T., 2003. Review of the effects of endocrine-disrupting chemicals in birds. Pure and Applied Chemistry 75, 2287-2303.

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

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

Goutte, A., Angelier, F., Bech, C., Clément-Chastel, C., Dell'Omo, G., Gabrielsen, G.W., Lendvai, Á.Z., Moe, B., Noreen, E., Pinaud, D., 2014. Annual variation in the timing of breeding, pre‐breeding foraging areas and corticosterone levels in an Arctic population of black-legged kittiwakes. MEPS 496, 233-247.

Goutte, A., Angelier, F., Welcker, J., Moe, B., Clément-Chastel, C., Gabrielsen, G.W., Bech, C., Chastel, O., 2010. Long-term survival effect of corticosterone manipulation in black-legged kittiwakes. General and comparative endocrinology 167, 246-251.

Goutte, A., Clément-Chastel, C., Moe, B., Bech, C., Gabrielsen, G.W., Chastel, O., 2011. Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes. The Journal of experimental biology 214, 2005-2013.

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

Groothuis, T.G., Carere, C., 2005. Avian personalities: characterization and epigenesis. Neuroscience & Biobehavioral Reviews 29, 137-150.

Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O., 2001. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environmental Science & Technology 35, 766-770.

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

Harding, A.M., Welcker, J., Steen, H., Hamer, K.C., Kitaysky, A.S., Fort, J., Talbot, S.L., Cornick, L.A., Karnovsky, N.J., Gabrielsen, G.W., 2011. Adverse foraging conditions may impact body mass and survival of a high Arctic seabird. Oecologia 167, 49-59.

Hario, M., Kilpi, M., Selin, K., 1991. Parental investment by the sexes in the Herring Gull: the use of energy reserves during early breeding. Ornis Scandinavica, 308-312.

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

Hekster, F.M., Laane, R.W., de Voogt, P., 2003. Environmental and toxicity effects of perfluoroalkylated substances, Reviews of environmental contamination and toxicology, Springer, pp. 99-121.

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

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

Hinzman, L.D., Bettez, N.D., Bolton, W.R., Chapin, F.S., Dyurgerov, M.B., Fastie, C.L., Griffith, B., Hollister, R.D., Hope, A., Huntington, H.P., 2005a. Evidence and implications of recent climate change in northern Alaska and other arctic regions. Clim. Change 72, 251-298.

Hinzman, L.D., Bettez, N.D., Bolton, W.R., Chapin, F.S., Dyurgerov, M.B., Fastie, C.L., Griffith, B., Hollister, R.D., Hope, A., Huntington, H.P., Jensen, A.M., Jia, G.J., Jorgenson, T., Kane, D.L., Klein, D.R., Kofinas, G., Lynch, A.H., Lloyd, A.H., McGuire, A.D., Nelson, F.E., Oechel, W.C., Osterkamp, T.E., Racine, C.H., Romanovsky, V.E., Stone, R.S., Stow, D.A., Sturm, M., Tweedie, C.E., Vourlitis, G.L., Walker, M.D., Walker, D.A., Webber, P.J., Welker, J.M., Winker, K., Yoshikawa, K., 2005b. Evidence and implications of recent climate change in northern Alaska and other arctic regions. Clim. Change 72, 251-298.

Holberton, R.L., Wingfield, J.C., 2003. Modulating the corticosterone stress response: A mechanism for balancing individual risk and reproductive success in arctic-breeding sparrows? Auk 120, 1140-1150.

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

Houde, M., De Silva, A.O., Muir, D.C., Letcher, R.J., 2011. Monitoring of Perfluorinated Compounds in Aquatic Biota: An Updated Review: PFCs in Aquatic Biota. Environmental science & technology 45, 7962-7973.

Isaksen, K., Bakken, V., 1995. Breeding populations of seabirds in Svalbard. Seabird populations in the northern Barents Sea. Source data for the impact assessment of the effect of oil drilling activity (Eds. Isaaksen, K and V. Bakken). Norsk Polarinstitutte Meddelelser, 11-35.

Jaspers, V.L., Rodriguez, F.S., Boertmann, D., Sonne, C., Dietz, R., Rasmussen, L.M., Eens, M., Covaci, A., 2011. Body feathers as a potential new biomonitoring tool in raptors: A study on organohalogenated contaminants in different feather types and preen oil of

West Greenland white-tailed eagles (*Haliaeetus albicilla*). Environ. Int. 37, 1349-1356.

Jaspers, V.L., Voorspoels, S., Covaci, A., Eens, M., 2006. Can predatory bird feathers be used as a non-destructive biomonitoring tool of organic pollutants? Biology letters 2, 283-285.

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

Jones, P.D., Hu, W., De Coen, W., Newsted, J.L., Giesy, J.P., 2003. Binding of perfluorinated fatty acids to serum proteins. Environ. Toxicol. Chem. 22, 2639-2649.

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Gobas, F.A., 2008. Bioaccumulation behaviour of polybrominated diphenyl ethers (PBDEs) in a Canadian Arctic marine food web. Science of the total environment 401, 60-72.

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Morin, A.E., Gobas, F.A., 2007. Food web–specific biomagnification of persistent organic pollutants. science 317, 236-239.

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

Key, B.D., Howell, R.D., Criddle, C.S., 1997. Fluorinated organics in the biosphere. Environmental Science & Technology 31, 2445-2454.

Kitaysky, A., Wingfield, J., Piatt, J., 1999. Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. Functional Ecology 13, 577-584.

Lanctot, R.B., Hatch, S.A., Gill, V.A., Eens, M., 2003. Are corticosterone levels a good indicator of food availability and reproductive performance in a kittiwake colony? Horm. Behav. 43, 489-502.

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

Letcher, R.J., Norstrom, R.J., Lin, S., Ramsay, M.A., Bandiera, S.M., 1996. Immunoquantitation and Microsomal Monooxygenase Activities of Hepatic Cytochromes P4501A and P4502B and Chlorinated Hydrocarbon Contaminant Levels in Polar Bear (*Ursus maritimus*). Toxicol. Appl. Pharmacol. 137, 127-140.

Letcher, R.J., Norstrom, R.J., Muir, D.C., 1998. Biotransformation versus bioaccumulation: Sources of methyl sulfone PCB and 4, 4'-DDE metabolites in the polar bear food chain. Environmental science & technology 32, 1656-1661.

Lohmann, R., Breivik, K., Dachs, J., Muir, D., 2007. Global fate of POPs: Current and future research directions. Environ. Pollut. 150, 150-165.

Lorenzen, A., Moon, T.W., Kennedy, S.W., Fox, G.A., 1999. Relationships between environmental organochlorine contaminant residues, plasma corticosterone concentrations, and intermediary metabolic enzyme activities in Great Lakes herring gull embryos. Environ. Health Perspect. 107, 179-186.

Lormée, H., Jouventin, P., Trouve, C., Chastel, O., 2003. Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies Sula sula. Ibis 145, 212-219.

Love, O.P., Shutt, L.J., Silfies, J.S., Bortolotti, G.R., Smits, J.E.G., Bird, D.M., 2003. Effects of dietary PCB exposure on adrenocortical function in captive American kestrels (*Falco sparverius*). Ecotoxicology 12, 199-208.

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

Macdonald, R., Barrie, L., Bidleman, T., Diamond, M., Gregor, D., Semkin, R., Strachan, W., Li, Y., Wania, F., Alaee, M., 2000. Contaminants in the Canadian Arctic: 5 years of progress in understanding sources, occurrence and pathways. Science of the Total Environment 254, 93-234.

Mackay, D., Shiu, W.Y., Ma, K.-C., 1997. Illustrated handbook of physical-chemical properties of environmental fate for organic chemicals. CRC press.

Martin, J.W., Mabury, S.A., Solomon, K.R., Muir, D.C., 2003. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 22, 189-195.

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

Martin, L.B., 2009. Stress and immunity in wild vertebrates: timing is everything. General and comparative endocrinology 163, 70-76.

Mehlum, F., 1991. Breeding population size of the Common Eider *Somateria mollissima* in Kongsfjorden, Svalbard, 1981-1987. Skrifter-Norsk Polarinstitutt, 21-29.

Mehlum, F., Gabrielsen, G., 1995. Energy expenditure and food consumption by seabird populations in the Barents Sea region. Ecology of fjords and coastal waters, 457-470.

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

Moe, B., Stempniewicz, L., Jakubas, D., Angelier, F., Chastel, O., Dinessen, F., Gabrielsen, G.W., Hanssen, F., Karnovsky, N., Rønning, B., 2009. Climate change and phenological responses of two seabird species breeding in the high-Arctic.

Murvoll, K.M., Skaare, J.U., Moe, B., Anderssen, E., Jenssen, B.M., 2006. Spatial trends and associated biological responses of organochlorines and brominated flame retardants in hatchlings of North Atlantic kittiwakes (*Rissa tridactyla*). Environ. Toxicol. Chem. 25, 1648-1656.

Nordstad, T., Moe, B., Bustnes, J.O., Bech, C., Chastel, O., Goutte, A., Sagerup, K., Trouve, C., Herzke, D., Gabrielsen, G.W., 2012. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (Rissa tridactyla) across their breeding cycle. Environ. Pollut. 164, 219-226.

Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., Erwin, K.N., Levin, E.D., 2009. The toxicology of climate change: Environmental contaminants in a warming world. Environ. Int. 35, 971-986.

O'Reilly, K.M., Wingfield, J.C., 2001. Ecological factors underlying the adrenocortical response to capture stress in arctic-breeding shorebirds. General and comparative endocrinology 124, 1-11.

Odermatt, A., Gumy, C., Atanasov, A.G., Dzyakanchuk, A.A., 2006. Disruption of glucocorticoid action by environmental chemicals: potential mechanisms and relevance. The Journal of steroid biochemistry and molecular biology 102, 222-231.

Oehme, M., 1991. Dispersion and transport paths of toxic persistent organochlorines to the Arctic—levels and consequences. Science of the total environment 106, 43-53.

Olsen, K.M., Larsson, H., 2004. Gulls of North America, Europe, and Asia. Princeton University Press Princeton, New Jersey.

Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. 37, 637-669.

Peakall, D., Tremblay, J., Kinter, W., Miller, D., 1981. Endocrine dysfunction in seabirds caused by ingested oil. Environmental research 24, 6-14.

Polen, P.B., Hester, M., Benziger, J., 1971. Characterization of oxychlordane, animal metabolite of chlordane. Bulletin of environmental contamination and toxicology 5, 521-528.

Post, E., Forchhammer, M.C., Bret-Harte, M.S., Callaghan, T.V., Christensen, T.R., Elberling, B., Fox, A.D., Gilg, O., Hik, D.S., Høye, T.T., 2009. Ecological dynamics across the Arctic associated with recent climate change. Science 325, 1355-1358.

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

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

Rahman, F., Langford, K.H., Scrimshaw, M.D., Lester, J.N., 2001. Polybrominated diphenyl ether (PBDE) flame retardants. Science of the Total Environment 275, 1-17.

Rector, M., Kouwenberg, A.-L., Wilhelm, S., Robertson, G., McKay, D., Fitzsimmons, M., Baker, C., Cameron-MacMillan, M., Walsh, C., Storey, A., 2012. Corticosterone levels of Atlantic puffins vary with breeding stage and sex but are not elevated in poor foraging years. General and comparative endocrinology 178, 408-416.

Reneerkens, J., Piersma, T., Ramenofsky, M., 2002. An experimental test of the relationship between temporal variability of feeding opportunities and baseline levels of corticosterone in a shorebird. J. Exp. Zool. 293, 81-88.

Rigét, F., Bignert, A., Braune, B., Stow, J., Wilson, S., 2010. Temporal trends of legacy POPs in Arctic biota, an update. Science of the total environment 408, 2874-2884.

Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19, 249-255.

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. A-Mol. Integr. Physiol. 140, 73-79.

Romero, L.M., Reed, J.M., Wingfield, J.C., 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. General and Comparative Endocrinology 118, 113-122.

Romero, L.M., Remage-Healey, L., 2000. Daily and Seasonal Variation in Response to Stress in Captive Starlings (*Sturnus vulgaris*): Corticosterone. General and comparative endocrinology 119, 52-59.

Rozman, K.K., Klaassen, C.D., 2001. Absorption, distribution, and excretion of toxicants. Cassarett and Doull's Toxicology The Basic Science of Poisons.

Sagerup, K., Helgason, L.B., Polder, A., Strøm, H., Josefsen, T.D., Skåre, J.U., Gabrielsen, G.W., 2009. Persistent organic pollutants and mercury in dead and dying glaucous gulls (*Larus hyperboreus*) at Bjørnøya (Svalbard). Science of the total environment 407, 6009-6016.

Sagerup, K., Henriksen, E.O., Skaare, J.U., Gabrielsen, G.W., 2002. Intraspecific variation in trophic feeding levels and organochlorine concentrations in glaucous gulls (Larus hyperboreus) from Bjornoya, the Barents Sea. Ecotoxicology 11, 119-125.

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

Sakshaug, E., Johnsen, G.H., Kovacs, K.M., 2009. Ecosystem Barents Sea. Tapir Academic Press.

Sandberg, R., Moore, F.R., 1996. Fat stores and arrival on the breeding grounds: reproductive consequences for passerine migrants. Oikos, 577-581.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55-89.

Silverin, B., Arvidsson, B., Wingfield, J., 1997. The adrenocortical responses to stress in breeding willow warblers *Phylloscopus trochilus* in Sweden: effects of latitude and gender. Functional Ecology 11, 376-384.

Smetacek, V., Nicol, S., 2005. Polar ocean ecosystems in a changing world. Nature 437, 362-368.

Sonne, C., Mæhre, S.A., Sagerup, K., Harju, M., Heimstad, E.S., Leifsson, P.S., Dietz, R., Gabrielsen, G.W., 2013. A screening of liver, kidney, and thyroid gland morphology in organochlorine-contaminated glaucous gulls (*Larus hyperboreus*) from Svalbard. Toxicological & Environmental Chemistry 95, 172-186.

Sovocool, G.W., Lewis, R.G., Harless, R.L., Wilson, N.K., Zehr, R.D., 1977. Analysis of technical chlordane by gas chromatography/mass spectrometry. Analytical Chemistry 49, 734-740.

Stockholm Convention, 2011. Stockhom Convention on Persistent Organic Pollutants (POPs). [Online] http://chm.pops.int/TheConvention/Overview/tabid/3351/Default.aspx [Accessed 15.04.2014].

Suja, F., Pramanik, B.K., Zain, S.M., 2009. Contamination, bioaccumulation and toxic effects of perfluorinated chemicals (PFCs) in the water environment: a review paper. Water Science & Technology 60.

Tombre, I.M., Mehlum, F., Loonen, M., 1998. The Kongsfjorden colony of barnacle geese: Nest distribution and the use of breeding islands, 1980-1997. Skrifter-Norsk Polarinstitutt, 57-66.

Tyler, C.R., Jobling, S., Sumpter, J.P., 1998. Endocrine disruption in wildlife: A critical review of the evidence. Crit. Rev. Toxicol. 28, 319-361.

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

Ulfstrand, S., 1972. DDT and PCB relocate when caged robins use fat reserves. Ambio 1, 36-40.

Umetrics, 2008. User guide to Simca-P+. Version 12. [Online] http://www.umetrics.com [Accessed 06.03.2014].

Van de Vijver, K.I., Hoff, P.T., Das, K., Van Dongen, W., Esmans, E.L., Jauniaux, T., Bouquegneau, J.-M., Blust, R., De Coen, W., 2003. Perfluorinated chemicals infiltrate ocean waters: link between exposure levels and stable isotope ratios in marine mammals. Environmental science & technology 37, 5545-5550.

Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W., Evans, N.P., 2010. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. General and comparative endocrinology 166, 25-32.

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

Verreault, J., Berger, U., Gabrielsen, G.W., 2007b. Trends of perfluorinated alkyl substances in herring gull eggs from two coastal colonies in northern Norway: 1983-2003. Environmental science & technology 41, 6671-6677.

Verreault, J., Gabrielsen, G.W., Bustnes, J.O., 2010. The Svalbard Glaucous Gull as Bioindicator Species in the European Arctic: Insight from 35 Years of Contaminants Research, in: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology, Vol 205, Springer, New York, pp. 77-116.

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

Verreault, J., Gebbink, W.A., Gauthier, L.T., Gabrielsen, G.W., Letcher, R.J., 2007c. Brominated flame retardants in glaucous gulls from the Norwegian Arctic: more than just an issue of polybrominated diphenyl ethers. Environmental science & technology 41, 4925-4931.

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

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

Verreault, J., Letcher, R.J., Ropstad, E., Dahl, E., Gabrielsen, G.W., 2006a. Organohalogen contaminants and reproductive hormones in incubating glaucous gulls (Larus hyperboreus) from the Norwegian Arctic. Environ. Toxicol. Chem. 25, 2990-2996.

Verreault, J., Skaare, J.U., Jenssen, B.M., Gabrielsen, G.W., 2004. Effects of organochlorine contaminants on thyroid hormone levels in arctic breeding glaucous gulls, *Larus hyperboreus*. Environ. Health Perspect. 112, 532-537.

Verreault, J., Verboven, N., Gabrielsen, G.W., Letcher, R.J., Chastel, O., 2008. Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the glaucous gull. General and comparative endocrinology 156, 569-576.

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

Villanger, G.D., Gabrielsen, K.M., Kovacs, K.M., Lydersen, C., Lie, E., Karimi, M., Sørmo, E.G., Jenssen, B.M., 2013. Effects of complex organohalogen contaminant mixtures on thyroid homeostasis in hooded seal (*Cystophora cristata*) mother–pup pairs. Chemosphere 92, 828-842.

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

Vonderheide, A.P., Mueller, K.E., Meija, J., Welsh, G.L., 2008. Polybrominated diphenyl ethers: causes for concern and knowledge gaps regarding environmental distribution, fate and toxicity. Science of the Total Environment 400, 425-436.

Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10, 506-513.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J., Fromentin, J.-M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. Nature 416, 389-395.

Weiser, E., Gilchrist, H.G., 2012. Glaucous Gull (*Larus hyperboreus*), The birds of the North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology Retrieved from the Birds of North America Online.

Wingfield, J.C., Oreilly, K.M., Astheimer, L.B., 1995. MODULATION OF THE ADRENOCORTICAL RESPONSES TO ACUTE STRESS IN ARCTIC BIRDS - A POSSIBLE ECOLOGICAL BASIS. Am. Zool. 35, 285-294.

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

Wold, S., Ruhe, A., Wold, H., Dunn, I., WJ, 1984. The collinearity problem in linear regression. The partial least squares (PLS) approach to generalized inverses. SIAM Journal on Scientific and Statistical Computing 5, 735-743.

Wold, S., Sjöström, M., Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. Chemometrics and intelligent laboratory systems 58, 109-130.

Wolkers, H., Van Bavel, B., Derocher, A.E., Wiig, Ø., Kovacs, K.M., Lydersen, C., Lindström, G., 2004. Congener-specific accumulation and food chain transfer of polybrominated diphenyl ethers in two Arctic food chains. Environmental science & technology 38, 1667-1674.

Yordy, J.E., Wells, R.S., Balmer, B.C., Schwacke, L.H., Rowles, T.K., Kucklick, J.R., 2010. Partitioning of persistent organic pollutants between blubber and blood of wild bottlenose dolphins: implications for biomonitoring and health. Environmental science & technology 44, 4789-4795.

Zala, S.M., Penn, D.J., 2004. Abnormal behaviours induced by chemical pollution: a review of the evidence and new challenges. Anim. Behav. 68, 649-664.

Appendices

Appendix A

Levels of corticosterone and sampling time

The plasma levels of corticosterone at first and second blood sample of female and male glaucous gull plasmas are presented in Table 2 and 3 respectively. Plasma was sampled during the breeding season of 2013 in Kongsfjorden, Svalbard.

Table 2: Plasma levels of corticosterone (ng/ml) and time of sampling (mm:ss) of female glaucous gulls breeding in Kongsfjorden, Svalbard 2013.

Females (n = 11)	**Mean ± S.D**	**Median**	**Range**
Baseline corticosterone (ng/ml)	50,74 ± 24,73	49,14	23,72 – 112,38
Stress-induced corticosterone (ng/ml)	108,39 ± 56,50	80,17	51,76 – 224,91
Increase corticosterone (ng/ml)	57,64 ± 38,83	44,97	24,81 – 144,86
Time of first sample (min)	08:30 ± 02:15	08:01	05:13 – 12:28
Time of second sample (min)	28:15 ± 05:41	26:30	21:20 – 38:12
Time of handling (min)	19:45 ± 04:05	18:33	13:19 – 25:44

Table 3: Plasma levels of corticosterone (ng/ml) and time of sampling (mm:ss) of male glaucous gulls breeding in Kongsfjorden, Svalbard 2013.

Males (n = 6)	**Mean ± S.D**	**Median**	**Range**
Baseline corticosterone (ng/ml)	36,69 ± 14,38	30,62	25,38 – 61,73
Stress-induced corticosterone (ng/ml)	103,45 ± 58,54	95,06	46,70 – 199,86
Increase corticosterone (ng/ml)	66,76 ± 47,52	60,10	18,44 – 138,13
Time of first sample (min)	08:03 ± 02:16	08:03	05:48 – 11:50
Time of second sample (min)	28:55 ± 07:33	30,04	20:10 – 38:48
Time of handling (min)	21:16 ± 06:32	21:05	14:20 – 30:02

Table 4: Plasma concentrations of corticosterone (ng/ml) and sampling time (mm:ss) of glaucous gulls breeding in Kongsfjorden, Svalbard 2013.

Glaucous gull (n = 17)	**Mean ± S.D**	**Median**	**Range**
Baseline corticosterone (ng/ml)	45.78 ± 21.58	38.33	23.72 – 112.38
Stress-induced corticosterone (ng/ml)	106.65 ± 53.77	80.17	46.70 – 224.91
Increase corticosterone (ng/ml)	60.86 ± 39.62	44.97	18.44 – 144.86
Time of first sample (min)	08:20 ± 02:12	08:01	05:13 – 12:28
Time of second sample (min)	28:29 ± 06:10	26:45	20:10 – 38:48
Time of handling (min)	20:08 ± 03:59	18:44	14:57 – 26:20

Individual concentrations of corticosterone hormones and time of sampling

Table 5: Individual concentrations in ng/ml of corticosterone in baseline (B-CORT), stress-induced (S-CORT) and increase (I-CORT) samples of male and female glaucous gull plasma, and the time of the B-CORT sample (T1), the S-CORT sample (T2) and handling time/I-CORT sample (dT) in European time (mm:ss) and as a numeric variable (min). Plasma was sampled during the breeding season of 2013 in Kongsfjorden, Svalbard.

Number	ID	B-CORT (ng/ml)	S-CORT (ng/ml)	I-CORT (ng/ml)	T1 (min)	T2 (min)	dT (min)	T1 (mm:ss)	T2 (mm:ss)	dT (mm:ss)
Males										
G04	AJD	45.72	126.87	81.14	8.77	38.80	30.03	08:46	38:48	30:02
G07	ADN	28.26	46.70	18.44	5.80	20.83	15.03	05:48	20:50	15:02
G09	AJH	61.73	199.86	138.13	8.75	33.52	24.77	08:45	33:31	24:46
G11	AAJ	25.38	124.96	99.57	5.83	20.17	14.33	05:50	20:10	14:20
G13	AJN	26.10	65.17	39.07	7.37	33.38	26.02	07:22	33:23	26:01
G18	AJS	32.97	57.15	24.18	11.83	26.75	14.92	11:50	26:45	17:25
Females										
G01	AJB	63.11	140.21	77.10	12.47	38.20	25.73	12:28	38:12	25:44
G02	AJC	112.38	224.91	112.53	7.33	25.20	17.87	07:20	25:12	17:52
G03	ADD	23.72	74.36	50.63	8.02	21.33	13.32	08:01	21:20	13:19
G05	AAT	56.21	94.36	38.15	5.22	23.77	18.55	05:13	23:46	18:33
G06	AFB	25.99	51.76	25.77	5.50	26.50	21.00	05:30	26:30	21:00
G08	AJF	35.21	80.17	44.97	10.08	34.08	24.00	10:05	34:05	24:00
G10	AJL	51.87	196.72	144.86	9.33	31.73	22.40	09:20	31:44	22:24
G12	ADL	38.33	63.14	24.81	7.95	22.05	14.10	07:57	22:03	14:06
G14	ABA	65.09	114.38	49.29	9.53	27.43	17.90	09:32	27:26	17:54
G15	AAD	49.14	74.68	25.55	11.03	35.20	24.17	11:02	35:12	24:10
G16	AFC	37.10	77.57	40.47	6.97	25.17	18.20	06:58	25:10	18:12

Appendix B

Organohalogenated contaminants analyzed

Table 6 : Polybrominated diphenyl esters (PBDEs) analyzed in blood plasma from glaucous gulls breeding in Kongsfjorden, Svalbard 2013.

Organobrominated contaminants		
Group	**Acronym**	**Analyte**
PBDEs[1]	BDE-28	2',4,4'-Tribromodiphenyl ether
	BDE-47	2,2',4,4'-Tetrabromodiphenyl ether
	BDE-66	2',3',4,4'-Tetrabromodiphenyl ether
	BDE-85	2,2',3',4,4'-Pentabromodiphenyl ether
	BDE-99	2,2',4,4',5'-Pentabromodiphenyl ether
	BDE-100	2,2',4,4',6'-Pentabromodiphenyl ether
	BDE-138	2,2',3',4,4',5'-Hexabromodiphenyl ether
	BDE-153	2,2',4,4',5,5'-Hexabromobiphenyl ether
	BDE-154	2,2',4,4',5,6'-Hexabromobiphenyl ether
	BDE-183	2,2',3',4,4',5',6'-Heptabromodiphenyl ether

[1] PBDE congeners numbered by the IUPAC system (International Union of Pure and Applied Chemistry).

Table 7: Organochlorinated contaminants analyzed in blood plasma from glaucous gull breeding in Kongsfjorden, Svalbard 2013.

Organochlorinated contaminants		
Group	**Acronym**	**Analyte**
CHLs	*trans*-Chlordane	*trans*-chlordane
	cis-Chlordane	*cis*-chlordane
	oxy-chlordane	*oxy*-chlordane
	trans-nonachlor	*trans*-nonachlor
	cis-nonachlor	*cis*-nonachlor
	Mirex	1,1α,2,2,3,3α,4,5,5,5α,5β,6-dodecachlorooctahydro-1*H*-1,3,4-(methanetriyl)cyclobuta[*cd*]pentalene
HCB	HCB	Hexachlorobenzene
HCH	*a*-HCH	1α,2α,3β,4α,5β,6β-hexachlorocyclohexane
	b-HCH	1α,2β,3α,4β,5α,6β-hexachlorocyclohexane
	g-HCH	1α,2α,3β,4α,5α,6β-hexachlorocyclohexane
DDTs	*o,p,'*-DDT	*o,p,'*-dichloro-α,α-diphenyl-β,β,β-trichloroethane
	p,p,'-DDT	*p,p,'*-dichloro-α,α-diphenyl-β,β,β-trichloroethane
	o,p,'-DDE	*o,p,'*-dichloro-diphenyl-dichloroethylene
	p,p,'-DDE	*p,p,'*-dichloro-diphenyl-dichloroethylene
	o,p,'-DDD	*o,p,'*-dichloro-diphenyl-dichloroethane
	p,p,'-DDD	*p,p,'*-dichloro-diphenyl-dichloroethane
PCBs[2]	PCB-28	2,4,4'-trichlorobiphenyl
	PCB-52	2,2',5,5'-tetrachlorobiphenyl
	PCB-99	2,2',4,4',5-Pentachlorobiphenyl
	PCB-101	2,2',4,5,5'-pentachlorobiphenyl
	PCB-105	2,3,3',4,4'-pentachlorobiphenyl
	PCB-118	2,3',4,4',5-pentachlorobiphenyl
	PCB-138	2,2',3,4,4',5'-hexachlorobiphenyl
	PCB-153	2,2',4,4',5,5'-hexachlorobiphenyl
	PCB-180	2,2',3,4,4',5,5'-heptachlorobiphenyl
	PCB-183	2,2',3,4,4',5',6-Heptachlorobiphenyl
	PCB-187	2,2',3,4',5,5',6-heptachlorobiphenyl
	PCB-194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl

[2] PCB congeners numbered by the IUPAC system (International Union of Pure and Applied Chemistry).

Table 8: Perfluorinated compounds analyzed in plasma from glaucous gull breeding in Kongsfjorden, Svalbard 2013.

Perfluorinated compounds			
	Acronym	**Analyte**	**Chemical formula**
PFCA			$C_nF_{2n+1}COOH$
	PFHxA	Perfluorohexanoic acid	$CF_3(CF_2)_4COOH$
	PFHpA	Perfluoroheptanoic acid	$CF_3(CF_2)_5COOH$
	PFOA	Perfluorooctanoic acid	$CF_3(CF_2)_6COOH$
	PFNA	Perfluorononanoic acid	$CF_3(CF_2)_7COOH$
	PFDcA	Perfluorodecanoanoic acid	$CF_3(CF_2)_8COOH$
	PFUnA	Perfluoroundecanoic acid	$CF_3(CF_2)_9COOH$
	PFDoA	Perfluorododecanoic acid	$CF_3(CF_2)_{10}COOH$
	PFTrA	Perfluorotridecanoic acid	$CF_3(CF_2)_{11}COOH$
	PFTeA	Perfluorotetradecanoic acid	$CF_3(CF_2)_{12}COOH$
PFSA			$C_nF_{2n+1}SO_3H$
	PFBS	Perfluorobutane sulfonate	$CF_3(CF_2)_3SO_3H$
	PFHxS	Perfluorohexane sulfonate	$CF_3(CF_2)_5SO_3H$
	PFOSA	Perfluorooctane amid	$C_8H_2F_{17}NO_2S$
	PFOS	Perfluorooctane sulfonate	$CF_3(CF_2)_7SO_3H$
	Iso-PFOS	Isomer Perfluorooctane sulfonate	$CF_3(CF_2)_7SO_3H$
	PFDcS	Perfluorodecane sulfonate	$CF_3(CF_2)_9SO_3H$
Other PFCs	6:2 FTS	6:2 fluorotelomer sulfonate	$C_6F_{13}CH_2CH_2SO_3^-$

Appendix C

Biometric measuremets

Table 9: Biometric data from male and female glaucous gulls breeding in Kongsfjorden, Svalbard 2013

	Mean ± S.E	Median	Range
Males (n = 6)			
Weight (gr)	1738.33 ± 53.40	1747.50	1535 – 1900
Total head length (mm)	149.77 ± 0.74	150	147.6 – 152
Bill length (mm)	63.95 ± 0.71	64	61 – 66.2
Gonis length (mm)	23.08 ± 0.25	23.15	22.2 – 23.9
Wing length (cm)	476.50 ± 7.14	480	454 – 498
Body condition index (BCI)	0.00 ± 0.37	0.18	-1.62 – 0.76
Total Plasma lipid content (mg/ml)	14.17 ± 0.45	14.6	12.03 – 15.05
% Lipid weight	1.42 ± 0.045	1.46	1.20 – 1.50
Females (n = 11)			
Weight (gr)	1420.45 ± 15.25	1440	1320 – 1490
Total head length (mm)	136.65 ± 0.63	137	134 – 141
Bill length (mm)	57.27 ± 0.39	57.5	55 – 60
Gonis length (mm)	20.76 ± 0.20	20.7	19.5 – 22
Wing length (cm)	458.00 ± 2.64	458	441 – 472
Body condition index (BCI)	0.00 ± 0.29	0.37	-1.84 – 1.27
Total Plasma lipid content (mg/ml)	15.25 ± 0.69	15.75	12 – 19.8
% Lipid weight	1.53 ± 0.07	1.57	1.2 – 1.98

Individual biometric measurements

Table 10: Individual biometric measurements of male and female glaucous gull breeding in Kongsfjorden, Svalbard 2013, included in the present study.

Number	ID	Weight (gr)	Total head lenght (mm)	Bill lenght (mm)	Gonys depth (mm)	Wing lenght (cm)	BCI
Males							
G04	AJD	1685	148	66.2	22.7	473	0.446
G07	ADN	1680	152	63.5	22.2	454	0.328
G09	AJH	1810	151	63.9	23.4	498	-0.103
G11	AAJ	1820	151	65	22.9	487	0.876
G13	AJN	1900	149	61	23.9	488	0.153
G18	AJS	1535	147.6	64.1	23.4	459	-1.701
Females							
G01	AJB	1345	137.2	56.7	20.7	472	-1.433
G02	AJC	1490	139	58	20.6	467	1.273
G03	ADD	1450	137	56.3	20.8	450	0.573
G05	AAT	1455	134	57.9	21.3	458	0.648
G06	AFB	1440	136	55	19.5	455	0.428
G08	AJF	1440	136	57.6	20.7	461	0.365
G10	AJL	1390	137	57.5	21	457	-0.581
G12	ADL	1450	137	56.2	21.2	458	0.554
G14	ABA	1320	134	57	20.5	452	-1.851
G15	AAD	1410	141	60	22	467	-0.295
G16	AFC	1435	134.9	57.8	20.1	441	0.319

Appendix D

Individual concentrations of detected OHCs

Table 11: Individual and summed concentrations (ng/ml) of perfluorinated compounds, detected in > 60% of samples, in male and female glaucous gull plasma, included in statistical analyses. Samples <LOD (*) have random generated values from 0-LOD.Plasma was sampled during the breeding season of 2013 in Kongsfjorden, Svalbard.

Number	ID	Lipid weight (%)	PFHxS (ng/ml)	PFOS (ng/ml)	PFOA (ng/ml)	PFNA (ng/ml)	PFDcA (ng/ml)	PFUnA (ng/ml)	PFDoA (ng/ml)	PFTrA (ng/ml)	PFTeA (ng/ml)	PFOSA (ng/ml)	ΣPFSA[3] (ng/ml)	ΣPFCA[4] (ng/ml)	ΣPFCs[5] (ng/ml)
Males															
G04	AJD	1.46	0.028	12.294	0.032	1.177	0.963	3.784	0.994	2.808	0.390	0.388	12.710	10.146	22.856
G07	ADN	1.20	0.024*	7.467	0.143	2.231	0.574	2.954	0.650	2.520	0.367	0.664	8.156	9.438	17.594
G09	AJH	1.48	0.500	5.977	0.116	2.147	0.822	2.974	0.817	3.147	0.518	0.778	7.255	10.541	17.796
G11	AAJ	1.40	1.604	13.418	0.128	1.414	0.562	3.162	1.073	3.702	0.522	0.403	15.426	10.563	25.989
G13	AJN	1.50	0.573	10.846	0.120	1.347	0.445	2.472	0.722	2.918	0.476	0.887	12.305	8.500	20.805
G18	AJS	1.46	0.223	6.205	0.060	1.524	0.890	3.813	0.994	3.679	0.417	0.287*	6.715	11.376	18.091
Females															
G01	AJB	1.31	0.696	19.024	0.326	1.361	0.526	2.716	0.746	2.036	0.206	0.530	20.250	7.918	28.168
G02	AJC	1.20	0.370	9.159	0.107	1.331	0.594	2.593	0.437	1.880	0.191	1.090	10.619	7.133	17.752
G03	ADD	1.67	2.352	28.640	0.215	3.593	0.921	3.582	1.087	2.458	0.004*	1.438	32.429	11.861	44.290
G05	AAT	1.57	0.148	4.897	0.420	2.158	0.513	1.937	0.258	1.397	0.160	0.797	5.842	6.842	12.684
G06	AFB	1.98	0.025*	6.702	0.338	2.053	0.621	3.185	0.721	2.299	0.387	0.797	7.523	9.604	17.128
G08	AJF	1.58	0.372	7.702	0.007*	1.947	0.721	2.848	0.601	2.077	0.316	0.619	8.693	8.516	17.210
G10	AJL	1.26	0.603	4.932	0.007*	0.295	0.306	1.281	0.206	1.117	0.069	0.478	6.014	3.280	9.294
G12	ADL	1.66	4.364	53.948	0.351	2.506	0.732	2.520	0.524	2.159	0.221	0.833	59.145	9.013	68.158
G14	ABA	1.48	9.359	183.211	0.302	2.250	0.733	2.528	0.650	1.751	0.063*	0.800*	192.650	8.277	200.927
G15	AAD	1.39	3.947	77.965	0.004*	1.066	0.681	2.883	0.611	1.936	0.239	0.724	82.637	7.420	90.056
G16	AFC	1.68	0.253	2.889	0.003*	1.231	0.475	1.525	0.253	1.198	0.059*	0.083*	3.225	4.745	7.970

[3] ΣPFSA consist of PFHxS, PFOS and PFOSA, and includes the random generated values between 0-LOD for concentrations <LOD.
[4] ΣPFCA consist of PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTrA and PFTeA, and includes the random generated values between 0-LOD for concentrations <LOD.
[5] ΣPFCs consist of PFHxS, PFOS, PFOA, PFNA, PFDcA, PFUnA, PFDoA, PFTrA, PFTeA and PFOSA, and includes the random generated values between 0-LOD for concentrations <LOD.

Table 12: Individual and summed concentrations (ng/ml) of chlordanes and chlorinated pesticides, detected in > 60% of samples, in male and female glaucous gull plasma, as well as lipid weight (%), included in statistical analyses. Samples <LOD (*) have random generated values from 0-LOD. Plasma was sampled during the breeding season of 2013 in Kongsfjorden, Svalbard.

Number	ID	Lipid weight (%)	*cis*-chlordane (ng/ml)	*oxy*-chlordane (ng/ml)	*trans*-nonachlor (ng/ml)	*cis*-nonachlor (ng/ml)	Mirex (ng/ml)	ΣCHL[6] (ng/ml)	*p,p'*-DDE (ng/ml)	β-HCH (ng/ml)	HCB (ng/ml)	ΣOCPs[7] (ng/ml)
Males												
G04	AJD	1.46	0.015	10.00	1.83	1.15	3.63	16.62	4.45	0.45	6.60	11.50
G07	ADN	1.20	0.011	4.74	1.38	0.71	3.59	10.43	2.16	0.28	5.62	8.06
G09	AJH	1.48	0.020	26.50	2.67	0.87	10.40	40.46	9.37	0.89	13.4	23.66
G11	AAJ	1.40	0.012	8.54	0.09	0.14	4.63	13.40	2.20	0.28	6.07	8.55
G13	AJN	1.50	0.021	6.38	0.76	0.48	2.79	10.43	2.62	0.35	8.69	11.66
G18	AJS	1.46	0.016	12.90	1.14	0.86	5.41	20.32	3.56	0.42	8.84	12.82
Females												
G01	AJB	1.31	0.009	3.59	0.67	1.04	1.49	6.80	1.21	0.18	5.47	6.86
G02	AJC	1.20	0.020	4.80	1.08	1.14	2.04	9.08	1.85	0.24	5.23	7.32
G03	ADD	1.67	0.010	3.92	0.43	0.31	1.31	5.99	1.24	0.16	4.08	5.48
G05	AAT	1.57	0.030	17.00	1.79	2.00	10.20	31.02	6.42	0.51	9.51	16.44
G06	AFB	1.98	0.037	9.21	1.83	1.74	3.31	16.13	4.51	0.69	8.62	13.82
G08	AJF	1.58	0.017	13.10	1.07	0.87	9.32	24.37	4.19	0.26	7.46	11.91
G10	AJL	1.26	0.027	17.20	0.87	0.61	4.18	22.89	4.31	0.34	7.88	12.53
G12	ADL	1.66	0.003*	3.00	0.26	0.40	1.43	5.09	1.21	0.12	4.56	5.89
G14	ABA	1.48	0.003*	4.75	0.41	0.98	1.69	7.84	1.79	0.24	6.93	8.96
G15	AAD	1.39	0.029	5.66	0.59	0.45	2.31	9.04	1.74	0.23	5.70	7.67
G16	AFC	1.68	0.060	13.60	2.53	1.46	3.78	21.43	4.54	0.52	9.35	14.41

[6] ΣCHL consist of *cis*-Chlordane, *oxy*-Chlordane, *trans*-Nonachlor, *cis*-Nonachlor and Mirex, and includes the random generated values between 0-LOD for concentrations <LOD.
[7] ΣOCPs consist of HCB, β-HCH and *p,p'*-DDE.

Table 13: Individual and summed concentrations (ng/ml) of PCB congeners, detected in > 60 % of samples, in male and female glaucous gull plasma. Samples <LOD (*) have random generated values from 0-LOD. Plasma was sampled during the breeding season of 2013 in Kongsfjorden, Svalbard.

Number	ID	Lipid weight (%)	PCB-28 (ng/ml)	PCB-52 (ng/ml)	PCB-99 (ng/ml)	PCB-105 (ng/ml)	PCB-118 (ng/ml)	PCB-138 (ng/ml)	PCB-153 (ng/ml)	PCB-180 (ng/ml)	PCB-183 (ng/ml)	PCB-187 (ng/ml)	PCB-194 (ng/ml)	ΣPCB[8] (ng/ml)
Males														
G04	AJD	1.46	0.26	0.04	6.55	0.31	11.00	22.80	36.70	12.90	2.16	4.17	1.28	98.18
G07	ADN	1.20	0.14	0.05	2.93	1.41	5.79	10.50	26.80	14.00	2.05	2.84	2.00	68.53
G09	AJH	1.48	0.42	0.04	13.60	5.43	23.10	43.30	98.40	43.00	6.08	8.58	6.46	248.42
G11	AAJ	1.40	0.15	0.002*	3.43	1.62	6.66	16.50	28.60	16.10	2.42	2.50	2.63	80.63
G13	AJN	1.50	0.20	0.04	3.93	2.10	7.97	13.80	22.00	9.81	1.62	2.89	1.17	65.53
G18	AJS	1.46	0.25	0.05	5.15	2.60	10.60	23.90	41.70	26.20	3.49	3.92	4.49	122.36
Females														
G01	AJB	1.31	0.14	0.30	2.08	1.05	3.49	8.43	13.70	6.89	1.05	1.47	1.08	40.19
G02	AJC	1.20	0.14	0.38	2.48	1.26	4.51	8.59	20.10	9.13	1.44	2.03	1.14	51.89
G03	ADD	1.67	0.08	0.003*	1.85	0.87	3.23	5.43	13.50	4.22	0.77	1.31	0.51	31.78
G05	AAT	1.57	0.30	0.75	7.65	4.03	17.20	31.00	87.00	52.80	6.59	4.91	7.32	220.87
G06	AFB	1.98	0.22	0.74	5.19	2.37	8.69	20.80	31.90	11.60	2.11	4.65	1.42	91.80
G08	AJF	1.58	0.15	0.25	0.51	2.65	12.10	24.90	72.50	48.70	5.54	2.13	7.57	177.46
G10	AJL	1.26	0.17	0.03	6.44	2.31	8.78	23.00	50.10	22.00	3.07	3.45	2.80	122.15
G12	ADL	1.66	0.09	0.09	1.69	0.88	2.92	7.15	8.43	4.17	0.71	1.23	0.71	28.14
G14	ABA	1.48	0.14	0.007*	2.97	1.43	4.79	11.00	12.70	5.43	1.02	2.18	0.65	42.32
G15	AAD	1.39	0.12	0.10	2.41	1.18	4.39	9.73	18.00	8.62	1.29	1.55	1.16	48.56
G16	AFC	1.68	0.24	0.71	6.05	2.10	9.89	19.20	28.10	11.80	2.32	5.25	1.21	88.50

[8] ΣPCBs consist of PCB congeners -28, -52, -99, -105, -118, -138, -153, -180, -183, -187 and -194, and includes the random generated values between 0 - LOD for concentrations <LOD.

Table 14: Indvidual and summed concentrations (ng/ml) of polybrominated biphenyls, detected in > 60 % of samples, in male and female glaucous gull plasma. Plasma was sampled during the breeding season of 2013 in Kongsfjorden, Svalbard.

Number	ID	Lipid weight (%)	BDE-47 (ng/ml)	BDE-99 (ng/ml)	BDE-100 (ng/ml)	BDE-153 (ng/ml)	BDE-154 (ng/ml)	ΣPBDE[9] (ng/ml)
Males								
M04	AJD	1.46	3.13	0.24	0.50	0.25	0.21	4.33
M07	ADN	1.20	1.26	0.0004	0.27	0.27	0.09	1.89
M09	AJH	1.48	3.77	0.42	0.68	0.57	0.24	5.68
M11	AAJ	1.40	1.43	0.07	0.27	0.39	0.08	2.24
M13	AJN	1.50	1.45	0.31	0.27	0.37	0.10	2.50
M18	AJS	1.46	1.97	0.20	0.54	0.32	0.18	3.21
Females								
M01	AJB	1.31	2.61	0.85	0.51	2.14	0.17	6.27
M02	AJC	1.20	1.93	0.39	0.51	0.68	0.12	3.64
M03	ADD	1.67	1.53	0.48	0.23	1.24	0.09	3.57
M05	AAT	1.57	2.48	0.10	0.51	0.33	0.17	3.58
M06	AFB	1.98	3.04	0.38	0.55	0.26	0.40	4.63
M08	AJF	1.58	1.11	0.002	0.18	0.49	0.02	1.81
M10	AJL	1.26	2.13	0.32	0.42	0.39	0.128	3.39
M12	ADL	1.66	1.42	0.33	0.32	1.11	0.05	3.23
M14	ABA	1.48	1.92	0.36	0.31	1.00	0.14	3.73
M15	AAD	1.39	1.69	0.43	0.26	1.12	0.11	3.61
M16	AFC	1.68	6.24	0.52	0.86	0.37	0.41	8.40

[9] ΣPBDE consist of BDE congeners -47, -99, -100, -153 and -154, and includes the random generated number between 0 – LOD for concentrations <LOD.

Appendix E

Limit of detection and limit of qualification of analyzed organochlorinated and -brominated contaminants

Table 15: Limit of detection (LOD) and limit of qualification (LOQ) for the organochlorinated and –brominated contaminants analyzed in glaucous gull plasma. LOD was calculated as 3x signal/noise, except for BDE-28, -47, -99 and -154 where LOD was set to 2x signal/noise. LOD for BDE -153 was set to 2xLOD blank and for BDE-100 an approximate value was estimated. LOQ was calculated as 3xLOD (except for BDE-28,-47, -99, -100, -138, -153 and -154 were LOQ was calculated as 2xLOD). Contamination in the blank samples was detected, and the LOD and LOQ values for these individual compounds detected are listed in the table. When the value of LOD blank exceeded LOD, LOD blank was used as limit of detection for the individual compounds. The LOD blank marked with bold letters were used as LOD for the selected compound. Note that the OHC values in the present study are represented in ng/ml, while LOD and LOQ values are presented in pg/ml.

Compound	**LOD** (pg/ml)	**LOQ** (pg/ml)	**LOD blank** (pg/ml)	**LOQ blank** (pg/ml)
BDE-28	50.00	100	-	-
BDE-47	40.00	80	-	-
BDE-66	465.00	1395	-	-
BDE-85	199.00	597	-	-
BDE-99	25.00	50	-	-
BDE-100	50.00	100	20.00	-
BDE-138	417.00	1251	-	-
BDE-153	150.00	300	74.00	-
BDE-154	125.00	250	-	-
BDE-183	250.00	750	-	-
PCB-28	2.62	7.87	**15.10**	**30.20**
PCB-52	8.33	25.00	-	-
PCB-99	18.8	56.4	-	-
PCB-101	21.4	64.3	-	-
PCB-105	20.5	61.6	-	-
PCB-118	17.7	53.2	-	-
PCB-138	595	1785	-	-
PCB-153	455	1364	-	-
PCB-180	19.4	58.3	-	-
PCB-183	14.7	44.0	-	-
PCB-187	17.5	52.4	-	-
PCB-194	183	550	-	-
a-HCH	10.90	32.70	-	-
b-HCH	21.70	65.03	-	-
g-HCH	5.17	15.52	**5.95**	11.90
HCB	0.37	0.41	**236.00**	**472.00**
trans-chlordane	2.00	6.01	**2.15**	4.30
cis-chlordane	2.75	8.24	**6.90**	**13.80**
oxy-chlordane	5.83	17.50	**125.00**	**250.00**
trans-nonachlor	1.75	5.24	1.50	3.00
cis-nonachlor	0.94	2.81	**5.55**	**11.10**
Mirex	4.76	14.28	**17.70**	**35.40**
o',p'-DDT	7.32	22.00	-	-
p',p'-DDT	10.70	32.20	-	-
o',p'-DDD	1.30	3.89	-	-
p',p'-DDD	1.30	3.90	-	-
o',p'-DDE	1.08	3.23	-	-
p',p'-DDE	1.56	4.67	**2.00**	4.00

Limit of detection and limit of qualification of analyzed PFCs

Table 16: Limit of detection (LOD) and limit of qualification (LOQ) for the PFC analyzed in glaucous gull plasma. LOD was calculated as 3x signal/noise. LOQ was calculated as 3xLOD. If there was contamination in the blank samples, the LOD and LOQ values are listed in the table for the individual compounds. Note that the OHC values in the present study are represented in ng/ml, while LOD and LOQ values are presented in pg/ml.

Compound	**LOD** (pg/ml)	**LOQ** (pg/ml)	**LOD blank** (pg/ml)	**LOQ blank** (pg/ml)
PFBS	50.00	150	-	-
PFHxS	30.00	90	-	-
Iso-PFOS	30.00	90	-	-
PFOS	30.00	90	-	-
PFDS	85.00	255	-	-
PFHxA	20.00	60	-	-
PFHpA	10.00	30	-	-
PFOA	10.00	30	-	-
PFNA	50.00	150	-	-
PFDcA	50.00	150	-	-
PFUnA	80.00	240	-	-
PFDoA	40.00	120	-	-
PFTrA	70.00	210	-	-
PFTeA	90.00	270	-	-
PFOSA	290.00	870	-	-

Appendix F

PCA loadings for both female and male glaucous gulls

Table 17: Loadings for the individual variables onto principal component one, two, three and four (PC1, PC2, PC3 and PC4) in the PCA analysis of both male and female glaucous gulls breeding in Kongsfjorden, Svalbard 2013. All principal components are significant.

Var ID (primary)	PC1 (38.3 %)	PC2 (13.1 %)	PC3 (0.098%)	PC4 (0.093 %)
B-CORT	0.0138811	-0.232407	-0.202573	-0.025088
S-CORT	0.0625627	-0.163097	-0.304813	-0.0881021
I-CORT	0.0773402	-0.0947608	-0.303321	-0.105895
T1	-0.054726	-0.0487324	-0.273976	-0.261788
T2	0.0453008	-0.082938	-0.222205	-0.224587
%LW	0.0368247	0.0489247	0.366893	-0.00584962
PFHxS	-0.135092	-0.0391915	-0.180036	-0.0508796
PFOS	-0.189015	0.0112515	-0.0382161	-0.134402
PFOA	-0.0474415	-0.0290594	0.229443	0.0486998
PFNA	-0.0607689	0.182958	0.253117	0.00595263
PFDcA	-0.0270553	0.221245	0.0864978	-0.296359
PFUnA	-0.0490341	0.290845	0.0902997	-0.277968
PFDoA	-0.0594635	0.28401	0.0471286	-0.322552
PFTrA	0.00637031	0.326118	0.0013519	-0.243031
PFTeA	0.0858268	0.268417	-0.0250694	-0.121296
PFOSA	-0.0562757	0.0944777	0.0738286	0.103232
c-chl	0.152031	-0.108954	0.00480396	0.0503606
oxy-chl	0.232824	0.0209485	-0.109165	-0.028273
t-nonachl	0.214009	-0.0812655	0.154609	-0.0599164
c-nonachl	0.141971	-0.175367	0.211893	0.0687664
Mirex	0.207673	0.149559	-0.100126	0.1064
p,p'-DDE	0.241679	0.0274616	-0.00275105	-0.0440341
b-HCH	0.226091	0.00523585	0.126844	-0.130596
HCB	0.229856	-0.000450453	0.000220838	-0.0824141
PCB-28	0.232575	0.0186312	0.0367328	-0.151289
PCB-52	0.106111	-0.169617	0.0983704	0.181732
PCB-99	0.168969	-0.0932596	0.0852811	-0.174169
PCB-105	0.169536	0.0253647	-0.05074	0.146776
PCB-118	0.236448	0.0839275	-0.0379779	-0.0380971
PCB-138	0.237866	0.080259	-0.0665632	-0.0510071

Table 18: Continuation of Table 17; Loadings for the individual variables onto principal component one, two, three and four (PC1, PC2, PC3 and PC4) in the PCA analysis of both male and female glaucous gulls breeding in Kongsfjorden, Svalbard 2013. All principal components are significant.

Var ID (Primary)	PC1 (38.3 %)	PC2 (13.1 %)	PC3 (0.098%)	PC4 (0.093 %)
PCB-153	0.228737	0.10464	-0.10993	0.0806438
PCB-180	0.212562	0.125046	-0.150015	0.128005
PCB-183	0.211838	0.119006	-0.105147	0.120172
PCB-187	0.23419	-0.00601216	0.0762852	-0.124927
PCB-194	0.194889	0.15416	-0.171497	0.129858
BDE-47	0.140797	-0.242102	0.17546	-0.142429
BDE-99	-0.0549975	-0.293245	0.0365486	-0.26535
BDE-100	0.16191	-0.235492	0.135447	-0.154385
BDE-153	-0.152828	-0.139435	-0.0816551	-0.155117
BDE-154	0.119957	-0.215493	0.221803	-0.229986
BCI	0.0207904	0.0774517	0.14021	0.250733

Appendix G

PCA loadings for female glaucous gulls

Table 19: Loadings for the individual variables onto principal component one, two and three (PC1, PC2 and PC3) in the PCA analysis of female glaucous gulls (breeding in Kongsfjorden, Svalbard 2013), resulting in three significant principal components.

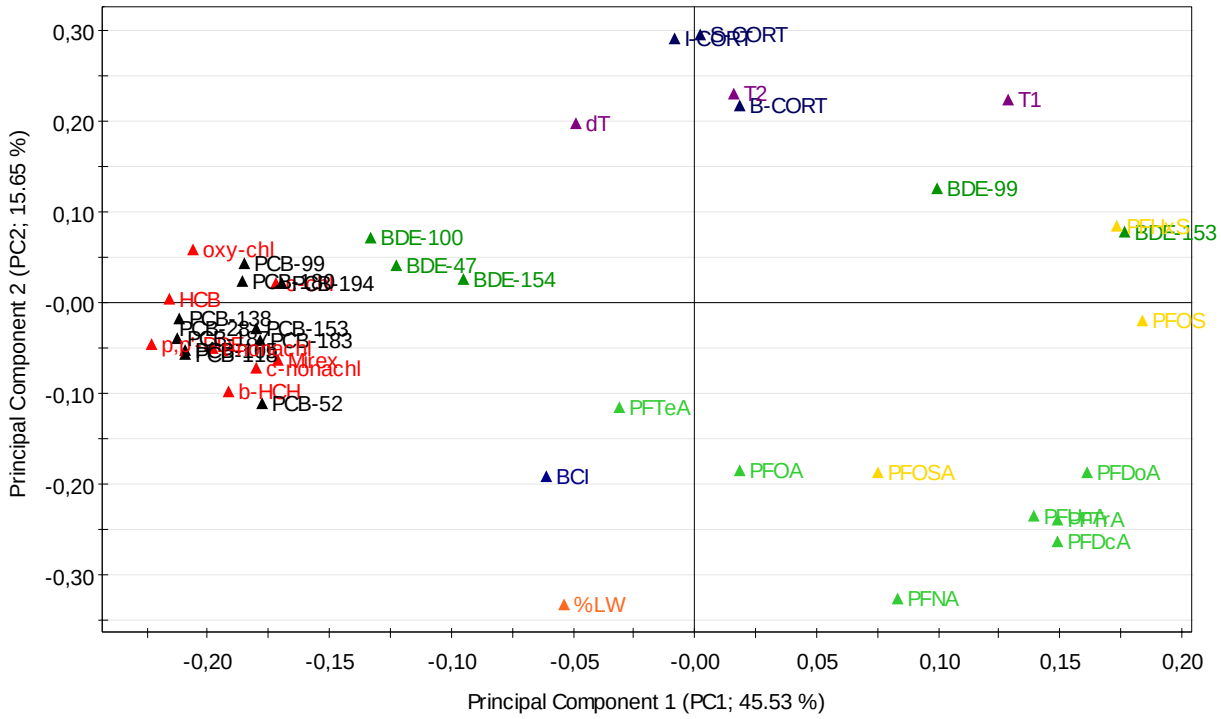
Var ID (Primary)	PC1 (45.5 %)	PC2 (15.6 %)	PC3 (13.9 %)
B-CORT	0.0183701	0.215718	-0.00411563
S-CORT	0.00228524	0.293755	0.0230197
I-CORT	-0.0083758	0.290045	0.036118
T1	0.129074	0.223427	0.118891
T2	0.0158214	0.230333	0.136691
dT	-0.0490966	0.197772	0.124919
%LW	-0.0536316	-0.331043	-0.101844
PFHxS	0.173272	0.0839414	0.0535891
PFOS	0.183718	-0.0205187	0.0223792
PFOA	0.0183066	-0.183822	-0.0677587
PFNA	0.0829032	-0.325635	0.0135472
PFDcA	0.148941	-0.261735	0.0623725
PFUnA	0.138889	-0.235085	0.045434
PFDoA	0.160765	-0.18676	-0.00306896
PFTrA	0.148579	-0.23971	0.0446219
PFTeA	-0.0310604	-0.114806	0.112914
PFOSA	0.0754165	-0.186163	0.0840512
c-chl	-0.171869	0.0223179	-0.174948
oxy-chl	-0.206364	0.0591039	0.104043
t-nonachl	-0.198232	-0.0504091	-0.152173
c-nonachl	-0.180452	-0.0716698	-0.106334
Mirex	-0.171224	-0.0631736	0.249073
p,p'-DDE	-0.222946	-0.0457872	0.0585254
b-HCH	-0.191577	-0.0979783	-0.136667
HCB	-0.215834	0.00479209	-0.0318149
PCB-28	-0.215339	-0.028159	-0.0685217
PCB-52	-0.177993	-0.111516	-0.143475
PCB-99	-0.184644	0.0432083	-0.136594
PCB-105	-0.209316	-0.0530355	0.128506
PCB-118	-0.209516	-0.0572328	0.132941
PCB-138	-0.211562	-0.0184523	0.13691
PCB-153	-0.179676	-0.0293458	0.243781

Table 20: Continuation of Table 19; Loadings for the individual variables onto principal component one, two and three (PC1, PC2 and PC3) in the PCA analysis of female glaucous gulls (breeding in Kongsfjorden, Svalbard 2013), resulting in three significant principal components.

Var ID (Primary)	PC1 (45.5 %)	PC2 (15.6 %)	PC3 (13.9 %)
PCB-180	-0.185946	0.0240402	0.232892
PCB-183	-0.178712	-0.0414087	0.239303
PCB-187	-0.212122	-0.0383555	-0.137541
PCB-194	-0.169879	0.0205335	0.266958
BDE-47	-0.122883	0.0413425	-0.304648
BDE-99	0.0995177	0.125275	-0.283225
BDE-100	-0.133446	0.0714604	-0.304805
BDE-153	0.176863	0.0785719	-0.0345423
BDE-154	-0.0946012	0.0248847	-0.341532
BCI	-0.0613118	-0.192032	0.0251064

PCA plot for female glaucous gulls

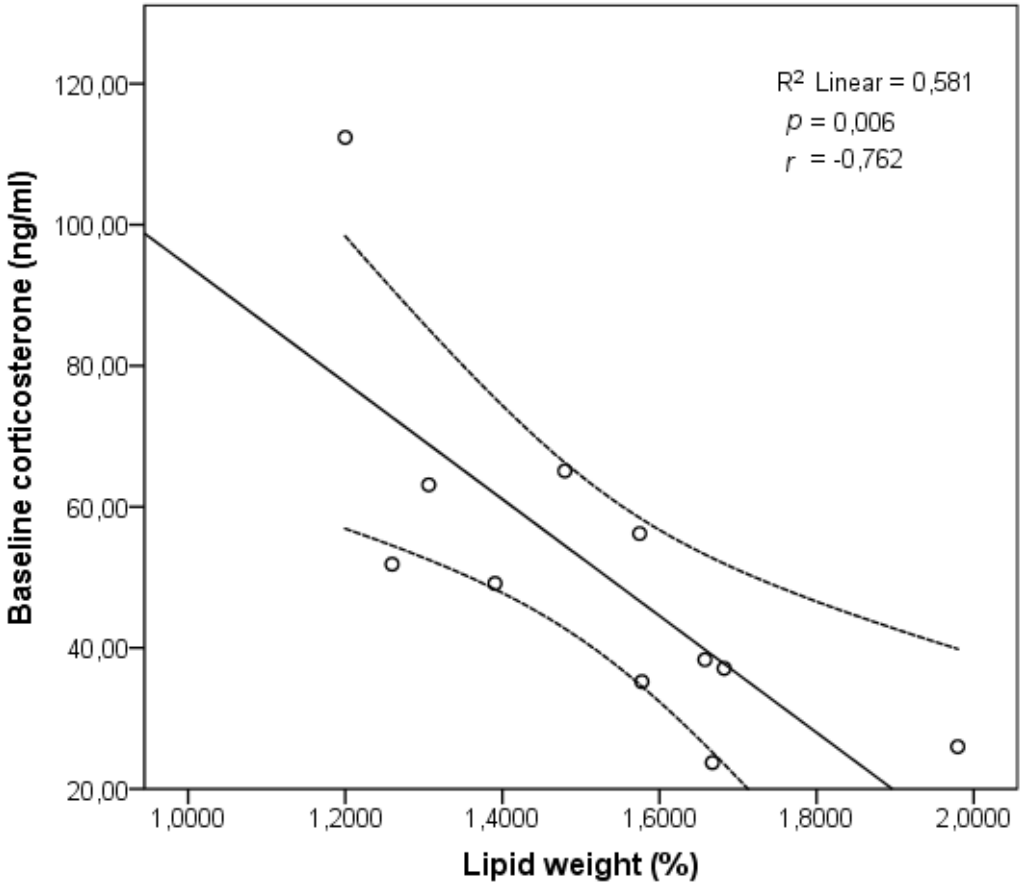
Figure 11: Biplot of loadings in PC1/PC2 dimension based on lipid weight (%LW), OHC and corticosterone hormone concentration (B-CORT, S-CORT and I-CORT) in plasma, sampling times (T1, T2 and dT) and calculated body condition index (BCI) of female glaucous gulls breeding in Kongsfjorden, Svalbard 2013.



Appendix H

Relationship between B-CORT and lipid weight (%) in female glaucous gulls

Figure 12: The linear relationship (± 95% confidence interval) between baseline corticosterone (ng/ml) and lipid weight (%) in plasma from female glaucous gulls breeding in Kongsfjorden, Svalbard 2013. The negative relationship was confirmed with Pearson's correlation coefficient, *r*, and significance, *p* (< 0.05). R^2-values for the linear regression are shown in the plot



Appendix I

PCA loadings for male glaucous gulls

Table 21: Loadings of the individual variables onto principal component one and two (PC1 and PC2) in the PCA analysis of male glaucous gull plasmas, resulting in one significant principal component (PC1).

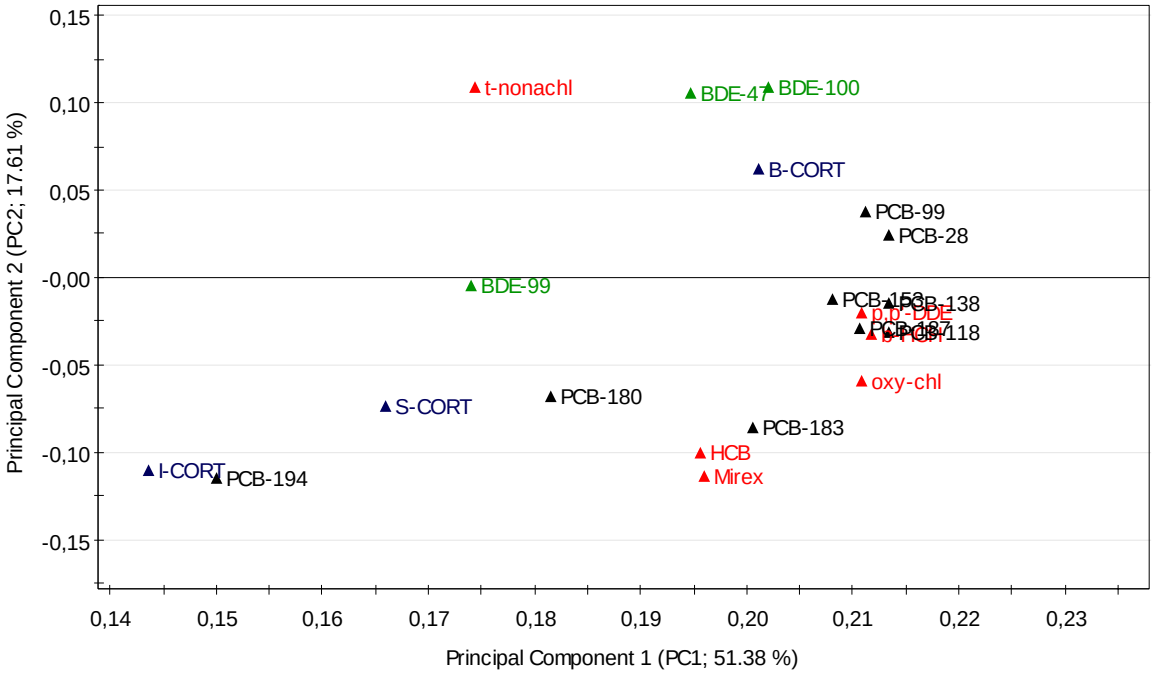
Var ID (Primary)	PC1 (51.4 %)	PC2 (17.6 %)
B-CORT	0.201175	0.0622398
S-CORT	0.166029	-0.0740653
I-CORT	0.143664	-0.110065
T1	0.114758	0.188896
T2	0.113162	0.182419
dT	0.0861857	0.137648
%LW	0.111245	0.0254065
PFHxS	0.0342489	-0.27796
PFOS	-0.122838	0.0162874
PFOA	-0.0530539	-0.323287
PFNA	0.0639815	-0.142291
PFDcA	0.130013	0.263266
PFUnA	0.0327121	0.275617
PFDoA	0.0181617	0.0882699
PFTrA	0.0332519	-0.0985464
PFTeA	0.0791611	-0.280419
PFOSA	0.0307294	-0.191798
c-chl	0.112937	-0.12563
oxy-chl	0.210865	-0.0591911
t-nonachl	0.174403	0.108587
c-nonachl	0.107587	0.296258
Mirex	0.196034	-0.114124
p,p'-DDE	0.210932	-0.0197058
b-HCH	0.21177	-0.0318955
HCB	0.195557	-0.100579
PCB-28	0.213373	0.0248184
PCB-52	0.0551768	0.190358
PCB-99	0.211294	0.0377717
PCB-105	0.0972888	-0.267171
PCB-118	0.213348	-0.0309182
PCB-138	0.213324	-0.014324
PCB-153	0.208023	-0.0123016
PCB-180	0.181564	-0.0676657
PCB-183	0.200622	-0.0863575
PCB-187	0.210689	-0.0287013
PCB-194	0.149996	-0.11496

Table 22: Continuation of Table 21; Loadings of the individual variables onto principal component one and two (PC1 and PC2) in the PCA analysis of male glaucous gull plasmas, resulting in one significant principal component (PC1).

Var ID (Primary)	**PC1 (51.4 %)**	**PC2 (17.6 %)**
BDE-47	0.194652	0.105391
BDE-99	0.174087	-0.00465483
BDE-100	0.20212	0.109003
BDE-153	0.158525	-0.245778
BDE-154	0.184832	0.186835
BCI	-0.0714771	-0.103556

PCA plot for male glaucous gulls

Figure 13: Selected variables from the biplot of loadings in PC1/PC2 dimension based on lipid weight (%LW), OHC and corticosterone hormone concentration (B-CORT, S-CORT and I-CORT) in plasma, sampling times (T1, T2 and dT) and calculated body condition index (BCI) for male glaucous gulls captured in Kongsfjorden, Svalbard 2013. The whole biplot of loadings in PC1/PC2 is shown in Figure 7.



Appendix J

PLS loadings for male glaucous gulls

Table 23: Loadings of the individual OHCs in the PLS model component explaining 79.6 % of the variation in B-CORT in male glaucous gull breeding in Kongsfjorden, Svalbard 2013.

Var ID (Primary)	PLS Component 1 (79.6 %)
PFDcA	0.159863
oxy-chl	0.243274
t-nonachl	0.213247
c-nonachl	0.138713
Mirex	0.225602
p,p'-DDE	0.247222
b-HCH	0.247155
HCB	0.221591
PCB-28	0.248938
PCB-99	0.247541
PCB-118	0.248324
PCB-138	0.24757
PCB-180	0.208603
PCB-183	0.231165
PCB-187	0.246806
BDE-47	0.232523
BDE-99	0.198477
BDE-100	0.237884
BDE-153	0.175089
BDE-154	0.220633