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Master's thesis in Biology

Supervisor: Åse Krøkje and Geir Wing Gabrielsen

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Preface

This is a sub-project of a research project funded by the Research Council of Norway (Norges Forskningsråd), project number 246112/E10: “Genotoxic Effects of Persistent Organic Pollutants and Metabolites in Arctic Sea Birds and eggs”.

This master thesis was written at the Department of Biology, at the Norwegian University of Science and Technology (NTNU), in collaboration with the Norwegian Polar Institute (NPI).

The project was initially started by Ola Tilset who collected data in the field and assisted with chemical analyses. In addition, Tilset conducted the initial stages of the DNA double-strand analyses and I carried out the analyses for glaucous gull and common eider. The lab work and all analyses for black-legged kittiwake were conducted by me.

Acknowledgements

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A special thanks to my friends and classmates for making my years in Trondheim fun and memorable. Thanks for long coffee breaks and for everything social. These past years would not have been the same without you!

Lastly, I want to thank my family for always believing in me, with this thesis and generally just in life.

Trondheim, July 2020
Marit Vågenes Michelsen

Abstract

Organohalogenated contaminants (OHCs) have been associated with adverse effects on DNA integrity, which may result in severe biological consequences on both an individual and population level. This study aimed to investigate the potential genotoxic effects induced by OHC exposure in terms of DNA double-strand breaks (DNA-DSBs) in three arctic seabirds species in Kongsfjorden, Svalbard: the common eider (*Somateria mollissima*), the black-legged kittiwake (*Rissa tridactyla*), and the glaucous gull (*Larus hyperboreus*).

DNA-DSBs in whole-blood cells were analysed using agaroses gel electrophoresis. Both the migrated fraction of total DNA (DNA-FTM) and median molecular length (MML) were quantified. There were significant differences between species in levels of DNA-FTM and MML. However, the different measurements gave contradicting results with respect to levels of DNA damage. Kittiwakes showed the highest levels of DNA-FTM, followed by eiders and glaucous gulls. In contrast the MML was lowest in eiders, followed by glaucous gulls and black-legged kittiwakes, indicating common eiders had the highest levels of DNA damage. Whole-blood cells from common eiders and glaucous gulls were analysed for 77 different OHCs. Glaucous gull males showed the highest contaminant levels followed by glaucous gull and common eider females.

No significant association was found between contaminants and DNA-FTM or MML in common eiders. For glaucous gull males, a significant negative association between contaminants (OCPs, PCB I+II, PCB III, PCB IV, and $MeSO_2$) and MML was found. This suggests that males with higher levels of contaminants also have higher levels of DNA-damage, whilst glaucous gull females seem to be less susceptible to contaminant-induced effects on DNA integrity. Moreover, a negative association between PCB I+II and DNA-FTM found in glaucous gulls indicates a favourable effect of PCB exposure on DNA integrity. The variability in the association between contaminants and DNA damage between species and between sexes within glaucous gull, could be explained by contaminants only having a genotoxic effect when above a certain concentration threshold. An alternative explanation is differences in both investment in and efficiency of cellular defence. Finally, adaptive responses to chronic contaminant exposure might influence the results presented in this study.

This study highlights importance of contaminant-associated effects on the genetic health of arctic seabird populations. Further studies should focus on elucidating the cellular mechanisms maintaining DNA integrity in seabird species exposed to multiple environmental stressors.

Sammendrag

Organohalogenerte miljøgifter (OHCs) har blitt assosiert med ugunstige effekter på DNA-integriteten, noe som kan føre til alvorlige biologiske konsekvenser både på individ-og populasjonsnivå. Hensikten med denne studien er å undersøke potensielle genotoksiske effekter i form av DNA dobbeltrådbrudd (DNA-DSBs) indusert av OHCs-eksponering i tre arktiske sjøfuglarter fra Kongsfjorden, Svalbard: ærfugl (*Somateria mollissima*), krykkje (*Rissa tridactyla*) og polarmåke (*Larus hyperboreus*).

Hele blodceller ble brukt til å analysere DNA-DSBs ved bruk av agarose gel-elektroforese. Både den migrerte fraksjonen av total DNA (DNA-FTM) og median molekylær lengde (MML) ble kvantifisert. Det var signifikante forskjeller mellom artene når det gjelder nivåer av DNA-FTM og MML. Imidlertid ga de forskjellige beregningene motstridende resultater med hensyn til nivåer av DNA-skade. Krykkje viste de høyeste nivåene av DNA-FTM, etterfulgt av ærfugl og polarmåke. I motsetning til dette var MML lavest hos ærfugl, etterfulgt av polarmåke og krykkje, noe som indikerer at ærfugl hadde de høyeste nivåene av DNA-skader. Hele blodceller fra ærfugl og polarmåke ble analysert for 77 forskjellige OHCs. Polarmåke hanner viste de høyeste forurensningsnivåene etterfulgt av polarmåke hunner og ærfugl hunner.

Det ble ikke funnet noen signifikant assosiasjon mellom miljøgifter og DNA-FTM eller MML i ærfugl. For polarmåke hanner, var en signifikant negativ assosiasjon mellom miljøgifter (OCPs, PCB I + II, PCB III, PCB IV og $MeSO_2$) og MML funnet. Dette antyder at hanner med høyere nivåer av miljøgifter også har høyere nivåer av DNA-skader, mens polarmåke hunner ser ut til å være mindre utsatt for miljøgift-induserte effekter på DNA-integriteten. En negativ assosiasjon mellom PCB I + II og DNA-FTM funnet i polarmåke hunner indikerer en gunstig effekt av PCB-eksponering på DNA-integritet. Variasjonen i assosiasjonen mellom miljøgifter og DNA-skade mellom arter og mellom kjønn i polarmåke, kan forklares ved at miljøgifter kun fører til en genotoksisk effekt over en viss konsentrasjonsgrense. En alternativ forklaring ligger i ulik investering og effektivitet av forsvarsmekanismer. Til slutt kan adaptive responser på kronisk miljøgifteksponering påvirke resultatene presentert i denne studien.

Denne studien belyser viktigheten av miljøgift-assosierte effekter på den genetiske helsen til arktiske sjøfuglbestander. Ytterligere studier bør fokusere på å belyse de cellulære mekanismene som er ansvarlige for å opprettholde DNA-integriteten i sjøfuglarter utsatt for flere miljøstressorer.

Abbreviations

Adj.	Adjusted
AMAP	Arctic Monitoring and Assessment Program
ANOVA	Analysis of variance
BCI	Body condition index
CE	Common eider
CI	Confident interval
Coeff. Value	Coefficient value
CV	Coefficient of variation
CYP1A	Cytochrome P450 1A
DCM	Dichloromethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DF	Detection frequency
DNA	Deoxyribonucleic acid
DNA-DSB	DNA double-strand break
DNA-FTM	DNA fraction of the total DNA, migrated into the gel
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
GC	Gas chromatography
GG	Glaucous gull
HFR	Halogenated flame retardants
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HPC	Halogenated phenolic compound
HR	Homologous recombination
ID	Individual
ISTD	Internal standards
Kbp	Kilo base pair
KW	Black-legged kittiwake
LMPA	Low melting point agarose
LOD	Limit of detection
Log	Logarithm
LOQ	Limit of quantification
$MeSO_2$-PCB	Methylsulfone-polychlorinated biphenyl
MML	Median molecular length
MS	Mass spectrometry
n	Number
Na_2SO_4	Natrium sulfate

N/A	Not analysed
ND	Not detected
NHEJ	Non-homologous end-joining
NILU	Norwegian Institute for Air Research
NPI	Norwegian Polar Institute
NTNU	Norwegian University of Science and Technology
OCP	Organochlorine pesticides
OHC	Organohalogenated contaminants
OH-PBDE	Hydroxy-PBDE
OH-PCB	Hydroxy-polychlorinated biphenyl
p-value	Probability of rejecting the null hypothesis
PBDE	Polybrominated diphenyl ether
PC	Principle component
PCA	Principle component analysis
PCB	Polychlorinated biphenyls
PFAS	Per- and polyfluoroalkyl substance
PFR	Organophosphorous flame retardants
PLSR	Partial least-squares regression
POP	Persistent organic pollutant
QQ	Quantile-quantile
R^2	R squared- coefficient of variance
rf	Response factor
Rf	Relative front
RiS	Research in Svalbard
ROS	Reactive oxygen species
rpm	Rounds per minute
SD	Standard deviation
SE	Standard error of the mean
TBE-buffer	Tris-borate-EDTA buffer
TE-buffer	Tris- EDTA buffer
TP53	Tumour protein 53
UV	Ultraviolet
ww	Wet weight

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1 INTRODUCTION

1.1 PERSISTENT ORGANIC POLLUTION

Anthropogenic activities are the source for a wide range of hazardous environmental contaminants, which with time, can get deposited and accumulate in the environment. Among these contaminants are a diverse group of compounds and their metabolites, called persistent organic pollutants (POPs). The main source of POPs is industrial activities, by-products from industrial production, and pesticide use (AMAP, 2004; UNEP, 2008a; Letcher, et al., 2010).

The Stockholm Convention is a global treaty founded in 2001 that aims to protect both human health and the environment from harmful persistent organic substances by evaluating, regulating, and eliminating POPs. The criteria for a compound to be categorized as a POP according to the Stockholm convention are: persistency in the environment, have a potential for long-range transport, can bioaccumulate in biota, and cause toxic effects (UNEP, 2008b). In 2004, the Stockholm Convention restricted twelve POPs, referred to as the legacy POPs, and as a result the emission of the compounds in question was restricted. These includes, among other compounds, dichlorodiphenyl-trichloroethane (DDT), mirex, heptachlor, chlordane, hexachlorobenzene (HCB), and polychlorinated biphenyl (PCBs) (UNEP, 2008a). The Stockholm Convention is constantly updated and new "emerging contaminants" have been added to the list, including numerous per- and polyfluoroalkyl substances (PFASs) and several brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs) (UNEP, 2017).

1.1.1 ORGANOHALOGENATED CONTAMINANTS

Among the most abundant subgroups of POP is the organohalogenated contaminants (OHCs), including the major contaminant groups: organochlorine pesticides (OCPs), PCBs, PBDEs, and their respective metabolites (AMAP, 2004). The OHCs have stable carbon-halogen bounds toward hydrolysis, and are therefore, to a degree, resistant to both biological and photolytic degradation (El-Shahawi et al., 2010). Numerous OHCs have a half-life of years or decades in soil/sediments and days in the atmosphere. Consequently, OHCs can persist in the environment for a long time, even if the original source of emission is removed (Jones & de Voogt, 1999; El-Shahawi et al., 2010). The OHCs have the potential to be transported at low concentrations by ocean current and over long distances in the atmosphere. Moreover, the contaminants can move from the air and water into soil and biota and in this way more easily accumulate and

cause toxic effects in animals and humans (El-Shahawi et al., 2010; Letcher, et al., 2010; Dietz et al., 2019). The majority of the contaminants are highly toxic in their parental form. Even so, other contaminants are harmless in their original form, yet can generate toxic metabolites. OHCs is a complex and expanding group of organic substances of special interest due to their capacity to cause damage to both human health and wildlife (Letcher et al., 2010; Watts et al., 2018; Dietz et al., 2019).

1.1.1.1 ORGANOCHLORINE PESTICIDES

OCPs were produced for use in agriculture and contribute greatly to the emission of pollution in the environment (Kaushik & Kaushik, 2007). In the 1930s, the first synthetic pesticides were produced and the use of insecticides, like DDT, revolutionized several agriculture processes. As a result, high concentrations of OCPs were emitted to the environment. Owing to their persistency and the potential to be long-range transported, OCPs are still found in high concentrations in the environment (Killin et al., 2004). OCPs were later found to be highly toxic for wildlife due to their structure and lipophilicity (Kaushik & Kaushik, 2007; Jayaraj et al., 2016). Organochlorinated pesticides, like DDT and chlordane, are particularly interesting because they, in addition to being harmful parent compounds, produce toxic metabolites as well. These metabolites include p,p-dichlorodiphenyldichloroethylene (DDE) and oxychlordane, which are found in high concentrations in wildlife, especially in top predators (AMAP, 2004).

1.1.1.2 POLYCHLORINATED BIPHENYL

PCBs consist of 209 congeners all made up of a biphenyl molecule with varying numbers of chlorine atoms attached and are identified based on their percentage of chlorine content and position of the chlorine atoms (Safe at al., 1987; El-Shahawi et al., 2010). Commercial PCBs were manufactured in large scale due to their broad range of physiochemical properties, including persistency, lipophilicity, chemical stability, and inflammability. PCBs were commonly used in commercial products, such as in organic diluents, dust-reducing agents, flame retardants, and heat transfer fluids. Moreover, their physiochemical properties made PCBs the preferable option for use in many industrial applications. Due to their persistency, PCBs still cause environmental and health problems worldwide. Once in the environment, PCBs degrade slowly and can be transported far away from their emission source. Furthermore, due to their lipophilicity, these chemicals can bioaccumulate in species and also show great

biomagnification potential with increasing trophic position (Safe, 1994; Borgå et al., 2005). In addition, studies have shown that PCB congeners can produce highly toxic metabolites, such as halogenated phenolic compounds (HPCs) and methylsulfone-PCBs ($MeSO_2$-PCBs) in vertebrate species (Letcher et al., 2010; Dietz et al., 2019).

1.1.1.3 POLYBROMINATED DIPHENYL ETHERS

PBDEs were initially produced in 1978 and are used mainly as flame retardants. Flame retardants are commonly used in products such as plastics and textiles and as a result of the widespread use of plastics in consumer products, are PBDEs found in high concentrations in the environment (Vonderheide et al., 2008). PBDEs are related to PCBs and share the same physical-chemical properties: they are highly stable, lipophilic, long range transportable, and persistent in the environment. Consequently, these compounds have the potential to persist in the environment and bioaccumulate in species (de Wit, 2002; Wolkers et al., 2004). PBDE can generate metabolites, such as hydroxy-PBDE (OH-PBDE). However, OH-PBDE have been given less attention compared to HPC and $MeSO_2$-PCBs, especially in vertebrate species (Verreault et al., 2007; de Wit et al., 2010).

1.2 EFFECTS OF ORGANOHALOGENATED CONTAMINANTS IN ARTIC SEABIRDS

The temperate regions are the primary areas of emission, production, and use of OHCs (UNEP, 2008a), whereas, in the Arctic region, local pollution sources are limited. Despite this, levels of OHCs have been measured in the Arctic region (Gabrielsen, 2007; Letcher et al., 2010; Dietz et al., 2019). Long-range atmospheric transport is generally considered the main route for pollutants accumulating in the Arctic region. In addition, but to a lesser extent, contaminants are transported to the Arctic region with ocean current, the polar ice, and through the arctic rivers (Braune et al., 2005; de Wit et al., 2006; de Wit et al., 2010). Transport of pollutants carried by animals migrating to the Arctic region may also have some significance (Blais et al., 2005). The physical-chemical properties of the compounds and a periodic movement between mobile and immobile media, often referred to as “the grasshopper effect”, facilitate the accumulation of pollutants in the Arctic region (Wania & Mackey, 1993; Gouin et al., 2004). Colder regions of the world work as an environmental sink of pollutants because the cold temperature makes the transported pollutants sink and deposit. Additionally, the colder temperature does not allow for the contaminants to break down easily (Burkow & Kallenborn, 2000; El-Shahawi et al., 2010).

Recent reports show a decrease in OCP, PCB, and PBDE levels in the Arctic (Hung et al., 2016; Rigét et al., 2019). Despite this, OCHs are still found in arctic seabird species (Letcher et al., 2010; Verreault et al., 2010; Dietz et al., 2019). OHCs are lipophilic, and thus accumulate in lipid-rich tissue, allowing for the contaminates to get transferred along with the lipid or energy transfer in the food web (Hop et al., 2002; Borgå et al., 2005). As a result of trophic transfer, the top predators in the marine food web, marine mammals and seabirds, are considered the two groups of species with the highest levels of contaminants in the Arctic region (Dietz et al., 2000; Borgå et al., 2001; 2004; Letcher, 2010). In a harsh arctic environment, lipids are normally stored as an energy source, which get utilized during the incubation period. This results in a redistribution of the lipophilic contaminants into the blood stream (Gabrielsen, 2009). Seabirds living in the Arctic region are therefore, especially during the breeding season, vulnerable to OHCs exposure. Their diet is considered the main route of contamination. Nonetheless, the seabirds' biotransformation capacity and their ability to eliminate the contaminants have some significance regarding the concentrations of contaminants found in the birds as well (Borgå et al., 2004; 2005). However, many seabirds show a low metabolic biotransformation capacity of OHCs (Borgå et al., 2001) and variation in biotransformation capacity between the different arctic seabird species have been found (Fisk et al., 2001; Borgå et al., 2005; Helgason et al., 2010).

Other biological factors may affect the accumulation, distribution, and biotransformation of contaminants, including migration route, reproductive strategy, body size, dynamic of lipids in organisms, seasonality, age, and foraging behaviour (Borgå et al., 2004; Leat et al., 2013; Guzzo et al., 2014). Moreover, during egg formation the females transfer contaminants to the egg together with essential lipoproteins. Through this mechanism, which is referred to as maternal transfer, females may decrease their blood contaminant concentrations by transferring a portion of their contaminant load to the eggs (Verreault et al., 2006a). Exposure of OHCs is known to cause severe biological consequences in arctic seabirds, including effects on the endocrine system, immune system, development, reproduction, and genotoxicity (Letcher et al., 2010; Dietz et al., 2019). Moreover, exposure to pollution may be measured as effects on a population level, such as breeding success and adult survival, which may affect the overall fitness of the population (Verreault et al., 2010; Erikstad et al., 2013).

Assessing a relationship between contamination and genetic damage *in situ* is difficult because natural populations are exposed to multiple stressors simultaneously, from both natural and anthropogenic sources. This includes predation, changes in food access, climate change, loss of habitat, as well as exposure to pollution (Bårdsen et al., 2018). In addition, are natural populations exposed to a mixture of chemicals and physical agents that potentially can interact (i.e., antagonistic or synergistic) (Eaton & Gilbert, 2013). Consequently, evaluating contaminant exposure in field studies is complex and difficult to carry out. A common solution is to extrapolate threshold levels obtained in laboratory studies. However, this may give inaccurate results because the threshold levels obtained in controlled laboratory studies are not fully considering multiple stressors, and therefore may be too high (Bårdsen et al., 2018). Moreover, most controlled laboratory studies overlook the chemical and physical variation found in the environment, and often investigate the effect of single chemicals separately (Matson et al., 2009). The knowledge about contaminants occurring as mixtures and the potentially interactions in combination with other stressors in nature emphasizes the need for standardized methods *in situ* studies (Wharfe et al., 2009).

1.3 GENETIC TOXICOLOGY

All living cells contain deoxyribonucleic acid (DNA) carrying its inherited information, and unwanted changes in the DNA structure and/or integrity may potentially cause severe biological consequences. A group of contaminants and physical agents, called genotoxicants, can modify DNA structure and/or function (Shugart, 1998). The field of genetic toxicology assesses the effect of genotoxicants on DNA and the genetic processes of living cells (Preston & Hoffmann, 2013). Genotoxic agents can cause different types of DNA-damage, including double -and single-stranded DNA break, DNA-protein -and DNA-DNA crosslinks, breaks in DNA backbone and various DNA adducts (Figure 1.1, Preston & Hoffmann, 2013).

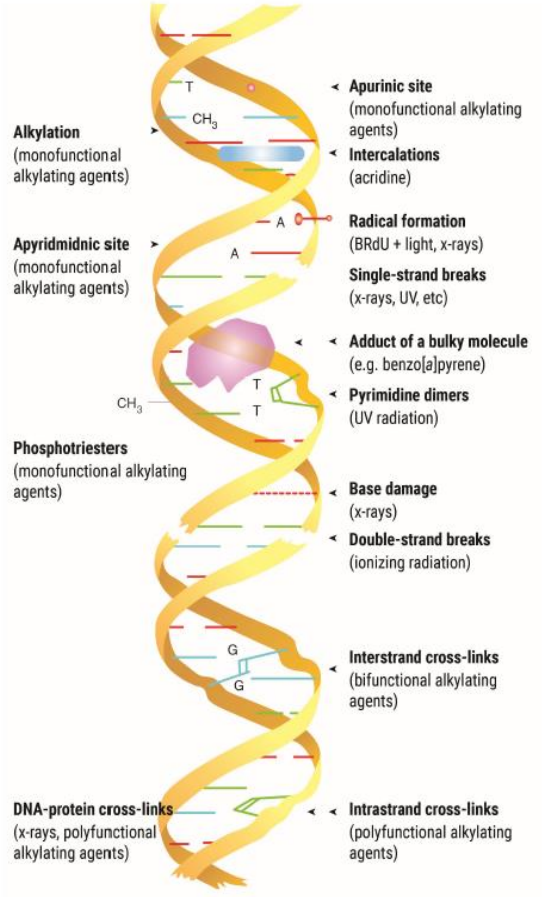

Figure 1.1: The different type of DNA damage induced by chemical and physical agents (Preston & Hoffmann, 2013).

Numerious OHCs, due to their physicochemical properties, have the potential to cause genotoxic effects (Shugart, 1988; Gonzalez-Mille et al., 2010). Various OHCs and their metabolites are alkylating agents that can bind covalently to the DNA bases, increasing DNA strand break frequency (Srinivasan et al., 2001). Moreover, variuous OHCs may induce oxidative stress (Costantini, et al., 2014). Increased oxidative stress may cause damage to cell structures, such as DNA, lipids, and proteins, and interfere with their functions (Birben et al., 2012). Among the reactive oxygen species (ROS), the hydroxyl radical is by far the most reactive byproduct capable of causing damage to the DNA (Chatterjee & Walker, 2018).

1.3.1 DNA DOUBLE-STRAND BREAKS

DNA integrity is frequently challenged by DNA lesions. The majority of such lesions are the consequences of normal endogenous processes, including transcription, recombination, and replication (Vamvakas, et al., 1997). In addition, strand breaks are associated with apoptosis, a mechanism for eliminating damaged cells (Vamvakas, et al., 1997). Under normal cellular conditions, the DNA is steadily changing between a stable conformation (double-stranded DNA) and a temporary state of instability and discontinuity (Shugart, 2000). During the latter state, the occurrence of alterations in DNA structure, such as strand-breaks, is more likely to occur (Shugart, 2000).

Strand-breaks can be induced directly by irradiation, exposure to xenobiotics, and increased production of ROS, or indirectly through incorrect DNA repair and extranuclear lesions (Vamvakas et al., 1997). DNA double-strand breaks (DNA-DSBs) are induced when the two complementary strands of the DNA double helix are broken concurrently at sites as close to one another that base-pairing and chromatin structure are deficient to keep the DNA strands in conjunction (Jackson, 2002). Strand-breaks can be single -or double-stranded, whereas double-stranded breaks generally are considered more severe, due to lack of undamaged complementary strand that can be utilized as a template during DNA repair (Jackson, 2002; Polo & Jackson, 2011).

Exposed DNA ends are vulnerable for single- and double-strand exonucleases, which potentially can result in loss of critical genetic information (Cromie, et al., 2001). Moreover, DNA-DSBs can induce DNA rearrangements (eg. deletions, translocations, and inversions). If DNA-DSBs are not repaired, the coding sequence of a gene can be interrupted. This may cause

damage on regulatory sequences, altered chromosome organization, damage to the systems ensuring correct DNA repair, chromosome segregation, and chromosome packing (Cromie et al., 2001).

1.3.2 DNA DAMAGE REPAIR

In any given cell, DNA-DSBs are occurring continuously at a low frequency. Under normal cellular conditions, the DNA repair system is relatively efficient in repairing DNA-DSBs (Vamvakas et al., 1997; Klaunig, 2013). The induction of DNA damage in the form of DNA-DSBs and increased ROS formation will activate the DNA repair system and the antioxidant defence to reverse the consequences of damage accumulated (Figure 1.2). If the damage is too extensive for the DNA repair system to handle, a signalling cascade inducing apoptosis will be activated (Figure 1.2, Norbury & Zhivotovsky, 2004). Moreover, DNA repair is costly and with limited resources apoptosis may be the preferable option for avoiding accumulation of DNA damage (Norbury & Zhivotovsky, 2004).

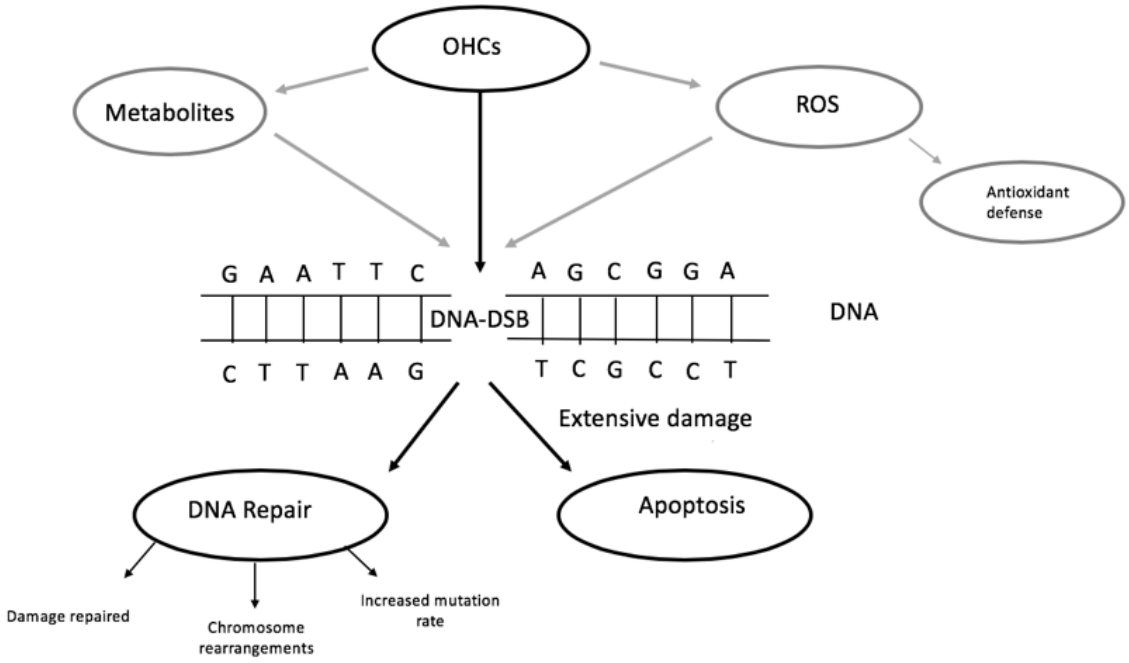

Figure 1.2: Exposure of OHCs, production of toxic metabolites, and ROS can induce DNA-DSBs. Consequently, damage accumulated will induce signalling cascades activating DNA repair system (NHEJ and HR) or if the damage is to extensive, apoptosis. Inaccurate repair can potentially cause increased mutation rate and chromosome rearrangements, which with time may lead to carcinogenic (not shown). Increased production of ROS can additionally increase antioxidant response which next may induce DNA repair and apoptosis (not shown). Modified from: Rodvelt (2018).

The two main DNA repair pathways for DNA-DSBs are homologous recombination (HR) and non-homologous end-joining (NHEJ). The two pathways work in a complementary manner to cope with DNA-DSBs. HR is mainly error-free, while NHEJ is error-prone (Van Gent et al., 2001; Peng & Lin, 2011). NHEJ ligates the broken DNA ends directly, and HR requires a homologous template DNA usually in the form of a sister chromatid. Consequently, HR works most efficient during DNA synthesis and the gap two phase of the cell cycle (Van Gent et al., 2001; Peng & Lin, 2011). On the other hand, NHEJ works throughout the entire cell cycle (Van Gent et al., 2001; Peng & Lin, 2011).

If the DNA repair system fails to repair the damage, it can result in increased mutation rate and chromosome rearrangement, which in turn may cause adverse biological consequences (Figure 1.2) (Pfeiffer, 1998; Jackson, 2002). Exposure to genotoxicants may cause DNA damage to both somatic and germ cells. Thus, exposure to genotoxicants are of special interest because the DNA damage induced in the germ cells has the potential for transmission to the next generation (Jha, 2008). These effects may, on a population level, be selected against and removed sooner or later from the gene pool (Jha, 2008). However, if not removed, bottlenecks, mutations, and selection caused by genotoxicant exposure may lead to changes in demographic, population structure, genetic variation, and reduce the overall fitness of a population (Bickham, 2000). These ecological impacts are of great concern in ecotoxicology, and studies investigating the association between contaminant exposure and DNA-DSBs frequency are of particular interest because DNA-DSBs show potential as an early biomarker of exposure (Haarr et al., 2018).

1.4 STUDY SPECIES

Several arctic seabird species breeding at Svalbard have been used in studies assessing the association between contaminant exposure and genetic damage (Østby et al., 2005; Krøkje et al., 2006; Fenstad et al., 2014; 2016a; Blévin et al., 2017a; Haarr et al., 2018). Arctic seabirds are good bioindicators of the arctic environment because they are relatively long-lived, occupies different trophic levels, the majority are migrating birds that regularly interact with other animals and human activity, and they show different life history and physiological adaptations (Durant et al., 2009; Le Bohec et al., 2013; Lescroël et al., 2016). Figure 1.3 shows the three study species, glaucous gull (a), black-legged kittiwake (b), and common eider (c) used in the present study.

Figure 1.3: The three study species used in the present study: a) glaucous gull, b) kittiwake, c) female eider. Photos: Geir Wing Gabrielsen/ Norwegian Polar Institute (npolar.no/en/species-archive/)

1.4.1 GLAUCOUS GULL (*LARUS HYPERBOREUS*)

Glaucous gull (Figure 1.3a), one of the largest gulls with circumpolar distribution, is the most studied avian species observed in the Arctic (Anker-Nilssen et al., 2000). The species nest along the coast and in open tundra in both colonies and dispersed (Anker-Nilssen et al., 2000). The glaucous gull is a top predator in the marine food chain, hence lives near to other seabird colonies. They predate on and consume a wide range of species, mainly seabird chicks and their eggs but also fish, crustaceans, carrion, berries, insects, rodents, seal pups and other marine organisms (Løvenskiold, 1964; Gabrielsen et al., 1995; Borgå et al., 2001). The glaucous, as a top predator, is particularity vulnerable to contaminant exposure due to trophic transfer (Gabrielsen, 2007; Helgason et al., 2010).

The glaucous gull is a migrating bird and arrives on Svalbard during the breeding season (from March-April until mid-September) after spending their winters in the northern part of the Atlantic Ocean (Anker-Nilssen et al., 2000). High levels of OHCs contamination have been measured in unhealthy and dying glaucous gull from Svalbard (Gabrielsen et al., 1995; Sagerup et al., 2009a). The Svalbard population of glaucous gull has been declining in the last decades, and the population status is near threatened. The population size, at Svalbard, is estimated to 4250 pairs in total (Fauchald et al., 2015). This population decline has been associated with high levels of pollution, particularly high OHCs levels (Bustnes et al., 2003; Verreault et al., 2010).

The glaucous gull is a bioindicator for the arctic environment (Verreault et al., 2010). Hence, the association between contaminant exposure and damage, on an individual level, has been studied in the glaucous gull (Løvenskiold, 1964; Verreault et al., 2010; Sonne et al., 2013). Several studies have documented that increasing contaminant concentrations result in numerous

adverse outcomes in the glaucous gull, including reproductive effects (Bustnes et al., 2003) and increased levels of DNA damage (Østby et al., 2005; Krøkje et al., 2006). Østby et al (2005) observed a significant increase in DNA adducts in exposed chicks compared to control chicks, while Krøkje et al (2006) reported increased chromosome aberrations and DNA-DSBs in exposed chicks compared to control chicks, although no significant relationship was found.

OHCs exposure is a stress factor known to affect, the seabirds not only on an individual level, but also on a population level (Dietz, et al., 2019). Genetic damage on germ cells may result in damage in future generations. With time, this may result in an effect on higher biological organizations, such as ecological effects on a population level (Bustnes et al., 2002; 2003; Erikstad et al., 2013). This includes, among other adverse effects, reduced adult survival (Bustnes et al, 2005), immune suppression (Bustnes et al., 2004; Sagerup et al., 2009b), low breeding success (Bustnes et al, 2003; Verboven et al., 2008), impaired embryonic development (Verboven et al. 2009), and hormonal dysfunction (Verreault et al., 2004; 2006a; 2008).

1.4.2 BLACK-LEGGED KITTIWAKE (*RISSA TRIDACTYLA*)

Black-legged kittiwake (hereafter kittiwake) (Figure 1.3b) is a long-lived colonial and monogamous seabird, breeding in the circumpolar zone (Mehlum & Gabrielsen, 1993; Helfenstein et al., 2004). Kittiwake is smaller in size than the glaucous gull and feeds mainly on small pelagic fishes and invertebrates. Hence the kittiwake feeds at an intermediate trophic level compared to the glaucous gull (Mehlum & Gabrielsen, 1993; Anker-Nilssen et al., 2000). There have been measured lower OHCs levels in kittiwake compared to the glaucous gull, presumably as a consequence of diet preferences and trophic transfer (Savinova et al., 1995; Borgå et al., 2005; Haarr et al., 2018).

The largest colony of kittiwake on Svalbard is at Bjørnøya (Bear Island). Most colonies are found on cliffs near the sea on islands or the mainland (Anker-Nilssen et al., 2000). The population on Svalbard is classified as near threatened and is listed at the red list of threatened species with population declines in all areas except for Bjørnøya (Fauchald et al., 2015). Even though the majority of colonies are declining on Svalbard, the total Svalbard population number is relatively stable and is estimated to 283 689 pairs in total (Fauchald et al., 2015; Anker-Nilssen et al., 2018).

The arctic kittiwake is chronically exposed to a mixture of organic pollution, which is known to be associated with decreased survival rate, lower breeding probability, and likely affecting population dynamics (Goutte et al., 2015; Blévin et al., 2016; 2017a; 2017b). Moreover, Blévin et al. (2016) showed a reduction in telomere length with increasing levels of OCH in the kittiwake. This suggests genotoxic potential of OHCs in kittiwake.

1.4.3 COMMON EIDER (*SOMATERIA MOLLISSIMA*)

Common eider (hereafter eider) (Figure 1.3c) is a migrating long-lived sea duck that breeds in the Arctic and boreal zones of the northern hemisphere, which includes a relatively large population on Svalbard. The eider normally breeds close to the ocean or on coastal islands to avoid predation by the arctic fox (Løvenskiold, 1964; Anker-Nilssen et al., 2000). The birds feed on a low trophic level, with a diet mainly composed of benthic invertebrates like molluscus and amphipods (Dahl et al., 2003). Lower OHCs levels are detected in eider compared to the glaucous gull and the kittiwake, presumably due to diet preferences and trophic position (Huber et al., 2014; Haarr et al., 2018).

The eider is by far the most abundant species at Svalbard (Prestrud & Mehlum, 1991) and is not as threatened as the glaucous gull and kittiwake populations. The eider population, on Spitsbergen, is estimated at approximately 17100 pairs in total (Fauchald et al., 2015). Despite this, abundant exploitation of eiders in the past have threatened to decrease the eider population on Svalbard. Consequently, conservation measures have been put into action to maintain population size (Prestrud & Mehlum, 1991), and the population has persisted relatively stable the last decade (Fauchald et al., 2015). However, data from 1981-2017 show some fluctuation and a recent population decrease of approximately 27 % from 2012-2017 (MOSJ, 2020).

The female eider incubates the eggs alone and does not feed during the incubation period (Gabrielsen et al., 1991). During the incubation period, the female eiders do not leave the nest for 24-26 days and experience extreme weight loss (~30-46 % of their body fat) (Bustnes et al., 2012; Fenstad et al., 2014). However, some females seem to handle breeding stress better than others (Korschgen, 1977). Studies show that during the incubation period, an increase in OHCs levels is associated with a decrease in body mass (Fenstad et al., 2014). Lipophilic compounds are redistributed in the bloodstream and may potentially induce additional stress on the already fasting female eiders (Fenstad et al., 2014).

1.5 OBJECTIVES

The objective of the original study was to determine levels of DNA damage, levels and patterns of OHCs, and investigate the potential genotoxic effect in terms of DNA-DSBs induced by OHCs exposure in the three different arctic seabird species: eider, kittiwake, and glaucous gull. Because of circumstance out of my control, the material for chemical analysis, to our current knowledge, appears to be lost for kittiwake.

It is expected to find differences in levels of DNA damage in the three different arctic seabird species. Moreover, arctic seabird species with high trophic position are expected to have higher levels of contaminants. As OHCs are known to either directly or indirectly result in DNA lesions, it is hypothesized to find a positive association between OHCs concentration in blood and levels of DNA-damage. Lastly, it is expected that high levels of contaminants, together with poor body condition, can increase levels of DNA damage, while individuals in better condition may detoxify and or eliminate the contaminants more efficiently, and therefore reducing the effect of contamination on DNA integrity.

2 MATERIALS AND METHODS

2.1 SAMPLE AREA AND FIELD PROCEDURES

The fieldwork was conducted in the area around Ny-Ålesund, Svalbard (78°´N, 12°´E, Figure 2.1) during the breeding season 2015 (from 5th of June to 7th of July). During the fieldwork, people from different projects on arctic seabirds were co-operating to keep the disturbance of the seabird colonies to a minimum. This includes minimizing the time used at each location and reducing the handling time per seabird. All procedures conducted were approved within the regulations of the Norwegian Animal welfare act and sampling was approved by the Governor of Svalbard (application reference number: 2014/00489-4). The fieldwork was registered in the database Research in Svalbard (RiS) with RiS-ID: 10186. Ola Tilset was responsible for the sampling of the material used in this project.

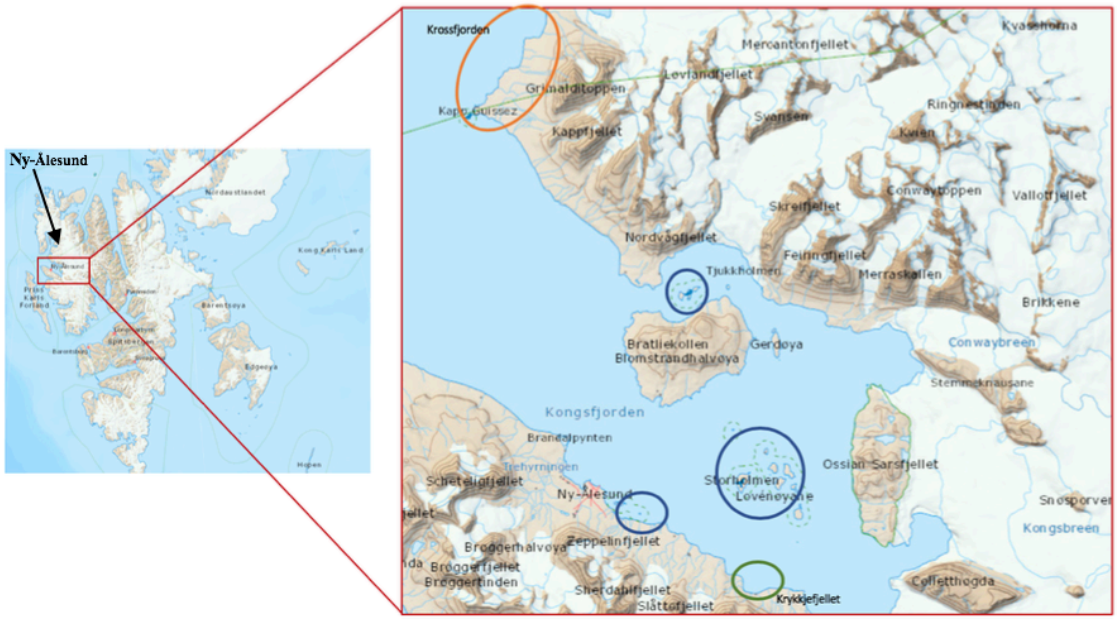

Figure 2.1: Location of the sampling site for fieldwork conducted during the breeding season in Ny-Ålesund, Svalbard, 2015. An overview map of Svalbard, showing Ny-Ålesund (left) and Kongsfjorden (right). All glaucous gull samples were obtained in Krossfjorden (orange), kittiwake samples were all from Krykkjefjellet (green), and eider samples were from the following islands: Prins Heinrich, Breøyene, and Storholmen (blue). Maps obtained and modified from the Norwegian Polar Institute (toposvalbard.npoloar.no)

The arctic seabird species, glaucous gull (n=14), kittiwake (n=19), and eider (n=20) were all captured and sampled once. All birds were caught on the nest, but different methods to capture were used, depending on the species. The glaucous gulls were caught with the use of a remotely triggered nest trap or a hand-held net gun, while kittiwakes and eiders were caught with the use of a fishing rod with a nylon snare at the end. Spark Body mass was recorded using a spring balance (Pesola Medio-Line 42500, Ecotone-Poland, 2500 g), skull length (head and bill) was

measured with a sliding caliper (±0.5mm), and the wing length (mm), i.e. the distance from the carpal joint to the tip of the longest primary, was measured using a ruler with a stop (±1mm).

All samples were collected from different locations in the sampling site in Kongsfjorden (Figure 2.1). Both female and male glaucous gull individuals were collected from different sites in Krossfjorden, an area spanning from Kapp Guisse to Fjortende Julibukta (Figure 2.1, marked with an orange circle). Female kittiwakes were all collected at Krykkjefjellet in Kongsfjorden (Figure 2.1, marked with a green circle). Female eiders were collected from three different islands Prince Heinrich, Breøyene, and Storholmen (Figure 2.1, marked with blue circles).

Blood samples (from two to ten mL, depending on the species) were drawn from the brachial vein (jugular vein for the eiders) using a syringe flushed with heparin with a 23/25 G needle. ID rings and global location sensing units (GLS loggers) which are used to track migration patterns in seabirds were positioned on individuals if they did not already have them placed from previous years.

A sample of 500 µL blood was transferred to an Eppendorf tube (1.5 ml) for later DNA-DSB analysis. The Eppendorf tube was immediately frozen in a thermos containing a mixture of ice and salt (~5 table spoons of salt/l ice, ~ -20°C), transported to the field station within six hours and transferred to a -80°C-freezer. After the field season the samples were transported in a dry shipper (-70 °C) from the laboratory in Ny-Ålesund, Svalbard to the Norwegian University of Science and Technology (NTNU) in Trondheim, Norway. Here, the samples were transferred to a freezer (-80°C) for storage at the Department of Biology. The remaining blood samples were transported to the laboratory at the Norwegian Institute for Air Research (NILU) in Tromsø, where the samples were kept in a freezer (-20 °C) until chemical analysis was conducted.

2.2 DNA DOUBLE-STRAND BREAK ANALYSIS

The analysis of DNA-DSBs was conducted on 53 blood samples (14 glaucous gulls, 19 kittiwakes, and 20 eiders) by agarose gel electrophoresis at the Department of Biology, NTNU. Gel electrophoresis is inexpensive, rapid, sensitive, and produces highly reproduceable results, and thus has become a well-established established method to detect DNA-DSB. Furthermore, it requires only microliters of sample material (Theodorakis et al., 1994; Krøkje et al., 2006).

The method used in the present study was first developed and used by Theodorakis and co-workers (1994) for analysing fish samples. The method was later modified for avian blood (Krøkje et al., 2006), and has been used on blood samples from glaucous gull (Krøkje et al., 2006) and several studies on eider (Fenstad et al., 2014; 2016a; Noori, 2018; McPartland, 2019). A list of chemicals and solutions used to perform the DNA-DSB analysis are presented in Appendix A, Table A1 and A2.

2.2.1 Principals of DNA double-strand break analysis

The principle of this procedure is to embed whole nucleated red blood cells containing DNA in low melting point agarose gel (LMPA) plugs to protect the DNA from procedural damage. The DNA is isolated by lysis and enzymatic digestion of the cells to remove nucleases and DNA-associated proteins, leaving the DNA as a nucleoid in the gel plug (Theodorakis et al.,1994; Shaposhnikov et al., 2008). The procedure is performed under natural pH conditions to not disrupt the supercoiled and duplex structure of the DNA (Collins et al., 2008; Shaposhnikov et al., 2008). Nuclear DNA is normally supercoiled chromatin, but when DNA DSBs are present, the supercoiled chromatin will relax, and increasing frequency of breaks will result in a more relaxed structure (Collins et al., 2008; Shaposhnikov et al., 2008).

During gel electrophoresis, the DNA, which is negatively charged, will travel towards the positively charged anode. Smaller DNA fragments will migrate further through the gel than larger fragments. An increase in DNA-DSBs will increase fragments traveling through the gel. Undamaged DNA unable to travel through the pores in the gel remains in the well (Lee et al., 2012). This method of separation allows for a comparison between the amount of undamaged DNA in the wells and the fragmented DNA in the gel (Appendix A, Figure A.1). The determination of the size of the DNA fragments is done by comparison with a known size ladder (Appendix A, Figure A.2 and A.3).

The quantification of the amount of DNA-DSBs can be done with the relative measures: the migrated DNA fraction of total DNA (DNA-FTM) and median molecular length (MML). The DNA-FTM is the fraction of the total DNA loaded into the well that has travelled through the gel (Fenstad et al., 2014). The MML is the median molecular length (kbp) of the migrated DNA and is a measure of the DNA fragment size distribution (Krøkje et al., 2006). An increase in the frequency of DNA-DSBs will result in more DNA fragments migrating through the gel,

consequently leading to an increase in DNA-FTM and lower MML. In other words, an increase in DNA-DSB is positively related to DNA-FTM and negatively related to MML.

2.2.2 Preparations of DNA plugs

Agarose plugs for electrophoresis were prepared according to the procedure described by Krøkje et al. (2006) and Fenstad et al. (2014). A small volume of whole blood (glaucous gull (2 µL), kittiwake (5-10 µL), and eider (8 µL)) was diluted in 500 µL of TE buffer (10 mM Tris base and 1 mM EDTA, pH 8) at 37 ºC, and then mixed with 500 µL of premelted 1 % LMPA at 37 ºC. To remove potential blood coagulation, the mixture was pulse centrifuged up to a speed of 7000 rpm and immediately placed back at the heat block. From this mixture, 50 µL plugs were cast in plug molds (BioRad, #170-3713). The plugs were set at 4 ºC for 1 hour to harden, before being placed into Theodorakis lysis buffer (NaCl (100 mM), Tris buffer (10 mM), EDTA (10 mM), SDS (0.5 %), pH 8) with freshly made proteinase K (1 mg/mL) added, and set for incubation at 55 ºC for 16 hours allowing the cells to lyse and being enzymatically digested.

2.2.3 Gel electrophoresis

After removed from the lysis buffer, the plugs were equilibrated to room temperature and inserted into the wells of the agarose electrophoresis gel (0.6 % agarose) in TBE running buffer (Sambrook & Russell, 2012). The plugs were sealed into the wells by adding premelted 1 % LMPA (37 ºC). Whole linearized lambda phage DNA and Hind III digested lambda phage DNA (2 µL) in TE buffer (43 µL) was used as the DNA ladder (positive control). Loading dye (15 µL) was added to the ladder mix designated to show the progress of the run. The gels ran in TBE buffer (90 mM Tris base, 90 mM boric acid, 2 mM EDTA) at 23 volt/cm for 15 hours.

2.2.4 Staining and quantification of DNA double-strand breaks

The gels were stained in ethidium bromide (EtBr) solution (~0.1mg/L TBE) for one hour and rinsed with tap water several times. Gel image data was acquired using the BioRad, Gel Doc 2000 system. EtBr stained DNA will fluorescence under UV-light, allowing for visualization. Densitometric data obtained from the gel image analysis were used in the calculation of MML of DNA fragments in the gel. The relative amounts of both the DNA left in the well and the DNA travelled into the gel were determined by the area under the respective DNA staining intensity curves (Fenstad et al., 2014).

To ensure that "gel" and "replicate" did not have any influence on the calculation of the relative DNA measures, the potential changes in conditions between the runs were minimized by running gels in parallel and running each sample multiple times at different days. Each sample (one individual) was represented with three lanes, and three intensity staining curves were made per lane, hence nine (3x3) curves were made per sample (Appendix A, Figure A.4). These intensity curves (Appendix A, Figure A.1) were used to calculate the amount of DNA-DSBs: represented byMML and DNA-FTM values for each sample.

The ladders known size markers and its corresponding Relative front (Rf)-value presents a standard curve. This is used to calculate MML (kbp) for each sample (Krøkje et al., 2006). The MML value corresponding to its Rf-value was extrapolated from the standard curve (Appendix A, Figure A.5). DNA-FTM (%) was calculated for each sample, according to Equation 1 (Fenstad et al., 2014).

$$\boldsymbol{DNA - FMT}\ (\%) = \frac{\boldsymbol{DNA\ in\ gel}}{\boldsymbol{DNA\ in\ gel + DNA\ in\ well}} \times \mathbf{100} \qquad \text{EQUATION 1}$$

2.3 CHEMICAL ANALYSIS

Analysis of OHC contaminants, funded by the Norwegian Polar Institute (NPI), was conducted in the laboratories at NILU, Fram Centre, Tromsø. Ola Tilset was involved in the preparation of the samples and the instrumental analysis and chemical quantification were conducted and coordinated by the NILU staff. All solvents used were purchased from Merck (Darmstadt, Germany), and the internal and labelled standards supplied by NILU were purchased from Wellington laboratories.

In total, 34 samples (14 glaucous gulls and 20 eiders) were analysed for 77 different contaminants including 17 OCPs, 12 PCBs, 7 PBDEs, 21 HPCs, 20 $MESO_2$-PCBs/DDE listed in Appendix A (Table A.3).

2.3.1 CHEMICAL ANALYSES OF CHLORINATED AND BROMINATED COMPOUNDS

2.3.1.1 EXTRACTION

Analysis was performed as described by Bustnes et al. (2008). The samples had to be defrosted at room temperature, mixed with a vortex mixer, and if necessary, spun down. 1-2 g of matrix (whole blood) was transferred to 15 mL glass vials. The extraction step was performed to release the lipids and lipid soluble compounds from the samples by running suitable solvents through the sample in the column. The samples were spiked with the ^{13}C internal standard (ISTD: POP I 20 µL, PBDE I 20 µL, HPC I 20 µL, $MeSO_2$-PCB I 50 µL) prior to the extraction enabling for quantification of the concentrations for the analysed compounds later.

The internal standard was added (100 µL, 2500 pg) and mixed by a vortex mixer. 2 mL of deionized water saturated with ammonium sulfate and 2 mL ethanol was added to enhance protein denaturation and phase separation. 6 mL of n-hexane was added, and the samples were mixed thoroughly for 45 seconds by a combination of manually shaking and by the use of a vortex mixer. The samples were left in the fume hood for a minimum of 15 minutes to allow the phases to separate. Empty glass vials were weighed before supernatant (n-hexane) was transferred. The samples were then treated with n-hexane for a second time, mixed and left in the fume hood for another 15 minutes. The second supernatant was then transferred to the glass vials containing the first supernatant.

Sample extracts were concentrated down to approximately 0.2 mL applying the RapidVap (LabConco RapidVap, Model 7900001, Kansas City, MO, US). To facilitate evaporation, a combination of low pressure, high-frequency vortex speed, and high temperature were used. During this process, all parameters had to be monitored and adjusted stepwise to prevent samples from being evaporated to dryness losing both organic material and analytes. When the sample volume was reduced to approximately 0.2 mL, the samples were cautiously blown with N_2 (N_2 purity of 99,995 %, quality 5.0, Yara Praxair AS, Porsgrunn, Norway) to a final volume of 50 µL. The glass vials with the sample extracts were weighed, re-dissolved in 0.5 mL n-hexane, capped, and transferred to the fridge (4°C) for storage.

2.3.1.2 CLEAN-UP

To avoid lipids and other compounds from the OHC extracts to interfere with the final measurement, these compounds were removed by absorption chromatography. Florisil (magnesium silicate, particle size 150-250 μm) and Na_2SO_4 powder was burnt for eight hours at 450 °C. Glass columns were packed with a bit of cotton cleaned with dichloromethane (DCM) in the tip of the columns, 1 g of florisil, and 1 g of sodium sulfate. The glass columns were placed on racks in a fume hood and each column was rinsed with 6 mL n-hexane. The florsil column was also rinsed with n-hexane. The sample was added to the column and the glass was rinsed with 1 mL n-hexane, which was also put through the florisil column. Next, 12 mL n-hexane with 10 % DCM was added to all the columns and the extract collected into clean 15 mL glass vials. All steps during this process had to be done with precision and caution to prevent the columns from going dry. 5-10 drops of isooctane were added to the sample extract, and the samples were evaporated down to 0.2 mL with the use of RapidVap. The concentrated sample extracts were transferred to gas chromatography (GC) vials with the use of glass capillary pipettes and n-hexane used to rinse the glass tubes was also added to the GC vials. To reduce sample volume to approximately 30 μL, N_2 evaporation was used. Finally, 10 μL of recovery standard (PCB 159; 213 pg/ μL) was added to the sample extract. The vials containing the sample extracts were transferred to the fridge (4°C) for storage.

2.3.1.3 INSTRUMENTAL ANALYSIS

Detection and quantification of PCBs, PBDEs, DDTs and MeSO-PCBs/DDE were conducted one by one using an Agilent 7890 gas chromatograph with a triple quadrupole mass spectrometer, Quattro Micro GC (Waters Corporation, Manchester UK) in electron ionization mode. The OCPs were analysed with the use of an Agilent 7890A GC equipped with a 5975C mass spectrometer (Agilent Technology, Boblingen, Germany) in negative chemical ionization (NCI) mode. 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) with precolumn (0.53 mm i.d deactivated) and restriction capillary column (0.18 m i.d) was used for separation. The column contained a split/spitless injector heated at 250 ºC for hydroxy-polychlorinated biphenyls (OH-PCBs), 280 ºC for PCBs, $MeSO_2$-PCBs/DDE, PBDEs, and OCPs and 220 ºC for DDTs. 1 μL of sample volume was introduced with spitless mode with helium (6.0 quality; Yara Praxair AS, Porsgrunn, Norway). In this way, helium worked as a carrier gas at a flow rate of 1 mL/min (under constant flow). The temperature was set to 70 ºC for 3 minutes for the majority of

samples and 2 minutes for the OCPs samples. This was followed by a temperature increase of 15 ºC per minute to 180 ºC. This was followed by a second temperature incline of 5 ºC per minute to 280 ºC where the temperature was held constant for 6 minutes (10 minutes for OPCs and 5 minutes for OH-PCBs).

2.3.2 QUANTIFICATION AND QUALITY CONTROL

2.3.2.1 QUANTIFICATION

Quantification of the concentrations of the individual compounds was performed using the internal standard method. The ISTD contains known concentrations of ^{12}C and ^{13}C labelled equivalents to the compounds analysed and were used to quantify the amount of the compounds in the sample. Baseline noise do occur during the process and need to be controlled. To do this, a blank sample is included when running the GC. In addition, as a quality control, a reference sample containing a known concentration of a compound was processed together with each sample batch. At the end of the sample preparation, recovery standards were added to each sample to measure the amount of internal standard that had been lost while preparing the samples. For this, the LCQuan software package (Thermo Fisher Scientific Inc., Waltham, MA, USA, Version 2.6) was used. The labelled standards were used to make a standard curve. The area under the standard peak was used to calculate unknown compound concentration in the sample extracts (Equation 2).

$$\boldsymbol{C_{Sample}} = \boldsymbol{rf}\frac{(\boldsymbol{C_{std}}\times\boldsymbol{Area_{sample}})}{\boldsymbol{Area_{std}}} \qquad \text{Equation 2}$$

In Equation 2, C_{sample} represent the unknown concentration of sample extract, C_{std} is the known concentration of the labelled standard added to the sample, $Area_{sample}$ is the area under the curve obtained from the chromatogram for the samples, and $Area_{std}$ represent the area under the standard curve. The response factor (rf) is calculated from the areas and concentration of the standard obtained in the chromatogram.

2.3.2.2 QUALITY CONTROL

Recovery standards were added to each sample prior to instrumental analysis. In this way recovery of the ISTD could be calculated to ensure quality of the method. When analysing blood samples, NILU uses reference material from the AMAP ringtest (organized by Institute National de Sante Publique du Quebec). These samples were included together with the solvent

blank sample in all analytical batches. In case of contamination of the blanks, the limit of detection (LOD) for the blank sample was set to three times the blank signal. In case of no contamination of the blanks, the LOD was set three times of the instrumental noise. The LOD for all the different samples are shown in Appendix A (Table A.4). NILU as a participant in the AMAP ringtest for human blood, verifies the quality of this chemical analysis.

2.4 DATA TREATMENT AND STATISTICAL ANALYSIS

In total, 53 blood samples (14 glaucous gulls, 19 kittiwakes, 20 eiders) were sampled and used in the DNA-DSB analysis. In addition, were 34 blood samples (14 glaucous gull and 20 eiders) analysed for contaminants. Excel (2020) was used for calculations of MML (kbp) and DNA-FTM (%). All statistical analyses were conducted using R, Version 3.6.2 (R Core Team, 2019). Level of significance was set to 0.05 (α=0.05). Chemical data were not available for kittiwake, and this species was excluded from all statistical analyses concerning contaminant data.

2.4.1 DATA TREATMENT AND LIMIT OF DETECTION

The detection frequencies (DF) for all the contaminants are listed in Appendix A, Table A.4. Compounds detected in less than 60 % of the samples (DF <0.6) for each species were considered too poorly represented in the dataset to have sufficient statistical power, and were excluded from further statistical analyses. 29 out of 77 compounds were excluded from further analyses. Missing values were removed for compounds with DF>0.6 by giving individual compounds under LOD a random number between 0 and the compound-specific LOD using the random number generator in R-studio. Consequently, 14 and 24 substitutions were made for glaucous gull and eider, respectively. Thus, the substitutions represent 2.13 % of the total dataset for glaucous gull and 4.29 % of the total dataset for the eider. In total for both species, the substitutions represent 3.12 % of the total dataset.

Parallel gels were run for each sample during gel electrophoresis, with a triplicate of each sample on each gel. In addition, each sample was run multiple times on different days. In this way, significance of both “gel” and “replicate was checked. Parallel gels were averaged together if the coefficient of variance (CV) were below 20 %. If one gel showed CV above 20 %, the best gel was chosen and used for calculation of MML and DNA-FTM.

2.4.2 DESCRIPTIVE STATISTICS

2.4.2.1 BIOMETRIC DATA AND BODY CONDITION INDEX

Body condition index (BCI) was used to determine the relative mass of an individual, which is the mass of an individual corrected for size. Pearson correlation tests were, due to the normality of the residuals, performed on the biometric data (weight (g), wing (mm), tars (mm), and head (mm)) to explore whether any of the following biometric variables: wing, tars, or head were predicting weight. Regression analysis was used to show standardized body mass as a function of standardized size. The body condition index was determined by the standardized residuals from the regression (i.e., differentiation between the observed body mass and the predicted size). A positive BCI (above zero) indicates an over average body condition for that individual, while a negative BCI indicates an under average body condition for that individual.

The sample size for eider was too small (n=10) to detect any significant relationship between the biometric data and body mass with which body conditions indices could be estimated. Therefore, additional biometric data from eiders sampled from the same colony in Kongsfjorden, Svalbard was included. The data used was taken from Haarr (2016). Head (mm) explained a significant part of weight (r=0.47, p=0.04). Linear regression was performed with weight as the dependent variable and head as the independent variable (lm, R^2=0.17, n=19, p=0.047). From the regression, the standardized residuals were extracted and used as BCI.

For kittiwake, biometric data was available for all 19 individuals. The sample size for kittiwake was too small (n=19) to detect any significant relationship between the biometric data and body mass with which body conditions indices could be estimated. Therefore, additional biometric data from eiders sampled from the same colony in Kongsfjorden, Svalbard was included. The data used was taken from Haarr (2016). Head explained a significant part of weight (lm, R^2=0.21, n=42, p=0.001). From the regression, the standardized residuals were extracted and used as BCI.

Because of known variations in size (sexual dimorphism), correlation analysis was conducted separately for glaucous gull males and females. No additional data was available for the glaucous gull. For glaucous gull females (n=10), no significant relationship between weight and the other biometric variables was found, likely due to the small sample size. Wing explained most of the weight, although no significant relationship was found (r=0.41, p=0.24). Linear

regression was performed with weight as the dependent variable and wing as the independent variable (R^2=0.06, n=10, p=0.25). From the regression, the standardized residuals were extracted and used as BCI. For glaucous gull males (n=4), tars explained a significant part of weight (r=0.96, p=0.04), despite the small sample size. Linear regression was performed, with weight as the dependent variable and tars as the independent variable (R^2=0.89, n=4, p=0.04). From the regression, the standardized residuals were extracted and used as BCI.

2.4.2.2 CORRELATION ANALYSIS

Correlation analyses, as part of the data exploration, were conducted. Shaprior-Wilk 's test was applied prior to correlation analysis to ensure normality of the residuals. If necessary, variables were log-transformed to fulfil the assumption of normally distributed residuals. When the data had normally distributed residuals, the correlation analysis were conducted with Pearson product-moment correlation coefficient. Spearman's rank test was used in cases of non-normality (monotonic relationship) because this test does not depend upon an assumption of normality.

2.4.2.3 GROUPING OF CONTAMINANTS

To reduce the number of variables, contaminants were grouped dependent on physicochemical properties as well as the correlation analysis. Hence, 77 contaminants were grouped into seven larger groups. Chlorinated pesticides were placed together in one group called OCPs. The PCB congeners were grouped according to Borgå et al. (2005): PCBI+II: PCB 153, -180, -183, -187, -194 (PCB I), -99, -138 (PCB II), PCB III: PCB 28, -105, -118, and PCB IV: PCB 52, -101. The grouping is based on persistency of the congeners. PCB I+II are treated as one group that includes the most persistent congeners due to lack of vicinal hydrogen atoms (PCB I) and lack of metabolism by di-ortho CI-substitution (PCB II). PCB III includes the congeners that have Cl-substitution in the ortho-meta position. This group can, owning to non- or mono-ortho Cl-substitution, be metabolized by cytochrome P450 1A (CYP1A). PCB IV have Cl-substitution in the meta-para position with two or less ortho-Cl substitutions. All PCBs pooled together as one major group is referred to as PCBs. PCB metabolites were grouped into two separate groups: HPCs and $MeSO_2$-PCBs. The brominated compounds were grouped as PBDEs. OCPs, PCB I+II, PCB III, PCB IV, and HPCs were detected in both species, while $MeSO_2$-PCBs and PBDEs were only detected in glaucous gull.

2.4.3 Comparison between species and sexes

2.4.3.1 DNA damage

Comparison between the three species with respect to levels of DNA damage were tested with a non-parametric alternative to one-way ANOVA test called Kruskal-Wallis rank test due to data with not normally distributed residuals.

2.4.3.2 Contaminant levels

Out of the 77 chemicals analysed, 27 chemicals were detected in both glaucous gulls (male and females) and eiders. Species-specific differences and sex-specific differences were investigated. The unpaired two-sample t-test was used to compare the mean of the two independent groups as long as the residuals were normally distributed, and the groups had equal variance. Shapiro-Wilk test was used to check for normally distributed residuals and F-test was used to check for equal variance between groups. Variables that did not have normally distributed residuals were log-transformed to obtain normally distributed residuals. Wilcoxon sign rank test was used on untransformed variables in cases where the assumptions for the unpaired two-sample t-test were not met. If the groups had normally distributed residuals, but unequal variance was an alternative to the unpaired two-sample t-test, called Welch t-test used.

2.4.4 Multivariate statistics

Multivariate analysis was performed using both MML and DNA-FTM as response variables for DNA damage. All factors (both dependent and independent variables) were log-transformed to fulfil model assumptions. For partial least squares regression (PLSR), all variables were scaled due to large differences in concentrations (ng/g ww).

2.4.4.1 Principal component analysis

Principal component analysis (PCA) is a multivariate statistical technique frequently used on complex dataset with many variables because large and complex dataset can be challenging to interpret (Jolliffe & Cadima, 2016). PCA utilizes linear transformation known as orthogonal transformation to convert multiple variables into groups of linearly uncorrelated variables called principal components. The first principal component (PC1) explains most of the variation followed by PC2, and so on (Abdi & Williams, 2010). In this manner, the dimensionality of the

dataset is reduced making the dataset more interpretable, and at the same time minimizes the information loss (Jolliffe & Cadima, 2016).

A PCA including all three species, was used for visualization of all variables together and for exploration of potentially relationships between the different variables. Furthermore, PCAs were also conducted for each species separately. All variables were log-transformed due to non-normality of the residuals and all data were scaled (divided by standard derivation (SD)) and mean centred (subtracted the mean from all observations) prior to the analysis (mean=0, SD=1).

2.4.4.2 PARTIAL LEAST SQUARES REGRESSION

PLSR was used to investigate potential linear relationship between DNA-DSB measurments and contaminants. DNA-FTM and MML was modelled as a function of the different contaminant groups (ΣOCP, ΣPCB I+II, ΣPCB III, ΣPCB IV, ΣPBDE, ΣHPC, $\Sigma MeSO_2$) and BCI. PLSR modelling can account for multicollinearity between contaminant groups and handle a small sample size. PLSR modelling was therefore chosen over other linear regression methods (Carrascal et al., 2009). This method analyses the variance in the dependent variable (DNA-FTM and MML) without losing degrees of freedom (df) of increasing parameters. In this way, the statistical power of the study is increased even though the sample size is relatively small.

All the variables were log-transformed and scaled to unit variance (mean=0, SD=1) and centred prior to analysis. The data from the PLSR modelling was used to make coefficient plots, which were used to determine if the predictor variables had a significant effect on the response variable (DNA-FTM or MML). The effect on DNA damage was determined as significant if the 95 % confident interval (95 % CI) did not include zero (error bars not overlapping zero).

2.4.4.3 LINEAR MODELS

Based on the result from the PLSR, linear models were run to confirm the association between the dependent DNA measurements (DNA-FTM and MML) and the independent variables (ΣOCPs, ΣPCB, ΣPBDEs, ΣHPCs, ΣMeSOs). Due to multicollinearity, all the PCB congeners were included as one group called ΣPCB. BCI and sex (glaucous gull) were also included in the model. Linear models were run separately for each species.

The most complex model (the global model) was simplified until all predictor variables were significant. The best model was chosen, based on the principle of parsimony. The most parsimonious model showing the largest significance and adjusted R squared (R^2-value) was considered the best model.

Quantile quantile (QQ)-plots and histograms of the residuals were checked to confirm the normality of the residuals and make sure that all the assumptions of linear regression were met.

3 RESULTS

3.1 BIOMETRIC ESTIMATES

The biometric estimates and BCI for all three species are presented in Table 3.1. Mean (± standard error (SE)) are shown for all the variables (weight (g), head (mm), tars (mm), and wing (mm), BCI). Biometric estimates for each individual are shown in Table B1 in Appendix B.

Table 3.1: Biometrical estimates: weight (g), head (mm), tars (mm), wing (mm), and body condition index (BCI) reported as mean (±standard error) for glaucous gull (n=14), kittiwake (n=19), and eider (n=20) from Kongsfjorden, Svalbard, sampled in the breeding season of 2015.

Biometric variables	**Glaucous gull (n=14)**		**Kittiwake (n=19)**	**Eider (n=20)**
	Males (n=4)	Females (n=10)	Females (n=19)	Females (n=20)
Weight (g)	1737.5 ±74.32	1401±13.86	403.68±7.04	1834.55±73.98
Head (mm)	151.5±1.94	137±0.58	90.23±0.90	118.09±0.73
Tars (mm)	73.6±2.42	68.59±0.6	34.64±0.33	50.81±0.51
Wing (mm)	482.5±2.60	460.5±3.35	314.53±2.10	284.83±1.55
BCI	-0.08 ±0.49	-0.073±0.35	0.40±0.25	0.12±0.26

The eider had the highest body mass (±SE) with 1834.55 g (±73.98), followed by glaucous gull males with 1737.5 g (±74.32), glaucous gull females with 1401 g (±13.86), and kittiwake with 403.68g (±7.04). The eider had significant higher body mass compared to glaucous gull (Wilcoxon test, p=0.001) and kittiwake (Wilcoxon test, $p=7.3e^{-06}$). The glaucous gull had significantly higher body mass compared to kittiwake (Wilcoxon test, $p=1.3e^{-06}$) (Kruskal-Wallis, $p=2.2e^{-08}$). The glaucous gull males were significantly heavier than the glaucous gull females (Wilcoxon test, p=0.006). However, no significant differences in BCI between the species were detected.

3.2 LEVELS OF DNA DOUBLE-STRAND BREAKS

The mean, SE, median, and range of the DNA-FTM (%) and MML (kbp) for glaucous gull (n=14), kittiwake (n=19), and eider (n=20) are presented in Table 3.2. The mean DNA-FTM and MML presented with SD and CV for each individual are found in Appendix B, Table B.2.

Table 3.2: DNA fraction of total DNA migrated (DNA-FTM) and median molecular length (MML) for glaucous gull (n=14), kittiwake (n=19), and eider (n=20) from Kongsfjorden, Svalbard, sampled in the breeding season of 2015. DNA-FTM (%) and MML (kbp) are presented as the mean, standard error (±SE), median, and range.

DNA-FTM (%)	**Mean**	**± SE**	**Median**	**Range**
Glaucous gull (n=14)	10.95	± 2.64	7.86	4.2-40.0
Kittiwake (n=19)	61.60	± 3.79	63.03	36.99-89.84
Eider (n=20)	12.67	±1.82	11.30	2.86-30.84
MML (kbp)				
Glaucous gull (n=14)	346.99	± 6.19	352.22	290.14-381.87
Kittiwake (n=19)	387.35	±14.54	395.78	230.64-503.72
Eider (n=20)	326.37	± 7.25	330.28	272.82-384.74

The mean DNA-FTM (±SE) was highest for kittiwake (61.60 % ±3.79), followed by eider (12.67 % ±1.82), and glaucous gull (10.95 % ±2.64). Significant differences in DNA-FTM was found between the species (Kruskal-Wallis, $p=1.6e^{-08}$). A significant difference in DNA-FTM were found between kittiwake and glaucous gull (Wilcoxon test, $p=4.9e^{-09}$) and between kittiwake and eider (Wilcoxon test, $p=2.9e^{-11}$) (Figure 3.1a).

The eider had the overall lowest mean MML (326.37 kbp ±7.25), followed by glaucous gull (346.99 kbp ±6.19), and kittiwake (387.35 kbp ±14.54). A significant difference was found between the species in MML (Kruskal-Wallis, p=0.00076). A significant difference between eider and glaucous gull (Wilcoxon test, p=0.047), between eider and kittiwake (Wilcoxon test, p=0.0003), and between glaucous gull and kittiwake (Wilcoxon test, p=0.024) was found (Figure 3.1b).

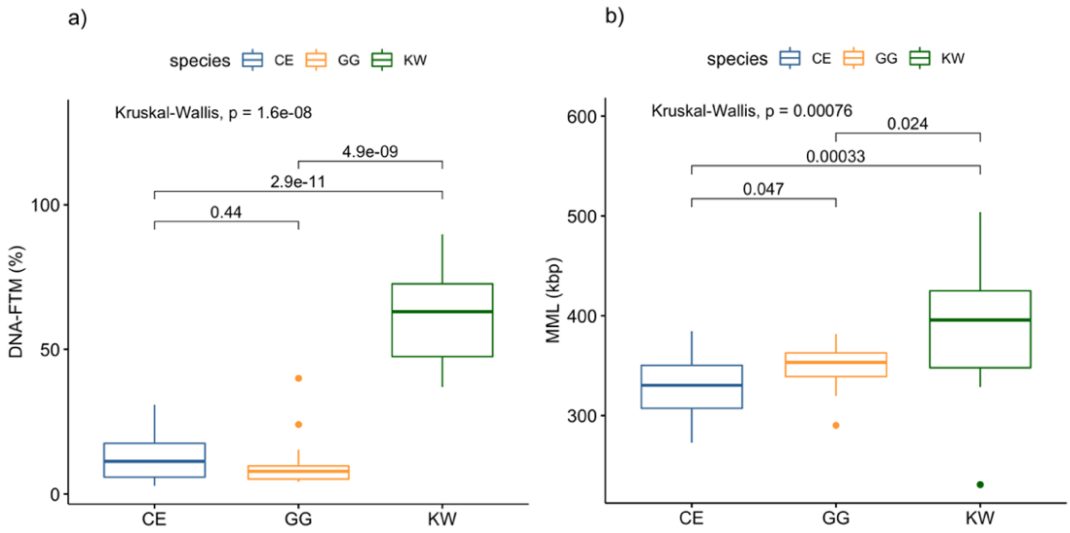

Figure 3.1: DNA measurements for DNA double-strand breaks (DNA-DSB) in eider (CE) (n= 20), glaucous gull (GG) (n=14), and kittiwake (n=19) sampled at Svalbard during the breeding season 2015. DNA measurement: a) DNA fraction of total migrated DNA (DNA-FTM (%) and b) median molecular length (MML (kbp)). The boxplot represents the median value of DNA measurement (horizontal bold line). The Kruskal-Wallis test was used to test for statistical differences in DNA-measurements between the species and p-value are shown in the figure. Significance level (α) is set to 0.05.

To determine if patterns of DNA damage were consistent between the two DNA damage measurements, the correlation between DNA FTM and MML within species were estimated. For glaucous gull, DNA-FTM and MML were negatively correlated (r=-0.60, p=0.02), while no significant correlation was found for eider (r=0.32, p=0.17) or kittiwake (r=-0.34, p=0.16).

Sex-specific differences in DNA-FTM and MML were investigated in glaucous gull. No significant differences between males and females, with respect to DNA-FTM (t-test, n=14, p=0.7) (Figure 3.2a), nor MML (t-test, n=14, p=0.87) (Figure 3.2b) were found.

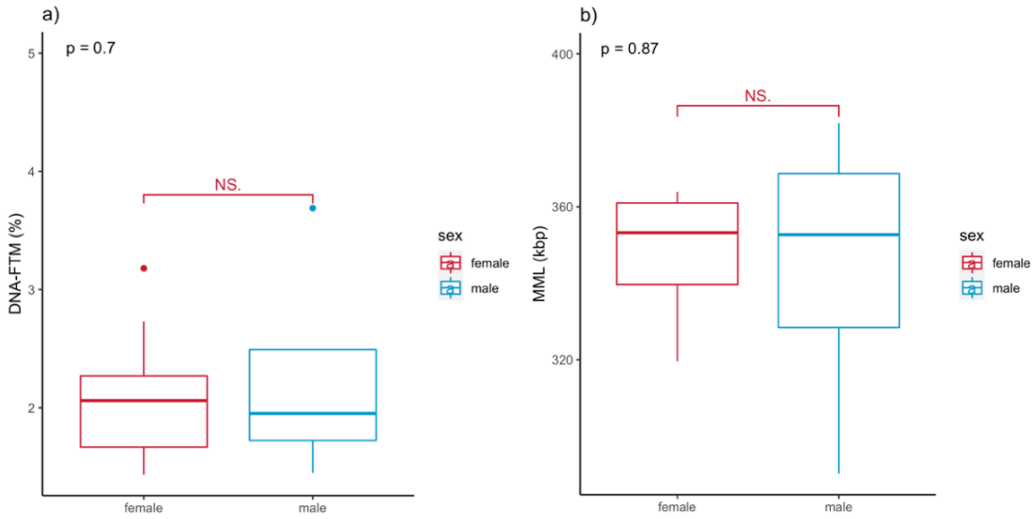

Figure 3.2: Differences between female (n=10) and male (n=4) glaucous gull (n=14) regarding DNA fraction of total migrated DNA (DNA-FTM (%)) (a) and median molecular weight (MML (kpb)) (b). DNA-FTM was log-transformed to fulfil model assumptions. The unpaired two-sample t-test (DNA-FTM) and Welch t-test (MML) was used to test for statistical differences between female and male. No significant differences in DNA-FTM (a), nor MML (b) were found. Significance codes are given as; *'NS'* =not significant, *'*'* =0.05, *'**'= 0.01*

3.3 CONTAMINANT LEVELS, PATTERNS, AND BIOTRANSFORMATION EFFICIENCY

3.3.1 CONTAMINANT LEVELS

Mean compound concentration (ng/g ww), SE, median, and range for all the compounds are listed in Appendix B (Table B.3 and Table B.4). Mean blood OHCs concentration (ng/g ww) in glaucous gull (males and females) and eider are presented in Table 3.3. Male glaucous gull showed the overall highest contaminant concentration (Σ_{47}OHC 453.03 ng/g ww) followed by glaucous gull females (Σ_{47}OHC 149.37 ng/g ww) and eider (Σ_{28}OHC 2.77 ng/g ww). The ΣPCB I+II was found in the highest concentration in the glaucous gull male (279.78±87.14 ng/g ww) and female (85.43±18.11 ng/g ww), whereas for eider, ΣOCP (1.08±0.15 ng/g ww) was the contaminant group found in the highest concentration.

Table 3.3: Mean blood organohalogen contaminants (OHC; ng/g ww) concentration from two different arctic seabirds; eider (n=20) and glaucous gull n=14). All samples are collected in Kongsfjorden, Svalbard, during the breeding season 2015.

	Eider (n=20)	**Glaucous gull (n=14)**	
	Females (n=20)	Males (n=4)	Females (n=10)
ΣOCP	1.08±0.15	129.93±37.16	46.98±6.47
ΣPCB I+II	0.71±0.07	279.78±87.14	85.43±18.11
ΣPCB III	0.32±0.02	30.47±9.25	11.22±1.81
ΣPCB IV	0.048±0.004	0.19±0.53	0.23±0.12
ΣPBDE	ND	6.42 ±1.73	3.48±0.40
ΣHPC	0.89±0.09	5.50±1.35	1.47±0.10
$\Sigma MeSO_2$	ND	0.35±0.07	0.10±0.01
ΣOHC	2.77	453.03	149.37

On species level, significant differences were observed with respect to contaminant levels (Figure 3.3). Glaucous gull had significantly higher levels of ΣOCP (Wilcoxon, $p=1.4e^{-09}$), ΣPCB I+II (t-test, $p=2.1e^{-14}$), ΣPCB III (Wilcoxon, $p=1e^{-06}$), ΣPCB IV (Wilcoxon, p=0.0002), and ΣHPC (Wilcoxon, $p=5.3e^{-05}$) compared to the eider (Figure 3.3). ΣPBDE and $\Sigma MeSO_2$ were not detected in the eider.

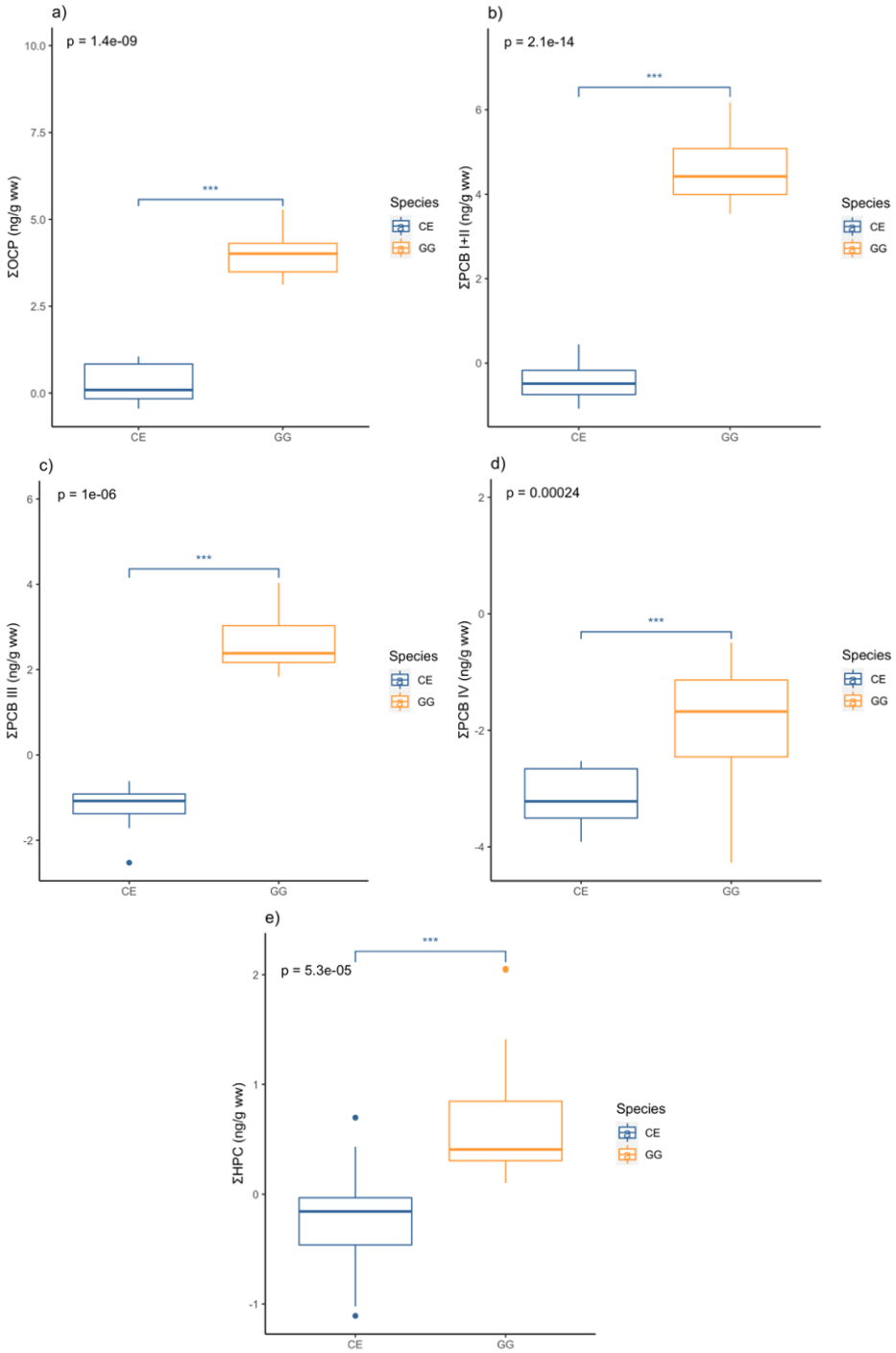

Figure 3.3: Concentration of contaminants (ΣOCP (a), ΣPCB I+II (b), ΣPCB III(c), ΣPCB IV (d) ΣHPC (e)) detected in the glaucous gull (GG) (n=14) and the eider (CE) (n=20). Unpaired two-sample t-test was used to test for significant differences between GG (orange) and CE (dark blue) if the assumptions of normality and equal variance were met. If not, alternative tests (Wilcoxon sign rank test or Welch t-test) were used to test for significant differences between GG and CE. Significance codes are given as; *'NS'* =not significant, *'*' =0.05*, *'**'= 0.01, '***'=0.001.*

For glaucous gull, males showed significant higher levels of Σ_8OCP (t-test, p=0.0067), Σ_7PCB I+II (t-test, p=0.056), Σ_3PCB III (t-test, p=0.051), Σ_{17}HPC (Wilcoxon test, p=0.002), and Σ_4MeSO_2 (Wilcoxon, p=0.008) compared to females. No significant differences were observed between females and males with respect to Σ_6PBDE (t-test, p=0.08) and Σ_2PCB IV (Wilcoxon test, p=0.24) (Figure 3.4).

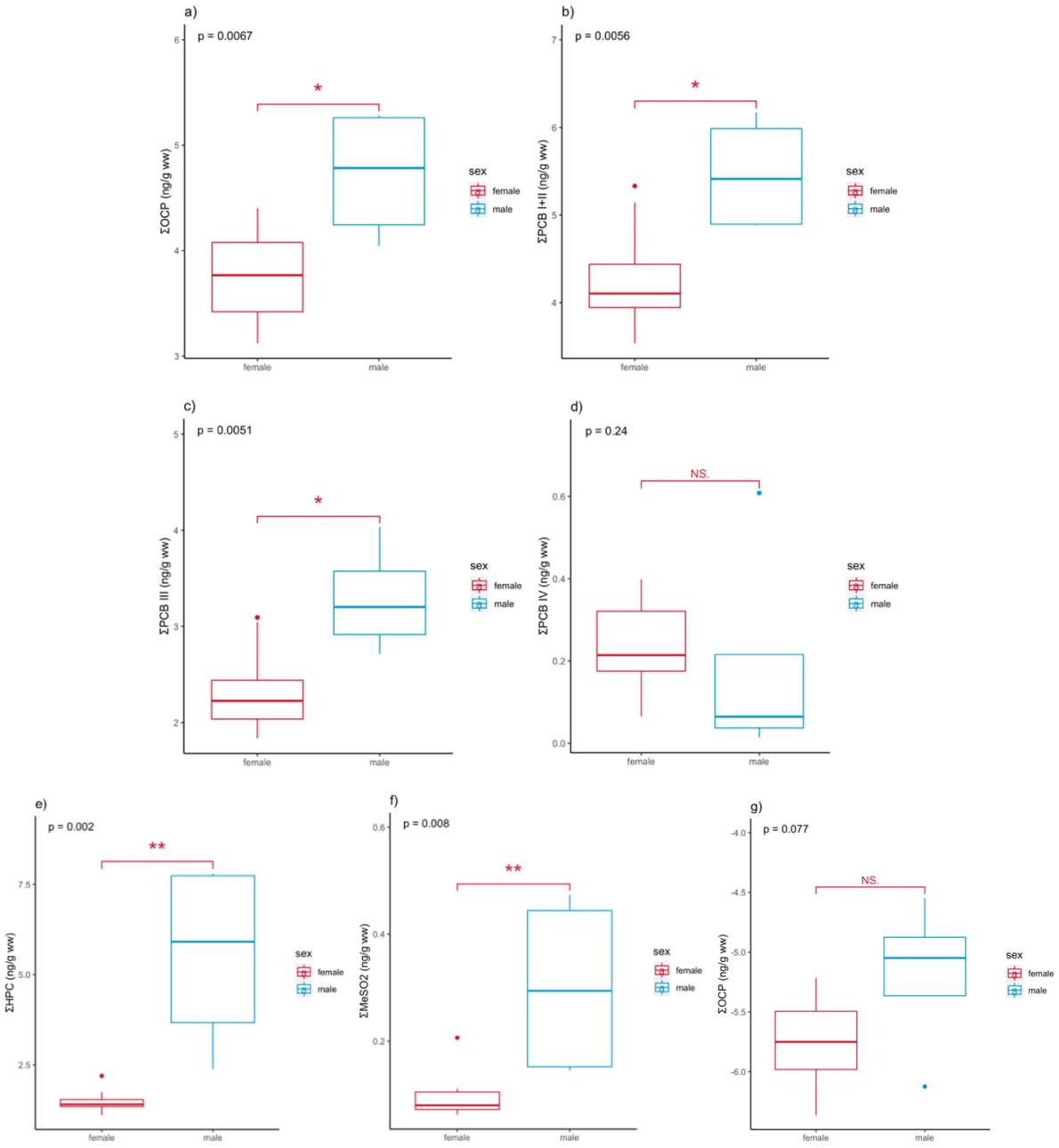

Figure 3.4: Sex-specific variation with respect to Σ_8OCP (a), Σ_7PCB I+II (b), Σ_3PCB III(c), Σ_2PCB IV, Σ_{17}HPC (e), and Σ_4MeSO$_2$ (f), and Σ_6PBDE (g) in glaucous gull individuals (n=14). Unpaired two-sample t-test was used to test for significant differences between male (light blue) and female (red) if the assumptions of normality and equal variance were met. If not, alternative tests (Wilcoxon sign rank test or Welch t-test) were used to test for significant differences between female and male. Significant differences in Σ_8OCP (a), Σ_7PCB II+II (b), Σ_3PCB III (c), Σ_{17}HPC (d), and Σ_4MeSO$_2$ (e) were found. No significant differences were between male and female in Σ_2PCB IV and Σ_6PBDE found. Significance codes are given as; *'NS'* =not significant, *'*'*=0.05, *'**'= 0.01.*

3.3.2 CONTAMINANT PATTERNS

The relative contribution and distribution of the contaminant groups differed between the species (Figure 3.5). The PCB I+II was the most abundant contaminant group in the glaucous gull males (61.81 %) and females (57.37 %). For glaucous gull males the relative contribution of the contaminant group decreased in the following order: Σ_7PCB I+II (61.81 %) > Σ_8OCP

(28.71 %) > Σ_3PCB III (6.73 %) > Σ_6PBDE (1.42 %) > Σ_{17}HPC (1.22 %) > Σ_4MeSO_2 (0.08 %) > Σ_2PCB IV (0.04 %). The relative contribution of Σ_{12}PCB to the total contaminant load was 68.58 %. For glaucous gull females the relative contribution of the contaminant groups decreased in the following order: Σ_7PCB I+II (57.37 %) > Σ_8OCP (31.55 %) > Σ_3PCB III (7.53 %) > Σ_6PBDE (2.34 %) > Σ_{17}HPC (0.99 %) > Σ_2PCB IV (0.15 %) > Σ_4MeSO_2 (0.06 %). The relative contribution of Σ_{12}PCB to the total contaminant load was 65.05 %. The OCP, was the predominant contaminant group in the eider. In eider the relative contribution of contaminant groups decreased in the following order: Σ_6OCP (42.16 %) > Σ_{16}HPC (26.10 %) > Σ_3PCB I+II (20.85 %) > Σ_2PCB III (9.48 %) > Σ_1PCB IV (1.41 %) > Σ_0MeSO_2 (ND) = Σ_0PBDE (ND). The relative contribution of Σ_6PCB to the total contaminant load was 31.74 %.

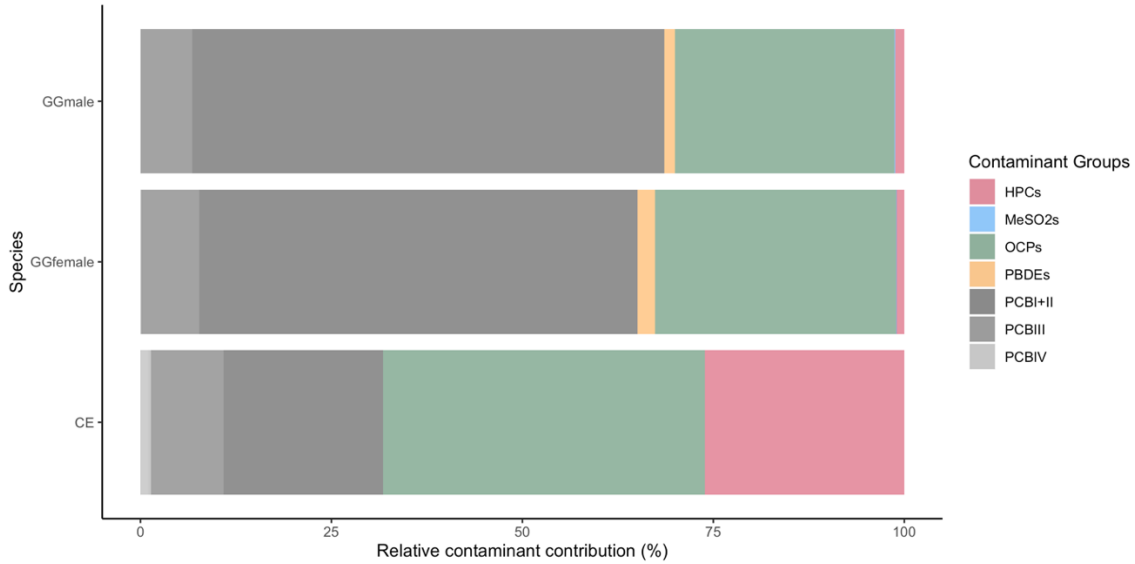

Figure 3.5: Relative contribution of the different contaminant groups (HPCs= light red, $MeSO_2$ = blue, OCPs= green, PBDEs= yellow, PCB I+II = dark grey, PCB III= grey, PCB IV= light grey) to the total contaminant load in the different species: glaucous gull male (GGmale), glaucous gull female (GGfemale), and eider (CE).

The OCP was the predominant group in eider (1.08±0.15 ng/g ww) and the second most abundant group in glaucous gull males (129.93±37.16 ng/g ww) and females (46.98±6.47 ng/g ww). ΣOCP contributed 42.16 %, 28.71 %, and 31.55 %, of the total contaminant burden, in eider, glaucous gull males, and glaucous gull females, respectively. For OCP (Figure 3.6a), the contaminant pattern among the species was relatively similar. However, β-HCH and c-chlordane were only detected in the glaucous gull. In glaucous gull males, glaucous gull females, and eider, the DDT metabolite, p.p'-DDE, was the predominant contaminant contributing to 71.62 %, 68.07 %, and 35.86 %, of the total OCP contaminant load, respectively. There was an increase in the relative contribution of p.p'-DDE to the total OCP burden and a decrease in HCB in species with increasing trophic position (Figure 3.6a). In addition, the

relative contribution of oxy-chlordane and mirex to the total OCP contaminant load did show a slight increase with trophic position and c-nonachlor and t-nonachlor showed a decrease with increasing trophic position.

The PCB I+II was the most abundant group in the glaucous gull male, glaucous gull female, and the second most abundant group in the eider. ΣPCB I+II constituting 61.81 %, 57.98 %, and 31.74 %, of the total contaminant burden in glaucous gull males, glaucous gull females, and eiders, respectively. The ΣPCB III and ΣPCB IV contributed less to the total contaminant load in both species. The relative contribution of ΣPCB III to the total contaminant load was 6.73 %, 7.61 %, and 9.48 % in glaucous gull male, glaucous gull female, and eider, respectively. Moreover, the relative contribution of ΣPCB IV was 0.04 %, 0.16 %, 1.41 %, in glaucous gull male, glaucous gull female, and eider, respectively. Several PCBs congeners were only detected in the glaucous gull (males and females). This includes PCB28, PCB52, PCB180, PCB183, PCB187, and PCB193. For both species, PCB 153 was the predominant contaminant in the group (Figure 3.6b). In the glaucous gull male, glaucous gull female, and eider, the PCB 153 contributed to 36.52 %, 33.12 %, and 35.45 %, of the total PCB burden, respectively. The relative contribution of PCB 153, PCB99, and PCB 138 did not show an increase with increasing trophic position. However, the less persistent PCB 118, PCB 105, PCB 101 did show a slightly decrease in relative contribution with increasing trophic position.

The HPC was the second, fifth, and sixth most abundant contaminant groups in eider, glaucous gull males, and glaucous gull females. ΣHPC contributing 26.10 %, 1.22 %, and 0.99 %, to the total contaminant load, in eider, glaucous gull males, and glaucous gull females, respectively. For the glaucous gull, the contribution of ΣHPC to the total contamination burden was relatively small in comparison to the ΣPCB I+II and ΣOCP. On the other hand, for the eider, the ΣHPC contributed to moderate levels (26.10 %) to the total contaminant burden in the species. The 4-OH-PCB 172 and 4-OH-PCB 193 was only detected in glaucous gull, while the 4-OH-PCB 130 was only detected in the eider. In the glaucous gull male and female, the 4-OH-PCB187 and 4-OH-PCB146 were the most abundant contaminants contributing to the total HPC contaminant load. The 4-OH-PCB187 and 4-OH-PCB146 contributed respectively 59.89 % and 20.45 % of the total HPC contaminant burden in glaucous gull males, and respectively 36.23 % and 13.64 % of the total HPC contaminant burden in glaucous gull females (Figure 3.6c). For eider, 4-OH-PCB 107 was predominant, contribution solely 19.08 % of the total HPC contaminant load (Figure 3.6c). The relative contribution of 4-OH-PCB187 and 4-OH-PCB146 to the total HPC

load showed an increase with trophic position, while the relative contribution of 4-OH-PCB107 showed a decrease with increasing trophic position (Figure 3.6c).

PBDEs were only detected in glaucous gull males and females (Figure 3.6d) and was the fourth most prominent group detected in both males and females. ΣPBDE contributed 1.42 % and 2.34 % to the total contaminant load, in males and females, respectively. The PBDE 47 was, in both males and females, the predominant PBDE, contributing 57.19 % and 51.73 %, to the total PBDE contaminant burden, respectively.

The $MeSO_2$ contaminants were only detected in the glaucous gull males and females (Figure 3.6e). $MeSO_2$ was the second and least prominent contaminant group detected in the males and females. Σ$MeSO_2$ contributed 0.08 % and 0.06 %, to the total contaminant load, in males and females, respectively. The $MeSO_2$ was detected but did not contribute greatly to the total contaminant load in the glaucous gull. In glaucous gull males and females, the relative contribution of 3-MeSO-PCB101 was 52.34 % and 42.43 % to the total $MeSO_2$ contaminant burden, respectively.

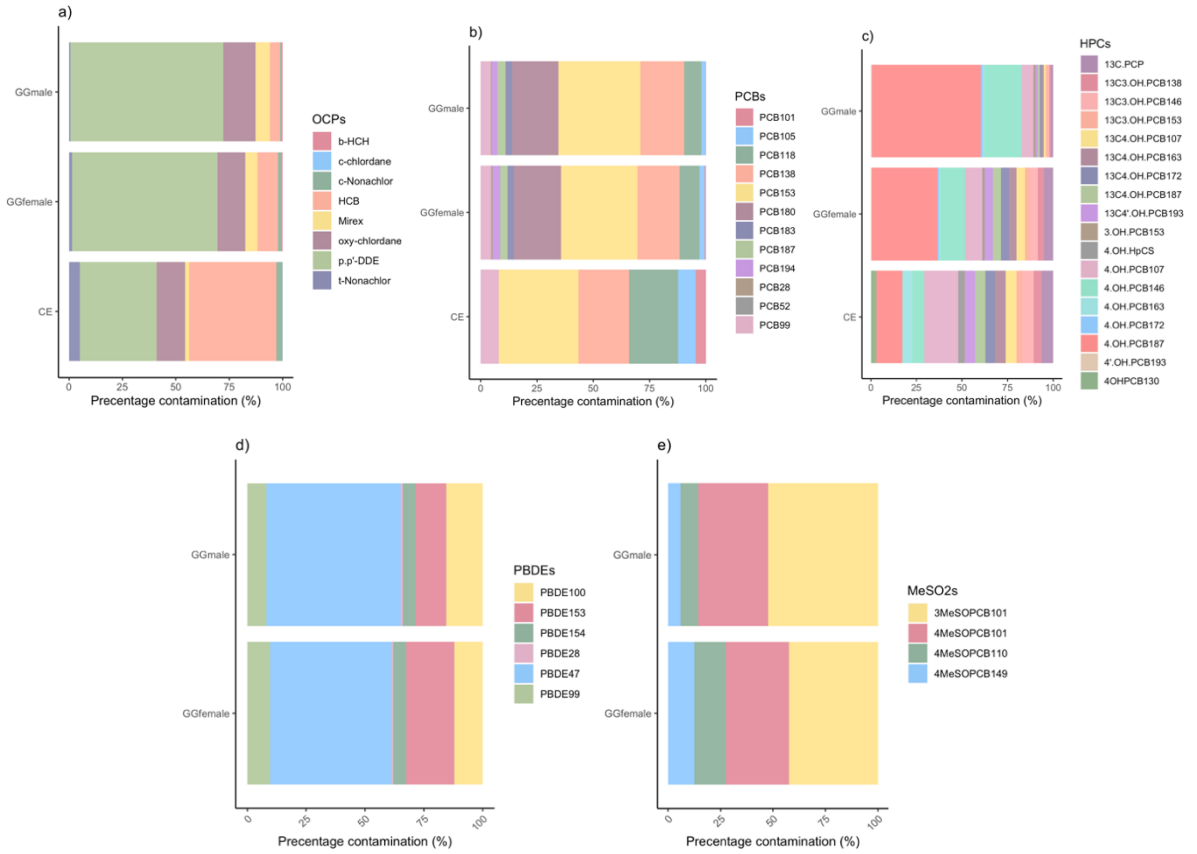

Figure 3.6: Contribution from single compounds to the different contaminant groups: OCPs (a), PCBs (b), HPCs (c) in common eider (CE) and glaucous gull male and female (GGmale and GGfemale, respectively). PBDE (d), and $MeSO_2$ (e) were only detected in the glaucous gull male and female. All individuals were sampled in Kongsfjorden, Svalbard during the breeding season of 2015.

3.3.3 BIOTRANSFORMATION EFFICIENCY

Mean biotransformation efficiency (ΣHPC to ΣPCB and $\Sigma MeSO_2$ to ΣPCB ratio) are presented in Table 3.4 for eider and glaucous gull. The metabolite to parent ratio was not significantly different between glaucous gull males and females, and both sexes were pooled together during analysis. For glaucous gull, ΣHPC to ΣPCB and $\Sigma MeSO_2$ to ΣPCB were found to correlate negatively with circulating ΣPCB concentrations (p=0.043 and p=0.044, respectively) (Figure 3.7a and 3.7b). In eider, no significant correlation between ΣHPC to ΣPCB ratio and circulating ΣPCB concentrations was found.

Table 3.4: The mean biotransformation ratio in eider (n=20), glaucous gull males (n=4), females (n=10) and both sexes pooled together (n=14). All individuals are sampled from Kongsfjorden, Svalbard during the breeding season 2015.

Species	ΣHPC to ΣPCB ratio	$\Sigma MeSO_2$ to ΣPCB ratio
Eider	0.8207	
Glaucous gull male	0.0237	0.001
Glaucous gull female	0.0187	0.001
Glaucous gull together	0.0201	0.001

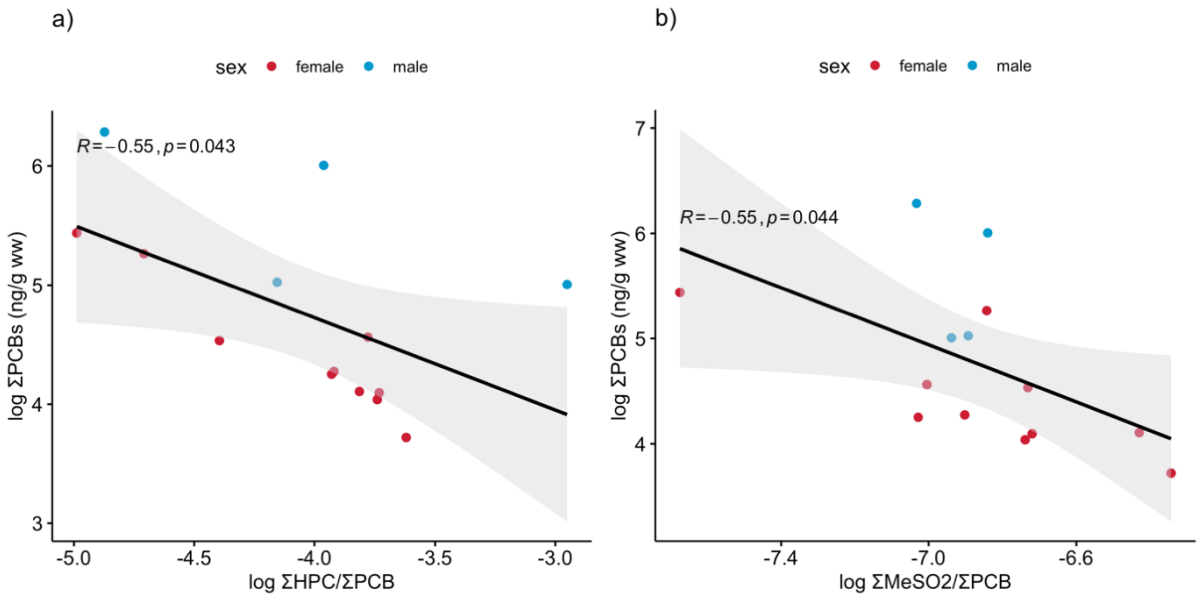

Figure 3.7: Correlation plots representing biotransformation efficiency for male (light blue) and female (red) glaucous gull (n=14) sampled from Kongsfjorden, Svalbard during the breeding season of 2015. Log-transformed circulating ΣPCB concentrations (ng/g ww) in relation to log-transformed ΣHPC to ΣPCB ratio (a) and log-transformed circulating ΣPCB concentrations (ng/g ww) in relation to log-transformed $\Sigma MeSO_2$ to ΣPCB ratio (b). R-value and corresponding p-value are shown in the figure.

3.4 ASSOCIATIONS BETWEEN CONTAMINANTS AND DNA DOUBLE-STRAND BREAKS

In the PCA and linear regression analysis ΣPCB I+II, ΣPCB III, and ΣPCB IV were pooled together as one major group called ΣPCB, due to multicollinearity. The PLSR can handle multicollinearity and small sample size, therefore ΣPCB I+II, ΣPCB III, and ΣPCB IV were analysed separately.

3.4.1 PRINCIPAL COMPONENT ANALYSIS

3.4.1.1 PCA COMBINED FOR ALL SPECIES

A PCA was conducted including all species for illustating the variance between the species with respect to all variables (OHCs, biometric data, and DNA measurements). The PC1 and PC2 explained 49.15 % and 19.83 %, of the total variation in the dataset, respectively (Figure 3.8).

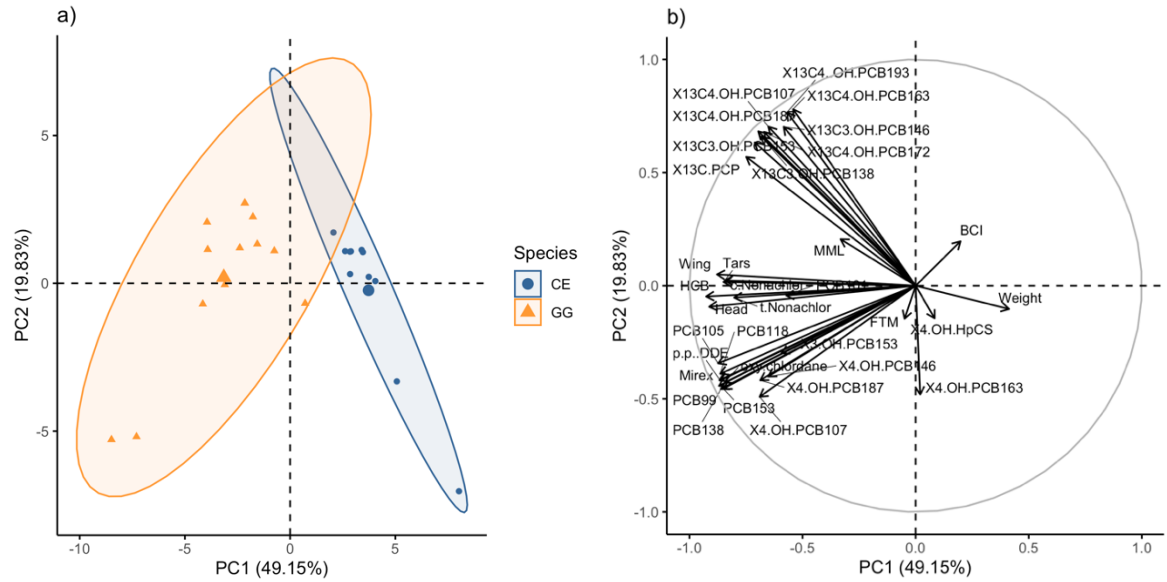

Figure 3.8: Score plot (a) and loading plot (b) for glaucous gull (GG) and eider (CE). The individuals are coloured according to species; GG= blue (n=13), CE= red (n=11) in the score plot. The loading plot indicates the orientation of the different variables (OHCs, biometric data, and DNA measurements). The score plot and the loading plot only include contaminants detected in both species. The principal components (PC1 and PC2) explained 49.15 % and 19.83 %, respectively, of the total variation in the dataset.

The score plot (Figure 3.8a) shows a clear grouping of individuals according to species, which indicates interspecies differences. As presented above, biometric data and the common contaminant groups (ΣOCP, ΣPCB, and ΣHCP) were statistically different between the species (section 3.1 and 3.3, respectively). The glaucous gulls and eiders are located along the negative side and the positive side, respectively, of the PC1 axis. Hence, the glaucous gulls are positioned

on the same side along the PC1 axis, as the common contaminant groups are located in the loading plot (Figure 3.8b). The glaucous gulls are therefore associated with high contaminant levels. Conversely, the eiders are located on the opposite side along the PC1, where none of the contaminant groups are located in the loading plot (Figure 3.8b). Eiders are therefore eiders associated with lower contaminant levels.

The grouping of species in the score plot, in combination with the differences in contaminant levels and biometric variables between the species suggest species-specific differences among most variables. Hence, analysing data from all species together may mask significant results or eliminate potential confounding factors due to species differences. Therefore, a PCA was conducted for each species separately.

3.4.1.2 PCA EIDER

In eider, the three major contaminant groups detected were OCP, PCB, and HPC. In the PCA for eider, PC1 and PC2 explained 36.07 % and 25.34 % of the variability in the dataset, respectively. Combined, PC1 and PC2 explained 61.41 % of the total variability in the dataset. These first two PC axes were plotted against each other in a loading plot (Figure 3.9). Contribution of each PC, each predictor variable, and each individual are shown in Appendix B (Figure B1, B2, and B3).

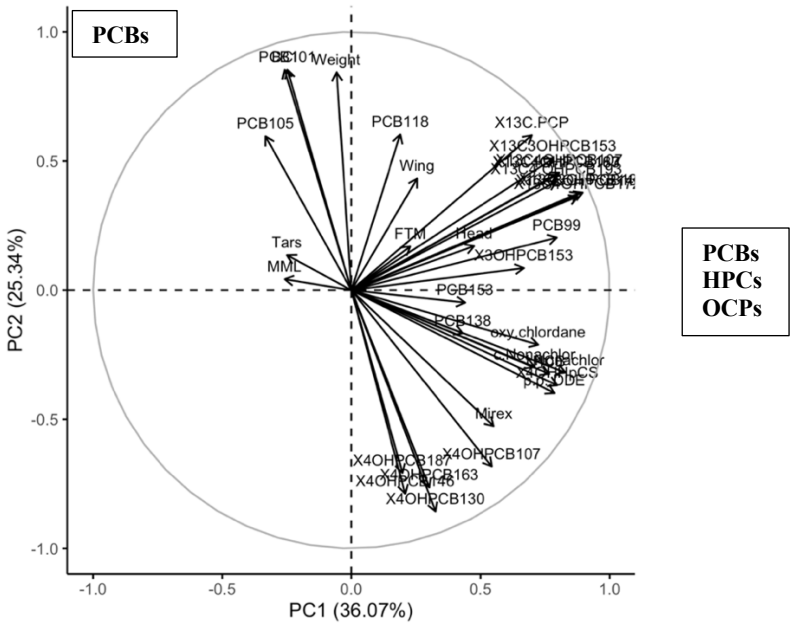

Figure 3.9: The principal components (PC1 and PC2) plotted against the loading plot for the eider individuals (n=11). The plot indicates the orientation of the OHC (OCP, PCB, and HPC), biometric variables, body condition index (BCI), and measurements of DNA damage (DNA-FTM and MML).

In the PCA, most contaminants were located in the same area in the plot (along the positive side of the PC1 axis). The PCA showed no clear clustering of contaminants according to the major contaminant groups, with exception of the OCP, which were all located in the right lower quadrate. The PCBs were spread in the loading plot, located in the left, upper quadrate, the right upper quadrate, and the right lower quadrate. The HPCs are spread out along the positive side of the PC1 axis. The OCP and HPC located in the right, lower quadrate indicates a positively association with each other. Moreover, the MML was located in the opposite quadrate, indicating a negative association between MML and OCP and MML and HPC. DNA-FTM is located together with the HPCs in the right upper quadrate, indicating a positive association.

Correlation tests were performed to investigate the relationships between variables indicated in the PCA. The correlation test confirmed a positive relationship between ΣOCP and ΣHPC ($r=0.69$, $p=0.02$). None of the other contaminant groups showed any sign of correlation with each other. Furthermore, none of the contaminant groups showed any association with MML or DNA-FTM. The correlation table for eider is presented in Appendix B, Table B.5.

3.4.1.3 PCA GLAUCOUS GULL

In glaucous gull, the five major contaminant groups detected were OCP, PCB, PBDE, HPC, and $MeSO_2$. In the PCA for glaucous gull, the PC1 and PC2 explained 44.33 % and 14.57 % of the total variability in the dataset, respectively. Combined, PC1 and PC2 explained 58.90 % of the total variability in the dataset. These first two PC axes were plotted against each other in a loading plot (Figure 3.10). Contribution of each PC, each predictor variable, and each individual are shown in Appendix B (Figure B4, B5, and B6).

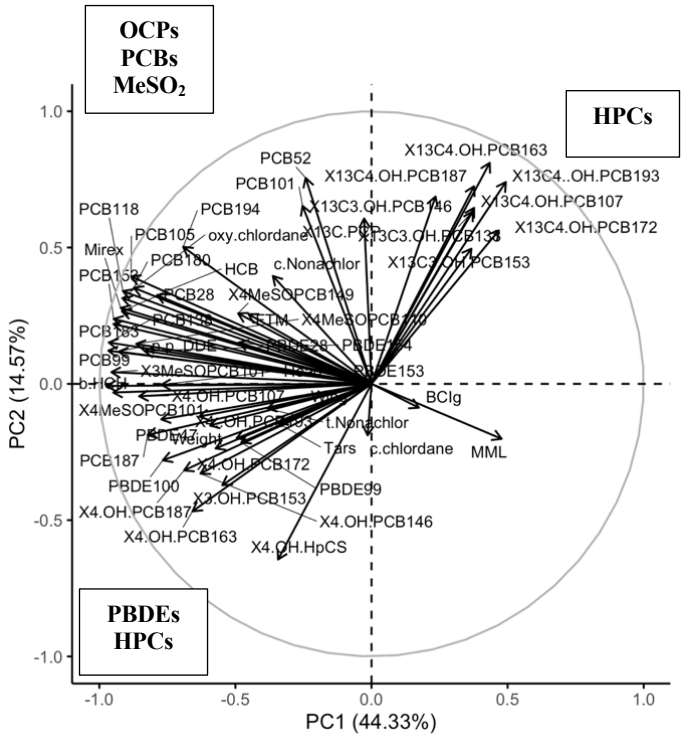

Figure 3.10: The principal components (PC1 and PC2) plotted against the loading plot for the glaucous gulls (n=11). The plot indicates the orientation of the OHCs (OCP, PCB, PBDE, HPC, and $MeSO_2$), biometric variables, body condition index (BCI), and measurements of DNA damage (DNA-FTM and MML).

In the PCA for glaucous gull, most contaminants were located in the same area in the plot (along the negative side of the PC1 axis), and no clear contaminant pattern was observed for the major contaminant groups. HPCs are located in the left lower quadrate and the right upper quadrate, indicating no strong association within the HPC group. The majority of PBDE were located in the left, lower quadrate and the remaining contaminant groups, OCP, PCB, and $MeSO_2$ were located mostly in the left, upper quadrate. This indicates a positive association between these contaminant groups. Moreover, DNA-FTM is located in the left, upper quadrate indicating a positive association with the contaminant groups located there. MML was located in the opposite quadrate (right, lower quadrate) indicating a negative association with the respective contaminant groups and the DNA-FTM.

Correlation tests confirmed a positive correlation between ΣOCP and ΣPCB ($r=0.96$, $p= 5.573e^{-08}$), ΣOCP and ΣHPC ($r=0.76$, $p=0.003$), ΣOCP and Σ$MeSO_2$ ($r=0.80$, $p=0.003$), and between ΣPCB and Σ$MeSO_2$ ($r=0.82$, $p=0.0005$). Furthermore, correlation between ΣPCB and ΣHPC ($r=0.78$, $p=0.002$), and between ΣHPC and Σ$MeSO_2$ ($r=0.65$, $p=0.01$) were found. This was not clearly indicated in the PCA. A significant negative association between MML and DNA-FTM was found ($r=-0.60$, $p=0.02$). The correlation table for glaucous gull (both sexes pooled together) is presented in Appendix B, Table B.6. These correlations could be due to sex-specific

differences, and thus, based on knowledge that the glaucous gull sexes are dimorphic were PCAs were conducted separately for each sex.

In the PCA for glaucous gull males (n=4), PC1 and PC2 explained 54.09 % and 27. 33 % of the total variability in the dataset, respectively. Combined, PC1 and PC2 explained 81.42 % of the total variability in the dataset. These first two PC axes were plotted against each other in a loading plot (Figure 3.11). Contribution of each PC, each predictor variable, and each individual are shown in Appendix B, (Figure B7, B8, and B9).

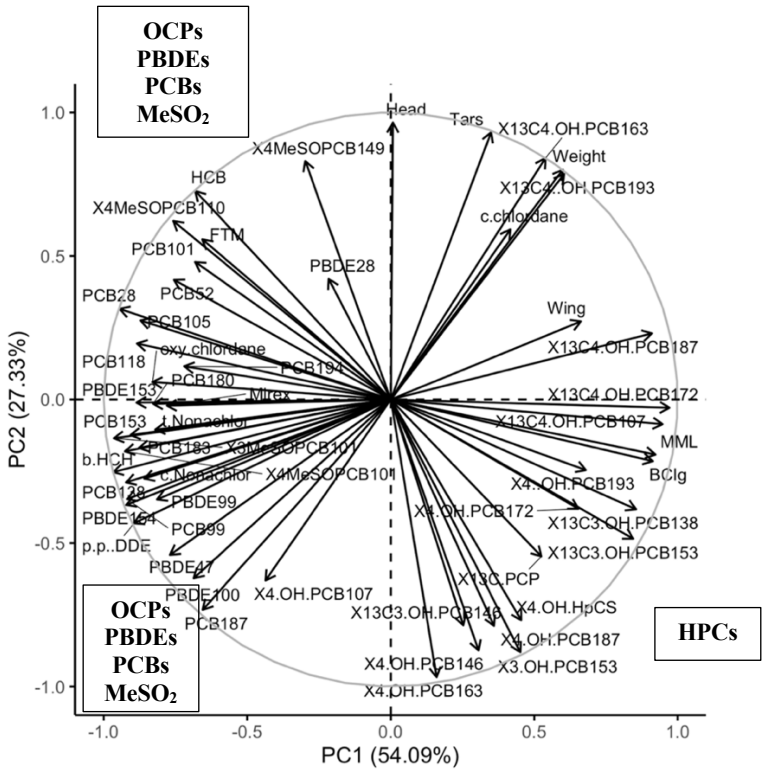

Figure 3.11: The principal components (PC1 and PC2) plotted against the loading plot for the glaucous gull males (n=4). The plot indicates the orientation of the OHCs (OCP, PCB, PBDE, HPC, and $MeSO_2$), biometric variables, body condition index (BCI), and measurements of DNA damage (DNA-FTM and MML).

The majority of the contaminant groups are located in the same area in the plot, along the negative side of the PC1 axis, with exception to the HPCs that are mainly located along the positive side of PC1. MML is located together with the HPC, indicating a positive association between HPCs and MML in male glaucous gull individuals. DNA-FTM, located in the left upper quadrate, indicates a negative association between DNA-FTM and HPCs and between DNA-FTM and MML. The other contaminant groups, located along the negative side of the PC1 axis, are spread out along the positive and negative side of the PC2 axis. These contaminants position indicate a positive association with DNA-FTM and a negative association with MML.

Correlation tests confirmed a positive correlation between ΣOCP and ΣPCB (r=0.96, p=0.04) and a negative association between ΣPCB and MML (r=-0.95, p=0.05). However, no other significant correlations were found (Appendix B, Table B.7).

In the PCA for glaucous gull females (n=10), PC1 and PC2 explained 37.47 % and 18.24 % of the total variability in the dataset, respectively. Combined, PC1 and PC2 explained 55.71 % of the total variability in the dataset. These first two PC axes were plotted against each other in a loading plot (Figure 3.12). Contribution of each PC, each predictor variable, and each individual are shown in Appendix B (Figure B10, B11, and B12).

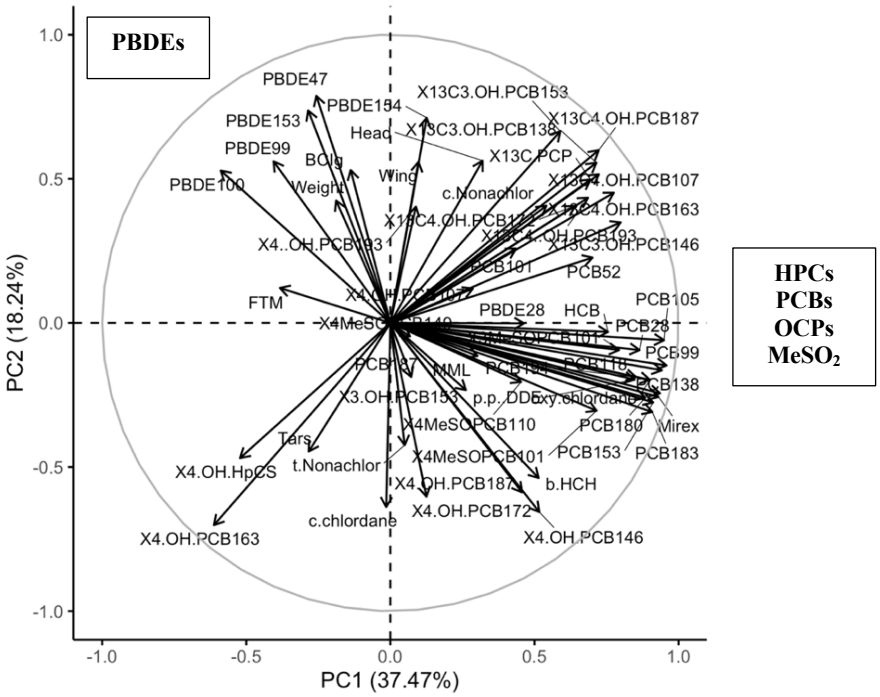

Figure 3.12: The principal components (PC1 and PC2) plotted against the loading plot for the glaucous gull females (n=10). The plot indicates the orientation of the OHCs (OCP, PCB, PBDE, HPC, and $MeSO_2$), biometric variables, body condition index (BCI), and measurements of DNA damage (DNA-FTM and MML).

In the PCA, the majority of the contaminants were located in the same area in the plot, along the positive side of the PC1 axis. However, the PBDEs were, together with DNA-FTM, located in the left upper quadrate, indicating a positive association between the PBDE and DNA-FTM. MML was, located along the positive side of the PC1 together with the majority of the other contaminant groups, indicating a positive association between the majority of the contaminant groups and MML. This suggest a negative association between the majority of the contaminates and PBDEs and the majority of the contaminants and DNA-FTM, as well as between MML and PBDE and between MML and DNA-FTM.

Correlation tests confirmed a positive correlation between ΣOCPs and ΣPCBs (r=0.92, p=0.0002), ΣOCP and $\Sigma MeSO_2$ (r=0.67, p=0.03), and between ΣPCB and $\Sigma MeSO_2$ (r=0.74, p=0.01). No significant correlation between the other contaminant groups or the DNA measurements were found (Appendix B, Table B.8).

3.4.2 PARTIAL LEAST-SQUARES REGRESSION

Partial least-squares (PLS) regression was conducted to analyse the best predictor variables for the response variables. ΣOCPs, ΣPCB I+II, ΣPCB III, ΣPCB IV, ΣPBDEs, ΣHPCs, $\Sigma MeSO_2$, and BCIs were included as predictor variables, while DNA-FTM and MML were included as the response variables. PLS regression was conducted for each species separately.

3.4.2.1 PLS EIDER

For eider (n=11), ΣOCP, ΣPCB II+I, ΣPCB III, ΣPCB IV, and ΣHPC and were included with BCI in the model. None of the predictor variables were significant in explaining DNA-FTM (Figure 3.13a), nor MML (Figure 3.13b).

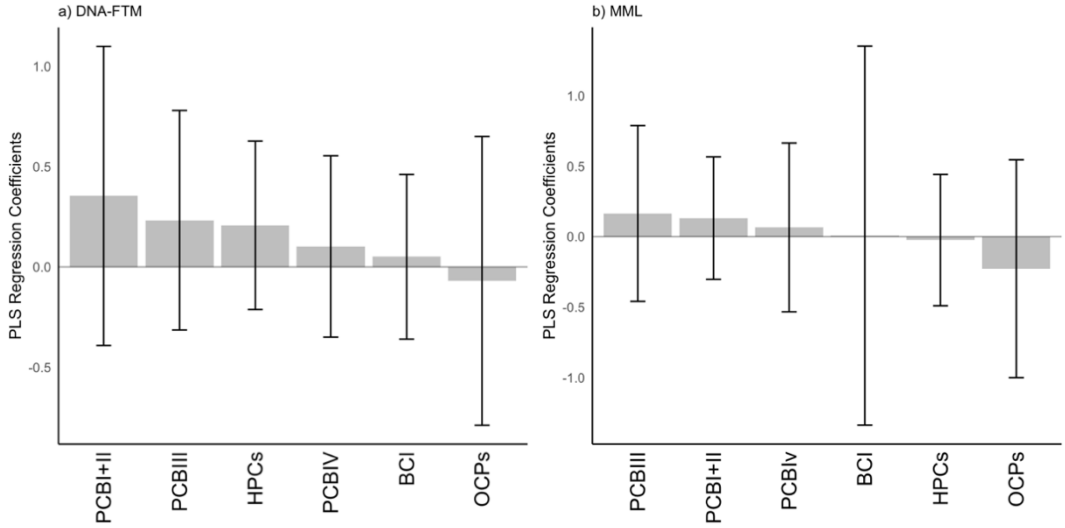

Figure 3.13: Partial least squares regression coefficients for eiders (n=11), sampled in Kongsfjorden, Svalbard during the breeding season 2015. DNA-FTM (a) and MML (b) are response variables. A positive association with the response variables is represented with boxes above zero, while a negative association with the response variables is shown with boxes below zero. When the 95 % confident intervals do not cross zero, the regression coefficient are significant. None of the predictor variables were significant in explaining the response variables.

3.4.2.2 PLS GLAUCOUS GULL

ΣOCP, ΣPCB I+II, ΣPCB III, ΣPCB IV, ΣPBDEs, ΣHPCs, $\Sigma MeSO_2$, and BCI were included in the model as predictor variables. None of the predictor variables were significant in explaining DNA-FTM (Figure 3.14a), nor MML (Figure 3.14b) when both sexes were pooled together (n=14).

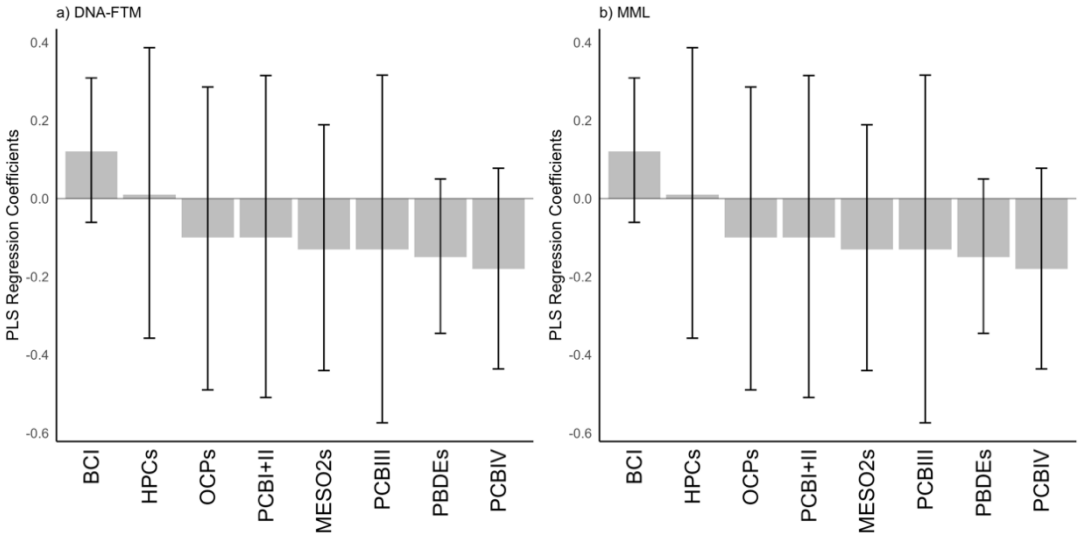

Figure 3.14: Partial least squares regression coefficients for glaucous gulls (n=14), sampled in Kongsfjorden, Svalbard during the breeding season 2015. DNA-FTM (a) and MML (b) are response variables. A positive association with the response variables is represented with boxes above zero, while a negative association with the response variables is shown with boxes below zero. When the 95 % confident intervals do not cross zero, the regression coefficient are significant. None of the predictor variables were significant in explaining the response variables.

Due to sexual dimorphism, PLS regressions were performed separately for males (Figure 3.15) and females (Figure 3.16). In the PLS model, including glaucous gull males (n=4), none of the predictor variables were significant in explaining DNA-FTM (a). However, a significant negative association between MML and ΣOCP (t=-5.3, p=0.013), ΣPCB III (t=-5.5, p=0.012), ΣPCB I+II (t=-7.3, p=0.005), ΣPCB IV (t=-5.4, p=0.012), and $\Sigma MeSO_2$ (t=-4.4, p=0.022) were found (Figure 3.15b). In addition, a significant positive association between MML and BCI was found (t=4.2, p=0.024) (Figure 3.15b).

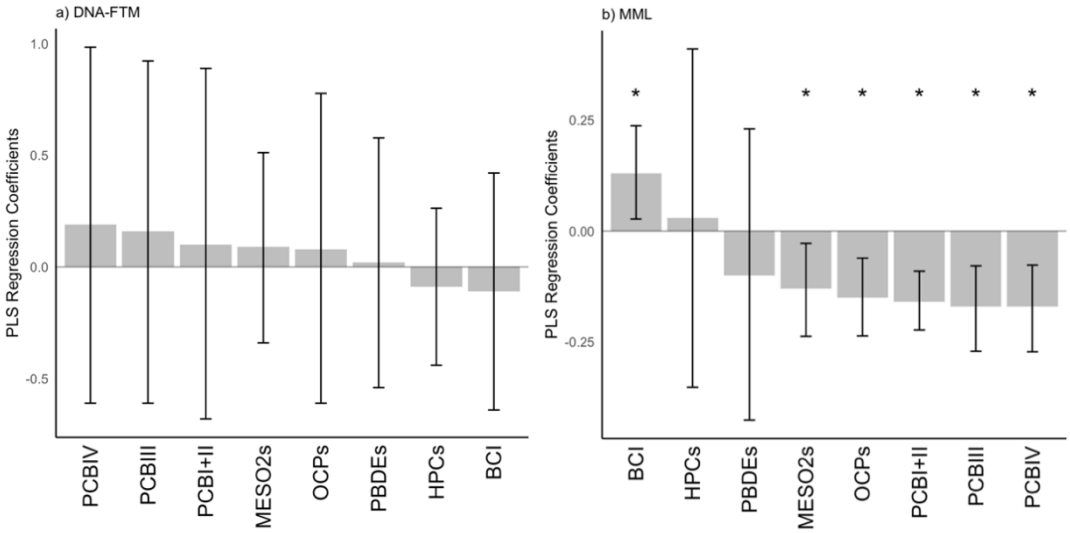

Figure 3.15: Partial least squares regression coefficients for glaucous gull males (n=4), sampled in Kongsfjorden, Svalbard during the breeding season 2015.DNA-FTM (a) and MML (b) are response variables. A positive association with the response variables is represented with boxes above zero, while a negative association with the response variables is shown with boxes below zero. When the 95 % confident intervals do not cross zero, the regression coefficient are significant. Significance is denoted with asterisks (*). Significant code is given as; '*'= 0.05.

In the PLS model including glaucous gull females (n=10), only ΣPCB I+II was significant in explaining DNA-FTM (t=-2.5, p=0.03) (Figure 3.16a). ΣPCB I+II was negatively related to DNA-FTM. None of the predictor variables were significant in explaining MML (Figure 3.16b).

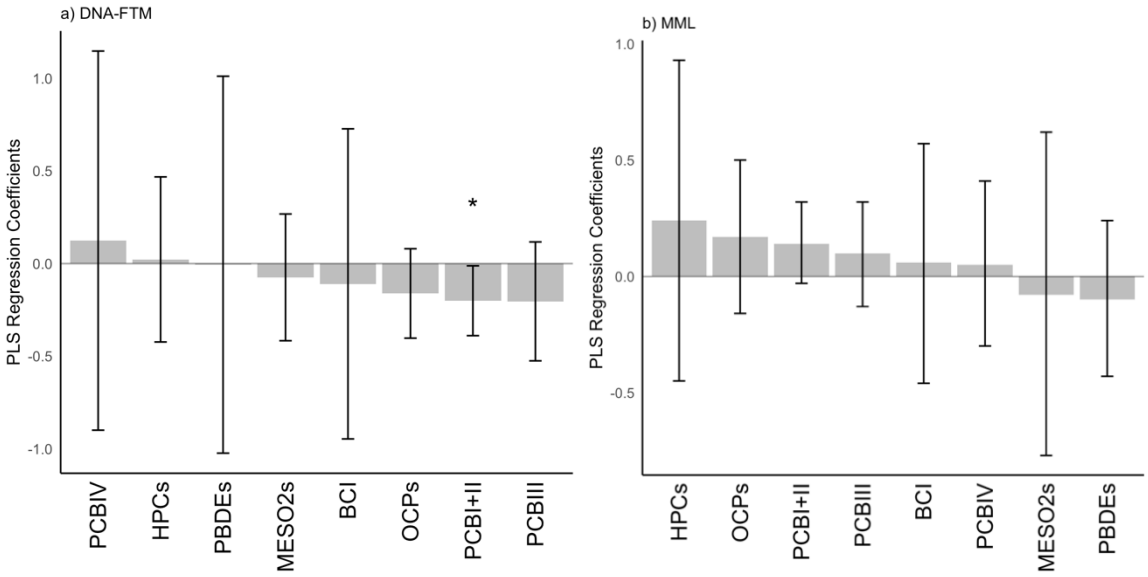

Figure 3.16: Partial least squares regression coefficients for glaucous gull females (n=10) sampled in Kongsfjorden, Svalbard during the breeding season 2015. DNA-FTM (a) and MML (b) are response variables. A positive association with the response variables is represented with boxes above zero, while a negative association with the response variables is shown with boxes below zero. When the 95 % confident intervals do not cross zero, the regression coefficient are significant. Significance is denoted with asterisks (*). Significant code is given as; '*'= 0.05.

3.4.3 LINEAR MODELLING

Linear models were performed to confirm associations between the contaminant groups and DNA measurements (DNA-FTM and MML) indicated in the PLS regression analysis. Due to multicollinearity, all the PCB congeners were pooled together as one group. For the glaucous gull, a model including both sexes (n=14) and a model including only females (n=10), were run. The sample size for glaucous gull males was too small to run a linear regression. Model estimates from the best model with SE, t-values, and p-values for all species are presented in Table 3.5 presented.

3.4.3.1 LINEAR MODEL EIDER

For eider (n=11), the linear models indicated that none of the contaminants were significant in explaining DNA-FTM, nor MML. These results are in agreement with the result of the PLS regression. The best model for both DNA-FTM (t=14.7, p=2.19e-07) and MML (t=187.3, p= <2e-16) was the null model, including only the intercept.

3.4.3.2 LINEAR MODEL GLAUCOUS GULL

A linear model including glaucous gull males and females (n=14) was conducted. The model included the interaction with sex to account for sexual dimorphism. For both DNA-FTM (t=12.16, p=$7.83e^{-12}$) and MML (t=255.8, p=$<2e^{-16}$) the best model was the null model, including only the intercept. This result is in conjunction with the result from the PLS regression.

A linear model regression including only female individuals (n=10) was conducted. For DNA-FTM (t=12.18, p=6.79e-07) the best model was the null model, including only the intercept. HPCs and BCI were positively associated with MML which is not in agreement with the results from the PLS regression. However, this result should be interpreted with caution due to HPC and BCI being correlated (Pearson, r=-0.67, p=0.03).

Table 3.5: The data was analysed using linear regression. Model estimates from the best linear model for DNA measurement (DNA-FTM and MML) are presented with standard error, t-value, and p-value. DNA measurement (DNA-FTM and MML) and Σcontaminants have been log-transformed to account for not normally distributed residuals.

Model	Species	Coefficients	Coeff. value	Standard error	t-value	p-value
DNA-FTM ~1	CE	Intercept	2.32	0.16	14.7	$7.83e^{-12}$
MML ~1	CE	Intercept	5.78	0.02	255.8	$<2e^{-16}$
DNA-FTM ~1	GG	Intercept	2.15	0.18	12.16	$1.79e^{-08}$
MML ~ 1	GG	Intercept	5.85	0.02	315.6	$<2e^{-16}$
DNA-FTM ~ 1	GG_F	Intercept	2.10	0.17	12.18	$6.79e^{-07}$
MML ~ 1	GG_F	Intercept	5.76	0.03	228.12	$8.21e^{-15}$
		+log(HPC)	0.25	0.07	3.85	0.01
		+BCI	0.04	0.01	3.01	0.02

4 DISCUSSION

There are only a limited number of genotoxic studies on arctic seabird populations. This research aimed to evaluate the genetic health of arctic seabirds by providing insight into contaminant levels, contaminant patterns, and cellular responses to contaminant exposure. Here, cellular and biochemical techniques were used to assess the association between contaminant levels and DNA damage in different arctic seabird species. For the kittiwake, no chemical data was available and contaminant levels and the association between contaminants and DNA damage will only be discussed based on previous studies. Therefore, the main parts of the discussion will focus on results obtained from eider and glaucous gull.

4.1 LEVELS OF DNA DOUBLE-STRAND BREAKS

Previous studies analysing DNA-DSBs (DNA-FTM and MML) in arctic eiders report dissimilar levels of damage. Bechmann (2016) and Fenstad et al. (2016a) have reported similar or lower levels of damage, while Fenstad et al. (2014) reported higher levels of damage compared to the levels of DNA-damage reported in the present study. Moreover, Rodvelt (2018) reported higher levels of DNA-DSBs in eider eggs. This suggests higher levels of DNA damage in eggs than in blood cells in arctic eiders. However, it should be taken into consideration when comparing these results that levels of DNA damage are measured in different matrixes and potential protocol variations may have been present. This may give false information regarding variation in levels of DNA-damage between the different studies. Fenstad et al. (2014) reported different levels of DNA-FTM in year 2008 and 2009. Furthermore, Bustnes et al. (2012) report yearly differences temperature during the breeding seasons of 2007-2009. This suggest that the variation in reported levels of DNA-FTM and MML in studies may be explained, to some extent, by seasonal weather variation. Moreover, Fenstad et al. (2014) reported how long the eiders had incubated when sampled. This was not reported in this study, neither in Bechmann (2016) and Rodvelt (2018). Therefore, incubation time could contribute to differences in levels of DNA damage between the studies. This demonstrate that comparing results from different field studies, in general, might be difficult due natural stresses, and seasonal and annual weather variation that can be difficult to control fully.

To our knowledge, this is the first study that have investigated levels of DNA-FTM and MML in blood samples from kittiwake, which mean no direct comparison can be made. Rodvelt

(2018) studied DNA-FTM and MML in arctic kittiwake eggs and found kittiwake eggs to have lower DNA-FTM and lower MML than reported in the present study. The two measurements show contradictory results, where the first suggests less damage, and the second indicates more damage in kittiwake eggs compared to blood cells. DNA-FTM are often consider a more precise measurement of DNA-damage because calculation of MML is subjected to individual choice (Fenstad et al., 2016a). However, since the different measurements give different results is it imperative that further research focus on levels of DNA damage in kittiwake.

As far as we know, only one study has investigated levels of MML in arctic glaucous gull (Krøkje et al., 2006). This study did not find a significant difference between male and female in total MML, which is in agreement with the results in the present study. However, a recent study on arctic char (*Salvelinus alpinus*) found males to have significantly higher levels of DNA-FTM, but not significant lower MML, than the females in the contaminated Lake Ellasjøen (Neerland et al., 2019). However, in the present study, no sex-specific differences in total levels of DNA-FTM was found either.

Previous studies have analysed, glaucous gull, kittiwake, and eider blood cells (Haarr et al., 2018) and eggs (Rodvelt, 2018) from the same colonies in Kongsfjorden, Svalbard. Neither found any significant differences between the glaucous gull, the kittiwake, and the eider with respect to levels of DNA damage. However, one should consider when comparing these results to the present results that DNA-damage are measured in different matrices (Rodvelt, 2018) and with the use of different methods (Haarr et al., 2018). In the present study, kittiwake showed significant higher DNA-FTM compared to glaucous gull and eider, indicating higher levels of DNA damage. Moreover, Rodvelt (2018) found kittiwake eggs to have lower DNA-FTM than both glaucous gull and eider eggs, although not significant. This is not in agreement with the results in the present study, indicating differences in stressors and/or mechanisms that cause DNA damage between the different life stages.

Eiders showed the highest levels of DNA damage when considering MML as the measurement of DNA damage. Rodvelt (2018) also found eider eggs to have lower MML than kittiwake and glaucous gull, indicating higher levels of damage in this species. The female eider incubates the eggs alone, while for glaucous gull and kittiwake both sexes incubate the eggs. Therefore, the female eiders undergo extreme weight loss during the incubation period. Thus, incubation period may have a greater impact on levels of DNA damage in eiders compared to kittiwake

and glaucous gull. In this study, information about how long the seabirds have incubated was not recorded for any of the species, but it may be important, particularly for the eiders. Previous studies have reported body mass loss to be one of the main sources of DNA damage in eiders (Fenstad et al., 2014; 2016a; Noori, 2018; McPartland, 2019). Moreover, eiders might be, to a higher degree, than glaucous gull and kittiwake, affected by the harsh arctic conditions, such as colder temperatures during the incubation period. This may potentially cause the eiders to allocate more resources towards survival mechanisms (Bustnes et al., 2012), reducing the amount of energy available for DNA repair. Moreover, metabolic capacity and environmental changes, such as climate change and food availability can result in differences in DNA damage (Fenstad et al., 2014). However, the contradicting results for DNA-FTM and MML in the present study warrant further research.

4.2 CONTAMINANT LEVELS, PATTERNS, AND BIOTRANSFORMATION

The different seabird species were expected to show different contamination levels due to different feeding strategies and trophic position. The glaucous gull, a top predator, showed higher level of contamination compared to the eider feeding on a lower trophic level, which is in conjunction with the species position in the food web (Hop et al., 2002; Borgå et al., 2004; Wold et al., 2011). The OHCs concentration was found to be within the range expected given the values reported in previous studies for glaucous gull and eider sampled from the same colony in Kongsfjorden, Svalbard (Haarr et al., 2018). The PCBs and OCPs were the main contributors to the total OHCs contaminant load in the different species. This is also in concurrence with previous studies on arctic seabird species (Verreault et al., 2005; Fenstad et al., 2016b; Haarr et al, 2018).

The levels of OHCs in eider were found to be at concentration lower than reported OHCs concentrations in eider from the Baltic sea (Fenstad et al., 2016b). As mentioned previously, the female eiders incubate the eggs alone and undergo extreme weight loss during the incubation period. Consequently, the time of incubation period may influence the association between contaminant burden and body condition in eiders. In the present study, big differences in body weight were observed (62.50 % increase from the lightest to the heaviest female), and rapid weight loss have been associated with a redistribution of contaminants from the lipid storage to the bloodstream (Fenstad et al., 2014). Moreover, previous studies have found an increase in contaminant levels at day 20 of the incubation period compared to day five in the

incubation period in female eiders (Bustnes et al., 2012; Fenstad et al., 2014). No information about how many days the eiders had incubated when sampled was recorded in this study, and therefore comparing blood contaminant concentrations with other studies might be difficult. However, variation in sampling time was relatively low in this study (1-2 days) so a major impact of incubation time on contaminant levels within the eiders used in this study was therefore considered unlikely.

In the present study, the highest contaminant levels were, as expected, found in the glaucous gull, which is in concurrence with the specie's trophic position. Generally, seabirds accumulate OHCs mainly through the diet, which may result in biomagnification of contaminants. Several studies have reported increased contaminant concentrations with increasing trophic position in avian species (Savinova et al., 1995; Hop et al., 2002; Haarr et al., 2018). However, species differences in breeding strategy, migration patterns, foraging, and physiology may have some significance (Borgå et al., 2004; Leat et al, 2013; Guzzo et al., 2014).

The glaucous gull sexes are dimorphic, and sex-specific differences in contaminant concentration were observed. This is in agreement with a previous study on glaucous gull (Melnes et al., 2017). In the present study, the male individuals showed significantly higher concentrations of all contaminant groups, with exception to ΣPBDE and ΣPCB IV. The sex-specific differences in contamination concentrations can be due to maternal transfer, different food strategies, diet quality, prey specialization, or differences in food intake. A previous study shows that maternal transfer in the glaucous gull females resulted in lower concentrations of less persistent compounds in the mother (Verreault et al., 2006b). Moreover, the study also shows that more persistent compounds were not as easily transferred to the eggs, and consequently remained in the mother (Verreault et al., 2006b).

Therefore, maternal transfer may explain the higher concentration of ΣPCB III in males but might not explain the higher concentrations of more persistent contaminants, such as ΣPCB I+II found in male individuals in the present study. This could possibly be explained by females having higher biotransformation capacity and/ or eliminate persistent contaminants better compared to males. However, this theory was not confirmed in the present study, whereas no significant sex-specific differences in biotransformation efficiency were found. Moreover, a previous study by Østby et al (2005) suggested males to be more sensitive to Aryl hydrocarbon receptor (AhR) mediated responses compared to females. Another potential explanation is that

male individuals may exhibit a bolder feeding strategy, leading to higher food intake. In addition, males may feed in more polluted areas compared to females. Higher food intake in male individuals can result in higher exposure to biotransformation inducers, but since no differentiation in biotransformation efficiency between the sexes were observed, it is more likely that males simply ingests a higher amount of contaminated food (Borgå et al., 2005).

The relative contribution of persistent contaminants is increasing with trophic position as a result of greater biomagnification potential, higher level of contaminants, and increased elimination of less persistent contaminants due to greater xenobiotic metabolic activity (Fisk et al., 2001; Borgå et al.,2004). Avian seabird species are homeotherms and thus have greater biotransformation capacity compared to poikilotherms. However, studies have reported differences in biotransformation capacity between arctic seabird species (Fisk et al., 2001; Borgå et al., 2005; Helgason et al., 2010). Several studies have reported higher relative contribution of persistent contaminants, such as p.p'-DDE, PCB 153, and PCB138, in the glaucous gull due to biomagnification (Hop et al, 2002; Haarr et al., 2018). A previous study has also reported a decrease in the relative contribution of persistent contaminant with decreasing trophic position (Borgå et al., 2001). In the present study, lower relative contribution of persistent contaminants was found in the eider, occupying a lower trophic level. Borgå et al. (2001) found persistent contaminants to be the prominent contributor to the total contaminant load in glaucous gull. Low relative contribution of less persistent contaminants in glaucous gull in the present study indicates that this species is more efficient in eliminating less persistent contaminants.

Furthermore, it was expected to observe a decrease in the contribution of less persistent contaminants, to the total contaminant load, with increasing trophic position. However, this trend was not observed in the present study. Several less persistent contaminants were under LOD in the eider, while detected in the glaucous gull. This indicates that biotransformation capacity alone does not explain the differences between contaminant patterns in the species. Higher food intake in the glaucous gull and different prey specifications may explain the differences.

In the present study, the ΣOCP levels were significantly higher in glaucous gull compared to eider. The DDT-metabolite, p,p'-DDE, was the prominent OCP contaminant in both species. Previous studies have reported high levels of p.p'-DDE in both glaucous gull (Haarr, et al.,

2018) and eider (Fenstad et al., 2016a). Moreover, arctic seabird species have shown high biomagnification potential of p.p'-DDE (Borgå et al., 2007). In the present study, an increase in the relative contribution of p.p-DDE to the total contaminant load with increasing trophic position was found. This might be explained by differences in diet or reflect higher biotransformation capacity of DDT in the glaucous gull compared to the eiders. Moreover, the HCH and chlordanes metabolites, respectively β-HCH and oxy-chlordane, have shown high biomagnification potential in seabirds (Borgå et al., 2001). This indicate differences in diet and/or high ability to metabolize and eliminate parent HCHs and chlordanes (Borgå et al., 2001). However, in the present study β-HCH and c-chlordane were only detected in glaucous gull. The opposite trend was observed for the HCB. The HCB's relative contribution to the total OCP contaminant load was decreasing with increasing trophic position. The HCB is a less persistent compound compared to the more persistent compound, p.p'-DDE, which may explain why the opposite association with trophic position was observed (Borgå et al., 2001). c-nonachlor and t-nonachlor also showed a decrease with increasing trophic position, indicating that these contaminants have low biomagnification potential.

The ΣPCB was, as expected, found in highest concentrations in the glaucous gull. The PCB 153 was the greatest contributor to the total PCB contaminant load in both species. This is in concurrence with previous studies reporting high concentrations of PCB 153 in arctic seabird species (Verreault et al., 2004; Sagerup et al., 2009b; Melnes et al., 2017; Haarr et al., 2018). PCB 153 was followed by PCB 138 and PCB180 in the glaucous gull. This is in agreement with previous studies on glaucous gull from Bjørnøya (Verreault et al., 2004) and Kongsfjorden (Haarr et al., 2018). In eiders, PCB 138 and PCB 118 were the second and third most abundant contaminants contributing to the total PCB contaminant burden. This is in concurrence with previously reported data on contaminant patterns in the eiders from Kongsfjorden (Evenset et al., 2016). The difference in contaminant patterns between the species may indicate different susceptibility to PCB congeners or different food intake between the species. Both species showed contaminant patterns dominated by persistent contaminants, indicating a high biotransformation capacity of less persistent contaminants. However, the eider contaminant pattern also includes a relatively large contribution of the less persistent PCB 138, indicating that the glaucous gull might be more efficient in the biotransformation of less persistent contaminants. This would suggest a decrease in less persistent PCBs (PCB 101 and -52) with increasing trophic position. Surprisingly, PCB 101 was only detected in low concentrations and

PCB 52 were under LOD in eider, while both were over LOD in the glaucous gull. However, PCB 101 did show a slightly decrease with increasing trophic position.

The ΣHPC levels were significantly higher in glaucous gull compared to eider. For glaucous gull, 4-OH-PCB187 and 4-OH-PCB146 were the most the prominent contaminants contributing to the total HPC contaminant load. In eiders 4-OH-PCB107 was the most prominent contaminant contributing to the total HPC contaminant load. The relative contribution of 4-OH-PCB187 and 4-OH-PCB146 to the total HCP load showed an increase with trophic position, indicating differences in food intake and/or a high biotransformation capacity of parent compounds in glaucous gull. Moreover, the relative contribution of 4-OH-PCB107 shows a decrease with increasing trophic position suggesting a lower biomagnification potential of this compound. The difference in contaminant patterns between the species suggest differences in biotransformation efficiencies.

The main source of HPC is biotransformation of parent PCB and a different pattern in HPC should reflect differences in PCB contaminant pattern. For glaucous gull, ΣHPC to ΣPCB were negatively correlated with ΣPCB concentration. This supports strong evidence of HPC as being metabolically derived in the glaucous gull. This relationship was not found in eider. This suggest interspecies differences in metabolic activity and/or elimination of metabolites. Enzymatic activity may exhibit short-term variation in response to physiological changes, such as body condition, potentially masking association between metabolic activity and metabolite concentrations. Moreover, other physiological factors, such as feeding strategy, may affect the result in the present study potentially masking associations between biotransformation activity and metabolite concentration.

PBDEs were only detected in the glaucous gull. Haarr et al. (2018) also detected PBDEs in glaucous gull, but not in eider from the same colony in Kongsfjorden. Previous studies have reported PBDE 47 to be the PBDE congener found in highest concentrations in the glaucous gull (Herzke et al., 2003; Verreault et al., 2007; 2018). This is in agreement with the present study, where PBDE47 is the most prominent contaminant contributing to the total PBDE contaminant load. However, Herzke et al. (2003) and Verreault et al. (2007) reported higher levels of PBDE in glaucous gull individuals from Bjørnøya compared to the levels reported in this study. Furthermore, the PBDE concentrations reported in glaucous gull are estimated to be over a hundred times lower than the PCB concentrations detected in the glaucous gull (Herzke

et al., 2003). This is also in concurrence with the results found in the present study. This suggests a low exposure and/or high xenobiotic metabolic activity of PBDE in arctic glaucous gull. A previous study has measured OH-PBDE in glaucous gull suggesting high metabolic activity of PBDE (Verreault et al., 2007). No PBDE metabolite data were available in the present study, and this will therefore not be discussed further.

It should be mentioned that the PBDE is, according to the Stockholm convention, an emerging POP (UNEP, 2017). This may explain why lower levels, in comparison to PCB, are found in arctic seabird species. In addition, other compounds, such as, halogenated flame retardants (HFR) and organophosphorous flame retardants (PFR) have been on the market concurrently with the PBDE, and have likely been used, at least to some degree, to replace PBDE (Vorkamp et al., 2019). HFR and PFR, have been determined in the Arctic, although in lower concentration than PBDE (Vorkamp et al., 2019). This highlights the potential threat of new emerging contaminant, made to replace the contaminants banned by the Stockholm Convention. An increasing concentration of new emerging contaminants in the Arctic may explain why the lower concentration of PBDEs have been detected in the glaucous gull and why PBDEs were not detected in eider.

$MeSO_2$-PCB was only detected in glaucous gull. $MeSO_2$ are generally shown to covary with its precursor PCB, and PCB biotransformation has been identified as an important source of $MeSO_2$-PCB in the glaucous gull (Verreault et al., 2005). In the present study, the $\Sigma MeSO_2$ to ΣPCB ratio was negatively correlated with ΣPCB blood concentration, supporting evidence of $MeSO_2$-PCB as metabolically derived. $MeSO_2$-PCB are not as lipophilic as their parent PCB and have a higher affinity to proteins (Letcher et al., 2000). The 3'- and 4'-$MeSO_2$-PCB-101 have been detected in high levels in the glaucous gull eggs (Verreault et al., 2005). This is in agreement with the result in the present study, reporting 3'- and 4'-$MeSO_2$-PCB-101 to be the main contributors to the total $\Sigma MeSO_2$-PCB contaminant load in the glaucous gull blood cells. However, it should be taken into consideration when comparing these studies that levels are measured in different matrices. It could be expected to find differences in contaminant levels between blood and egg samples. Less chlorinated $MeSO_2$-PCBs are more rapidly transferred to the eggs, whereas higher-chlorinated $MeSO_2$-PCBs are more resilient to transfer (Verreault et al., 2005). However, Verreault et al. (2006) did not find $\Sigma MeSO_2$-PCB concentration in eggs to be positive associated with female blood concentrations, and $\Sigma MeSO_2$-PCB concentration in eggs was found at concentrations six-fold lower than in female plasma. Moreover, in the same

study ΣPCB, ΣOCP, and ΣPBDE did show significant indication of maternal transfer. This suggests that different biochemical mechanisms may be involved in $MeSO_2$-PCB *in ovo* deposition in the glaucous gull. This may be related to the $MeSO_2$-PCB lower octanol-water partition coefficient and higher protein affinity (Letcher et al., 2000).

4.3 Association between contaminants and DNA double-strand breaks

The primary aim of the present study was to investigate the association between blood OHCs concentration and levels of DNA-damage in different arctic seabird species. Contaminants can induce cellular mechanisms, such as oxidative stress, that potentially can result in increased levels of DNA-damage. Based on this, it was expected that levels of DNA damage would increase with increasing blood OHC concentrations. On the contrary, contaminant exposure can induce adaptive responses, such as more efficient defence mechanisms that might have an impact on the association between blood OHCs concentration and levels of DNA-damage.

In the present study, it was expected that increased levels of contaminants would be associated with increased DNA-FTM and decreased MML. However, no association was found between contaminants and DNA-FTM, nor MML in the eiders. The lack of association found in the present study was somewhat unexpected and contrary to existing knowledge that contaminants can induce DNA-DSBs directly or indirectly through the production of ROS and toxic metabolites. Fenstad et al. (2016a) investigated the association between contaminant levels and DNA-damage in an arctic eider population from Kongsfjorden and an eider population from the Baltic sea. The high-exposed eiders from the Baltic sea showed higher levels of contamination, and a positive association between DNA-DSB frequency, OCPs and PCBs was found in this study (Fenstad et al., 2016a). In comparison, in the low-exposed eider population from Svalbard, no association between contaminant levels and DNA-DSB frequency was found. This is in agreement with the result presented in the present study. The OHC contaminant levels detected in Kongsfjorden may be under a threshold concentration for genotoxic effects in eiders. This might explain why Fenstad et al. (2016a) found an association between contaminants and DNA-DSB frequency in the high-exposure Baltic sea eiders and not in the low-exposure arctic eiders.

Other contaminants not included in the present study may interfere with the DNA integrity in arctic eiders and pose as better predictors of DNA damage. A study on an eider colony from Christiansø, Denmark found ΣPFAS and Mercury (Hg), in addition to body mass to significantly explain DNA-FTM (McPartland, 2019). Surprisingly, McPartland (2019) found that as ΣPFAS and Hg blood concentration increased, DNA-FTM decreased. Constantini et al. (2019) found a significant positive association between PFAS concentrations and oxidative stress, indicating genotoxic potential of PFAS. However, Haarr et al. (2018) did not find ΣPFAS to be a significant predictor of DNA damage in eiders sampled from the same colony in Kongsfjorden as the eiders used in the present study. Fenstad et al. (2016a) found Hg to be positively related to DNA-DSB frequency in high-exposed Baltic eiders, but not in arctic eiders. This suggests that the concentration of PFAS and Hg might be under a threshold for causing a genotoxic effect in the Arctic, and consequently are not good predictors for DNA damage in arctic eiders. However, based on previous studies reporting genotoxic potential of these contaminants, are further genotoxic studies including these contaminants warranted (Fenstad et al., 2016a; Constantini et al., 2019).

Fenstad et al. (2016a) found no significant difference in levels of DNA-DSBs between the high-exposed Baltic sea eiders and the low-exposed arctic eiders. Contaminants are only one of several potential predictors of DNA damage in arctic eiders. It has been shown that harsh conditions, in particular lower temperatures, can lead to higher weight loss during the incubation period (Bustnes et al., 2012). The lower critical temperature in arctic eiders have been found to be 7 ºC. Below this temperature the female eiders will allocate more energy towards maintaining normal body temperature (Gabrielsen et al., 1991; Bustnes et al, 2012). This could possible explain the similar levels of DNA damage between the eider populations from the Arctic and the Baltic Sea. However, a recent study by McPartland (2019) reported greater body mass loss in eiders from the Baltic Sea (Christiansø, Denmark) compared to eiders from both northern Norway (Bustnes et al., 2012) and the Arctic (Fenstad et al., 2014). The higher body mass decrease was also compatible across years, suggesting this was not a consequence of yearly variability in temperature in the Baltic Sea (Noori, 2018; McPartland, 2019). Lam et al. (2020) also found a significant decrease in body mass from start to end in the incubation period in common eiders from the Baltic sea. This provide strong evidence that lower temperature in the Arctic do not result in higher body weight loss during the incubation period in arctic eiders compared to eiders from the Baltic Sea. It should be mention that, the body weight at the start of the incubation period can have an impact on the decrease in body

weight during the incubation period. The mean body weight (±SE) at the start of the incubation period was higher in the eiders from the Baltic Sea (2181.39g±29.87, McPartland, 2019) compared to eiders from the Arctic (1752.00g±1.81, Fenstad et al., 2014). Moreover, the mean body weight at the end of the incubation period was lower in the arctic eiders (1348g±1.80, Fenstad et al., 2014) compared to eiders from the Baltic Sea (1553.60g±32.12).

A previous study on incubating arctic eiders reported a significant decrease in body mass and an increase in DNA-damage during the incubation period (Fenstad et al., 2014). In the present study, high variability in body weight between the female eiders was found. However, body condition did not have a significant effect on DNA-damage. How many days the eiders have incubated when sampled might have an effect on the association between contaminants and levels of DNA damage in the eiders. In the present study, the mean body weight was 1834.55g±73.98, indicating that the eiders might not have experienced very high fasting stress. This suggest that the eiders had not been incubating for many days when sampled, which might have had an impact on the association between levels of contaminants and DNA damage measured in this study. However, this will not be further discussed since no information is available regarding how many days the eiders had incubated when sampled in this study. Another explanation for the lack of association between body condition and DNA-damage may be due to a small statistical sample size or simply because eider individuals investigated in the present study were in good condition.

To increase survival, cells have evolved natural cellular responses to DNA-DSBs. This includes detoxification of contaminants, antioxidant defence, DNA repair systems, and apoptosis (Jenkins, 2010). With low levels of damage, the antioxidant system and DNA repair system will function optimally, and damage will be repaired until a certain level of damage is reached. Then, the system is saturated and unable to repair the accumulated damage (Jenkins, 2010). The antioxidant defence is exceeding when a cell is subjected to ROS formation to protect the cell against oxidative stress and strand breaks. Fenstad et al. (2016c) demonstrated an upregulation of the antioxidant defence in the Baltic sea eiders compared to the artic eiders. The upregulation of the antioxidant defence may be a result of high contaminant exposure, likely linked to increased ROS production. Increased oxidative stress can have a negative effect on reproduction, growth, and survival in vertebrates (Alonso-Alvarez et al., 2004; Bize et al., 2008). Increased antioxidant defence may indicate a higher tolerance for ROS and a reduction in levels of oxidative stress. In other words, upregulation of the antioxidant defence may

decrease levels of DNA-DSBs measured in the eiders. However, for incubating eiders, low energy resources are available at the end of the incubation period, which may result in a decrease in antioxidant defence because of limited resources available (Fenstad et al., 2014).

Adaptive responses to ionization radiation have been shown both *in vitro* and *in vivo* (Mitchel, 2006). For instance, Galvan (2014) reported an increase in antioxidant levels, as well as a decrease in oxidative stress and levels of DNA damage, as a response to chronic exposure to low background levels of ionizing radiation. Similarly, chronic exposure to genotoxic compounds can result in the upregulation of DNA repair mechanisms eventually leading to decreased levels of DNA damage as suggested by Maness & Emslie (2007) and Collins (2009). More efficient DNA repair systems have been suggested as an adaptation to high levels of contamination in royal terns (*Sterna maxima)* (Maness & Emslie, 2007), human cells (Wang et al., 2012), polar bears (Gilmore, 2015), and arctic char (Inderberg, 2019). Wang et al. (2012) found that low levels of exposure to PBDE 47 to human cells was not related to any obvious DNA damage or induction of apoptosis. Moreover, Gilmore (2015) found at low levels of contamination levels of DNA-damage decreased in polar bears and suggested upregulation of DNA repair genes as a possible explanation for the adaptation. The thioredoxin reductase gene, a gene related to oxidative stress, was positively related to OHC levels in polar bears (Gilmore, 2015). In a study by Inderberg (2019) six DNA repair genes were significantly upregulated in arctic char form the contaminated Lake Ellasjøen compared to the control lake, Lake Laksvatn. Furthermore, the transcript level of the genes was positively associated with the OHC levels and DNA-FTM. Based on these findings, further studies should use endpoints that enable quantification of upregulation of DNA repair genes and antioxidant defence when studying the association between contaminant levels and DNA-damage in arctic seabird species.

Contemporary evolution allows for a population to adapt in a rapidly changing environment by fixation of adaptive alleles within a population (Carroll et al., 2007; Bickham, 2011). High contaminant exposure has been ascribed to favour adaptive alleles resulting in changes in population-wide genetic diversity. For instance, Whitehead et al. (2017) showed reduced biotransformation capacity in Atlantic killifish (*Fundulus heteroclitus*) in a highly polluted estuary in The United States. Contemporary evolution as a response to selection caused by exposure of genotoxic compounds may favour more efficient DNA repair mechanisms, such as upregulation of DNA repair genes, as seen in polar bears (Gilmore, 2015) and arctic char (Inderberg, 2019). Moreover, fixation of adaptive alleles might eventually lead to evolutionary

trade-offs explaining why eiders had high levels of DNA damage, without any of the predictor variables being significant in explaining DNA-FTM and MML. Adaptive responses are costly, and it is possible that adaptation to some stressors will result in the eiders being vulnerable to other type of stressors. However, without further testing for the upregulation of DNA repair genes in the arctic seabird species, is it impossible to ascribe fixation of adaptive alleles to the lack of association between contaminants and levels of DNA damage found in the present study.

Activation of the Tumour protein (TP53) gene as a response to damage, such as strand breaks, will cause the cell to undergo apoptosis (Alberts et al., 2014). This presents another explanation for the lack of association in the present study: increased rates of apoptosis may mask DNA damage. DNA repair mechanisms are costly for an organism to maintain and a transition point for switching from DNA repair to apoptosis defined by an evolutionary optimum exists (Breivik & Gaudernack, 2004; Nowsheen & Yang, 2013). It is possible that, with increasing contaminant concentrations, oxidative stress becomes more extensive resulting in apoptosis playing a bigger part in maintaining genetic homeostasis. This will have an impact on the levels of DNA-DSBs in the cells. In addition, since eiders lose a large percentage of their body weight during the incubation period, is it not unlikely that they favour apoptosis due to the high energy demand of reproduction (Nowsheen & Yang, 2013). This is, however, a speculative assumption. Without further testing for apoptotic markers, it is impossible to distinguish between which cellular processes that determine the levels of DNA damage found in this study.

Lastly, the lack of a significant association could be explained by lack of variation in the data. If no variation in the data set regarding either contaminant levels or DNA-DSBs exists, it would not be possible to detect any kind of association. Low or no variation in the data set would indicate that the sample may not be representative for the bigger population it represents, and that the probability for the replicates being pseudoreplicates increases. This is of major concern in science because the consequence is unreliable replicates. However, in the present study variation in the data set was found, suggesting the samples being representative for the bigger arctic eider population it represents.

No chemical data was available for the kittiwakes included in the present study. Therefore, the discussion will only be based on results from other studies. Haarr et al. (2018) reported kittiwake to have higher levels of OHCs compared to eiders and lower OHCs levels than the

glaucous gulls. Based on this, the result in the present study showing highest levels of DNA-damage in kittiwake is surprising. This suggests that other stressors might be better predictors of DNA damage in kittiwakes. Moreover, Haarr et al. (2018) did not find any association between OHC contaminants and DNA-damage in kittiwake supporting this theory. However, a study by Blévin et al. (2016) reported a negative association between oxychlordane and telomere length in female breeding kittiwakes from Kongsfjorden, suggesting genotoxic potential of OHCs.

In previous studies genotoxic effects of PFAS exposure on DNA damage in kittiwakes have been investigated (Blévin et al., 2017a; Costantini et al., 2019), showing contradicting results. Blévin et al. (2017a) suggested PFAS to have a positive effect on telomeres, consequently leading to a higher survival rate. Moreover, Constantini et al. (2019) suggested that higher oxidative stress might be a result of long-chain PFAS exposure. Another potential predictor of DNA damage in kittiwakes is Hg. Previous studies have shown Hg to cause an adverse effect on survival and reproduction in kittiwakes (Tartu et al., 2013; 2016). However, the potential effect of both OHCs and other contaminants and chemical elements, such as PFAS and Hg, on DNA damage in kittiwake need further investigation.

For glaucous gull, no association between the different contaminant groups and MML and DNA-FTM was found when both sexes were pooled in the PLS model or the linear regression analysis. The glaucous gull is dimorphic, and sex-specific differences are expected. ΣOCP and ΣPCB I+II, ΣPCB III, ΣPCB IV, and $\Sigma MeSO_2$ were significantly and negatively associated with MML when only males were included in the PLS analysis. In addition, body condition was positively associated with MML. The result from the PLS regression indicates that with increasing contaminant levels of OCP, PCB, and $MeSO_2$ will MML tend to decrease in glaucous gull males. Furthermore, individuals in good condition have significantly less damage (measured as MML). High levels of contamination together with poor body condition can increase levels of DNA damage, while individuals in better condition may detoxify and/or eliminate the contaminants more efficiently and therefore reducing the effect of contamination on DNA integrity (Helberg et al., 2005). The present study shows, as expected, a negative effect of contaminant exposure on the DNA integrity in glaucous gull males. This suggests that OHCs contaminants are a good predictor of DNA damage in glaucous gull males.

However, the sample size for glaucous gull males in the present study is small. With a smaller sample size, the probability of false-negative (Type II errors) increases. Consequently, the results have a low statistical power. The association between contaminant levels and DNA-damage could be a statistical artefact. Due to small sample size, no linear regression was run, and the result from the PLS regression should be interpreted with caution. Therefore, the results should be regarded as a mere indicator of significance. However, the result still suggests a positive association between contaminant levels and DNA damage but warrants confirmation from further studies conducted on a larger sample size.

For glaucous gull females the PLS regression and linear regression did not show the same result concerning MML or DNA-FTM. In the PLS regression none of the predictor variables were significant in explaining MML, while in the linear regression the HPCs and BCI was significant predictors of MML. The linear regression showed as HPC and BCI increases the mean of MML also tend to increase, suggesting a positive effect of HPC and BCI on DNA integrity. However, a problem with the dataset in the present study, and with chemical data in general, is that multicollinearity between the predictor variables makes it difficult to distinguish which predictors being responsible for the observed effect on the dependent variable, in this case, DNA damage. Multicollinearity and a relatively small sample size are likely the reason different associations were found in the PLS regression and the linear regression. HPC and BCI were significantly correlated and it is therefore likely a result of multicollinearity or it is a statistical artefact.

For glaucous gull females, ΣPCB I+II was negatively associated with DNA-FTM. This indicates that an increase in ΣPCB I+II results in a decrease in DNA-FTM. This result is unexpected because it suggests a favourable effect of contaminant exposure on DNA integrity in glaucous gull females. It should be mentioned that this result was only found in the PLS regression, and the significance should therefore not be interpreted uncritically, but more as an indicator of significance.

It is possible that DNA damage only will be significant after a threshold level of contaminant concentration is reached. Moreover, the effect of contaminant exposure on DNA integrity might be different at lower contaminant concentrations. The DNA repair system and/or the antioxidant defence might be working optimally, and in this way reducing the levels of DNA-DSB. Erikstad et al. (2013) showed that glaucous gull females reduce survival at lower contaminant

concentrations, compared to males. Therefore, it is possible that females rather induce apoptosis in scenarios where males activate DNA repair to maintain cellular homeostasis. This can possible mask an association between contaminants and levels of DNA damage in females.

Alternatively, males may increase survival mechanisms when exposed to contaminants, to a greater extent, than females. This could explain why the males showed higher contaminant levels than females, without that resulting in significant differences in total levels of DNA damage. However, this would indicate a positive association between contaminates and DNA damage in females. This was not supported by this study findings, which suggests other stressors could be responsible for the DNA-damage observed in females.

To evolve higher tolerance to contamination might be maladaptive, eventually reducing the male's fitness. It has been suggested that adaptation to contaminants can result in trade-offs between contaminant resistant and supressed immune responses in glaucous gulls (Sagerup et al., 2000). Therefore, it is highly recommended that longitudinal studies are conducted on this arctic seabird population. This will allow investigation of potential trade-offs associated with adapting to contamination, which is not possible to investigate in the present study.

Moreover, chonic contamination exposure may, on a population level, selected against the most sensitive individuals, especially among females which reduce survival at lower contaminant concentrations. This suggests a strong selection towards high-quality over less sensitive phenotypes (Erikstad, et al., 2013). This is not unlikely considering that the glaucous gull population on Svalbard have been exposed to OHCs contamination for over four decades (Sagerup et al., 2009a). It is possible that a selection towards females with high-quality phenotypes may explain the unexpected negative association between ΣPCB I+II and DNA-FTM reported in the present study. Furthermore, the lack of a positive association between the different contaminant groups and DNA damage in female glaucous gull, may also be explained by selection against the most sensitive individual. Long term exposure of contaminants may have resulted in a population with high-quality phenotypes, better equipped to handle contaminant-induced stress on DNA integrity.

4.4 METHOD DISCUSSION

The method used in the present study is non-invasive and objective, meaning no animal has to be sacrificed, and no solely subjective measures, such as counting of chromosomes has to be conducted. The liver is the main organ for biotransformation, and consequently liver samples have been used in many genotoxic studies. However, using liver samples require the test animal to be sacrificed. Previous studies report that blood OHC concentrations are equivalent to concentrations in both liver and muscles (Henriksen et al., 1998; Kocagöz et al., 2014). Moreover, the avian karyotypes have micro chromosomes, difficult to see in microscopes, making the present method especially useful for monitoring the genetic damage in avian species.

Furthermore, the present method allows for longitudinal studies. The fact that this method does not require the animal to be sacrificed and needs only small blood volumes opens up for taking multiple measurements of the same test animal over a shorter or longer time period. This may be extremely valuable in a highly seasonal environment with yearly variability in climate, food availability, and predation. Furthermore, Fenstad et al. (2014) found blood contaminant concertation to be affected by sampling year, indicating yearly variation in contaminant levels in arctic seabird species.

One of the main problems with the present method lay in the calculation of the relative DNA measurements. The calculation requires individual judgments which can result in variation in the relative DNA measurements depending on the person doing the calculation, particularly for MML. MML is not normally distributed but tend to skew to the low median molecular length. This in combination with a small sample size may provide false significant results and it is therefore highly recommended to increase the sample size to obtain a higher statistical power. However, a pervious study has found DNA-FTM to be highly reproducible and DNA-FTM is considered as a more precise measurement (Krøkje et al., 2006). Consequently, DNA-FTM is often chosen over MML (Fenstad et al., 2014; Neerland et al., 2019).

4.5 FUTURE PROSPECTS

It is imperative that the genotoxic potential of OHCs in arctic seabird populations are further studied. The lack of association between the contaminants analysed in this study and DNA damage in eiders suggests other contaminants might be better predictors of DNA damage.

Further studies should therefore include additional chemical elements and contaminants, such as, Hg, PFAS, and PBDE metabolites, to encompass a wider range of chemical elements and contaminants.

It is also recommended that the different cellular responses to OHCs exposure, and how they are related, are further investigated in arctic seabird species. In this study, the levels of DNA-DSBs are measured in red blood cells (erythrocytes) lacking metabolic capacity. However, it is believed that damage to red blood cells is representative of the total level of damage in the rest of the organism. If so, DNA-DSBs in red blood cells can be used as a cell-wide biomarker for DNA repair, antioxidant responses, and apoptosis. This can fill information gaps concerning the evolutionary balance between the different cellular responses and how this affects the levels of DNA-DSB in the cells. A better understanding of cellular responses will provide a broader understanding of the association between contaminant levels and DNA damage and the cascade of cellular alteration that sooner or later affects the overall fitness of an organism.

In a harsh environment, like the Arctic region, natural stressors such as breeding, food availability, climate change, and predation may play a big part in the overall fitness of an individual. This type of natural stress may be associated with the overall genetic health of an individual. It is highly recommended that further studies, to achieve a broader perspective of the evolutionary aspects of a population's genetic status, include endpoints more relative to population dynamics. Moreover, further studies may profit from a longitudinal approach to determine the effect of potential seasonal and annual climate variation in the Arctic region, as well as potential evolutionary trade-offs associated with chemical-induced adaptation.

5 CONCLUSIONS

Levels of DNA damage were significantly different between the three species. However, the DNA-FTM and MML showed contradicting results, indicating the highest levels of DNA damage in kittiwake and eider, respectively. Moreover, levels of OHC contaminants varied between the species. Glaucous gull, as expected, showed significantly higher levels of contaminants compared to eider. Both the overall contaminant levels and relative contributions of the various contaminants were within the range expected given the values reported in previous studies for these arctic seabird species.

This study found varying relationships between contaminants and DNA damage at a species level, as well as sex-specific differences in glaucous gull. In common eiders, no association between contaminants and levels of DNA damage was found. It is possible that OHCs contaminant levels are too low to induce genotoxic effect, and thus other stressors, such as breeding stress, might be better predictors of DNA damage in the eiders. Other possible explanations are that the DNA-repair system is working optimally under low or moderate OHCs exposure, eiders have adapted to chronic contaminant exposure, or that increased rates of apoptosis may mask levels of DNA-damage measured in the present study.

For glaucous gull males, the contaminants included in this study seemed to be a good predictor of DNA damage. However, for female glaucous gull OHC contaminants did not appear to have a major impact on DNA integrity. These sex-specific differences might be due to contaminant levels being too low to cause genotoxic effect in females or because females inducing apoptosis to a higher degree compared to males. Lastly, contaminant-induced selection against the most sensitive females may have resulted in a population with high-quality females well-equipped to handle contaminant-induced effects on DNA integrity.

This study highlights importance of contaminant-associated effects on the genetic health of arctic seabird populations. Further studies should focus on elucidating the cellular mechanisms maintaining DNA integrity in seabird species exposed to multiple environmental stressors.

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APPENDICES

APPENDIX A- MATERIALS AND METHOD

Table A.1: Chemicals used in the DNA-DSB analysis. Each chemical is presented with product number and producer.

Chemical	Product number	Producer
Boric acid (H_3BO_3)	B7901	Sigma
Trizma base	T6066	Sigma
Ethylenediaminetatraacetic acid (EDTA)	E5134	Sigma
Sodium chloride (NaCl)	S3014	Sigma
Sodium dodecyl sulphate (SDS)	L-3771	Sigma
Tris HCl	T-3253	Sigma
Low melting point Agarose (LMPA)	162-0019	BIO-RAD
Agarose	A9539	Sigma
Proteinase K	P2305	Sigma
DNA Gel Loading Dye (6x)	#P0611	Fermentas
HindIII-digested lambda DNA	#SM0101	Fermentas
Lambda DNA	#SD0011	Fermentas
Ethidium Bromide	161-0433	SIGMA

Table A2: Solution, concentrations, and masses used in the DNA-DSB analysis.

Solution	Amount of chemicals
TE buffer	10 mM Tris (pH=8)
	1 mM EDTA (pH=8)
Theodorakis lysis buffer	1000 mM NaCl
	100 mM Tris (pH=7.6)
	500 mM EDTA
	SDS (0.5 %)
TBE buffer	450 mM Tris base
	900 mM Boric Acid
	5 mM EDTA
Agarose Gel (0.6 %)	0.6 g Agarose
	100 mL TBE buffer (0.5x)
LMPA (1 %)	20 mL TE
	200 mL LMPA
Lambda DNA ladder	43µL TE buffer
	15 µL DNA loading dye
	2 µL lambda DNA

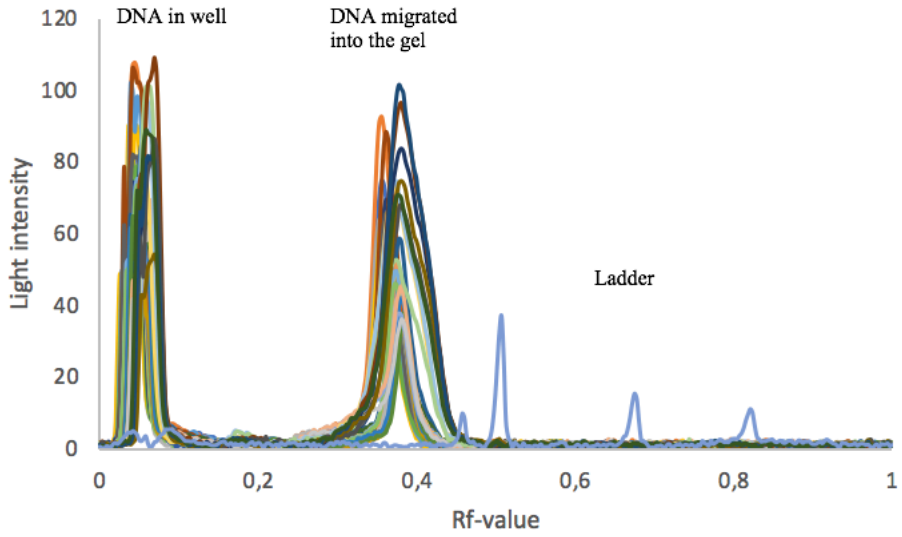


Figure A.1: Light intensity peaks in relation to the Rf-value of the biological samples. The light intensity peaks of the Lambda/Hind III ladder (labelled ladder) are shown for comparison. Light intensity is measured by the Gel documentation machine. The intensity peaks are marked corresponding to its position in the gel (well or migrated into the gel).

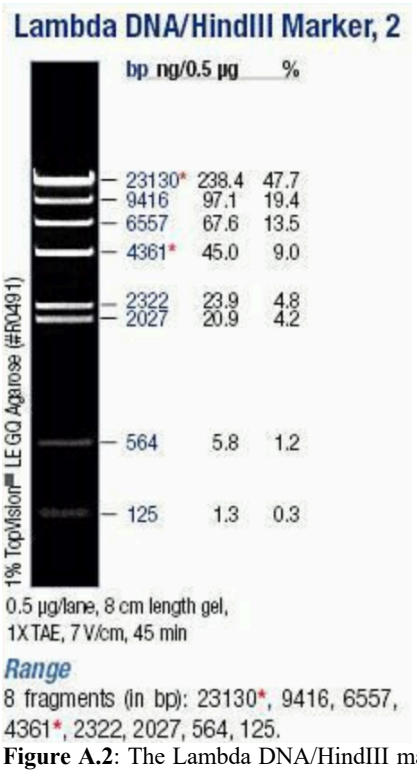
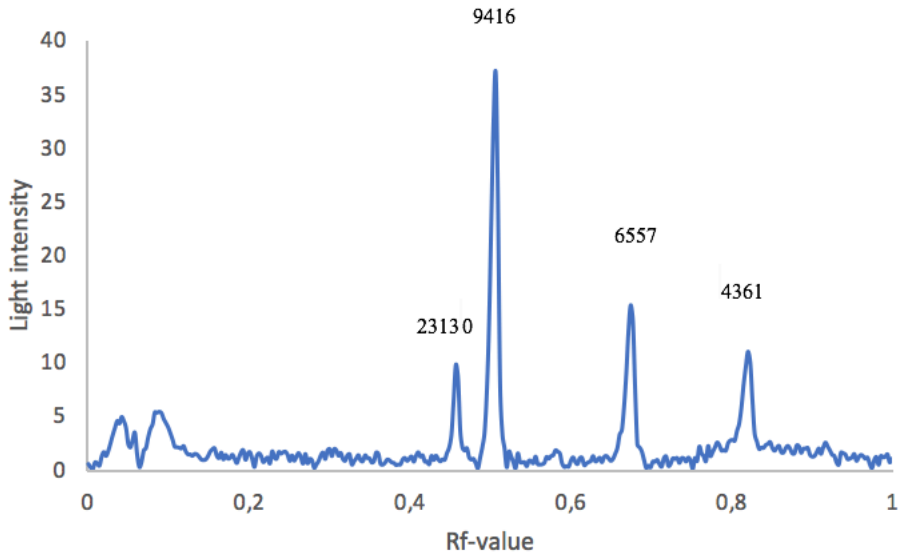


Figure A.2: The Lambda DNA/HindIII marker. Show the standard base pair (bp) for the ladder: lambda (λ) DNA: 483337, Hind III digested λ DNA: 23130, 9416, 6557, 4361, 2322, 2027, 564, 125 bp. The size marker is used as a reference to measure MML from the unknown length of the DNA fragment migrated through the gel.



Figrue A.3: Light Intensity peaks in relation to the Rf-value of the Lambda/Hind III Ladder. Light intensity is measured by the Gel documentation machine. Each peak is marked with corresponding size marker (bp).

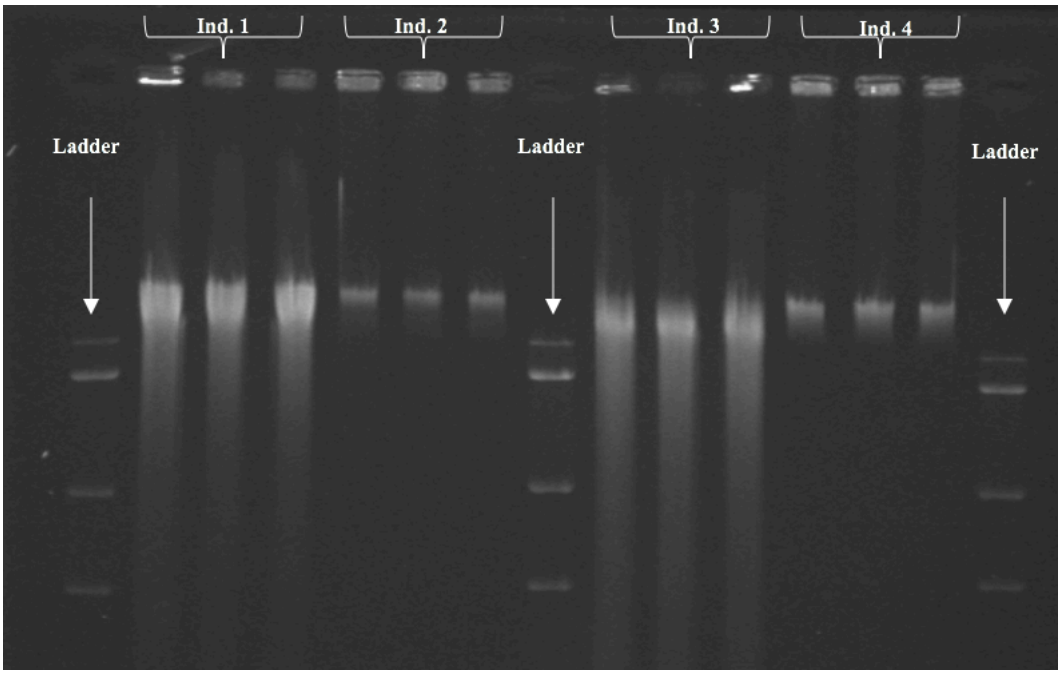


Figure A.4: Agarose gel electrophoresis set up. Four different samples (labelled; Ind.1, Ind.2, Ind.3, and Ind.4) was loaded into the gel in triplicates as shown in the figure. The Lambda/Hind III ladder was loaded in the first, middle and last well. Gels were run in parallel and each sample was run at two different days to ensure “gel” and “replicate” significance.

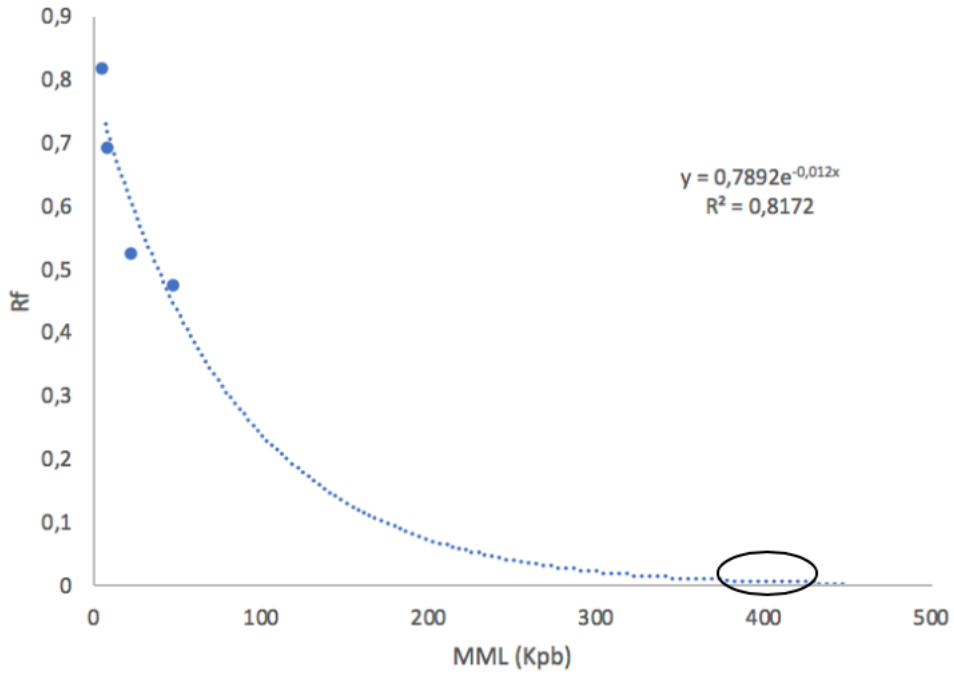


Figure A.5: Standard curve obtained from the Lambda/Hind III ladder with one extrapolated point on 400 Kbp (marked with a circle). The standard curve equation (shown in the figure) and the rf-value provided from the Gel documentation machine was used to quantify the MML value (Kbp).

Table A.3: The acronym and structure of all the contaminants analysed in glaucous gull and eider sampled from Kongsfjorden, Svalbard during the breeding season 2015.

Compound group		Acronym	Structure
OCPs		HCB	Hexachlorobenzene
		α-HCH	1α,2α,3β,4α,5β,6β-Hexachlorocyclohexane
		β-HCH	1α,2β,3α,4β,5α,6β-Hexachlorocyclohexane
		γ-HCH	1α,2α,3β,4α,5α,6β-Hexachlorocyclohexane
		Heptachlor	
		oxy-chlordane	
		t-chlordane	trans-chlordane
		c-chlordane	cis-chlordane
		t-Nonachlor	trans-nonachlor
		c-Nonachlor	cis-nonachlor
		Mirex	
		o,p'-DDT	o,p,'-Dichloro-α,α-diphenyl-β,β,β-trichloroethane
		p,p'-DDT	p,p,'-Dichloro-α,α-diphenyl-β,β,β-trichloroethane
		o,p'-DDD	o,p,'-Dichloro-diphenyl-dichloroethane
		p,p'-DDD	p,p,'-Dichloro-diphenyl-dichloroethane
		o,p'-DDE	o,p,'-Dichloro-diphenyl-dichloroethylene
		p,p'-DDE	p,p,'-Dichloro-diphenyl-dichloroethylene
PCBs	(III)	PCB 28	2,4,4'-Trichlorobiphenyl
	(IV)	PCB 52	2,2',5,5'-Tetrachlorobiphenyl
	(II)	PCB 99	2,2',4,4',5-Pentachlorobiphenyl
	(IV)	PCB 101	2,2',4,5,5'-Pentachlorobiphenyl
	(III)	PCB 105	2,3,3',4,4'-Pentachlorobiphenyl
	(III)	PCB 118	2,3',4,4',5-Pentachlorobiphenyl
	(II)	PCB 138	2,2',3,4,4',5'-Hexachlorobiphenyl

Compound group	Acronym	Structure
(I)	PCB 153	2,2',4,4',5,5'-Hexachlorobiphenyl
(I)	PCB 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl
(I)	PCB 183	2,2',3,4,4',5',6-Heptachlorobiphenyl
(I)	PCB187	2,2',3,4',5,5',6'-Heptachlorobiphenyl
(I)	PCB 194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
PBDEs	PBDE 28	2',4,4'-Tribromodiphenyl ether
	PBDE 47	2,2',4,4'-Tetrabromodiphenyl ether
	PBDE 99	2,2',4,4',5'-Pentabromodiphenyl ether
	PBDE 100	2,2′,4,4′,6'-Penta-bromodiphenyl ether
	PBDE 153	2,2',4,4',5,5'-Hexabromobiphenyl ether
	PBDE 154	2,2',4,4',5,6'-Hexabromodiphenyl ether
	PBDE 183	2,2',3',4,4',5',6'-Heptabromodiphenyl ether
HPCs	PCP	Pentachlorophenol
	4-OH-HpCS	4'-OH-hydroxyheptachlorostyrene
	4-OH-PCB 120	4'-OH-2,3',4,5,5'-pentachlorobiphen
	4-OH-PCB 107	4'-OH-2,3,3',4',5-pentachloro-4-biphenylol
	3-OH-PCB 153	3'-OH-2,2',4,4',5,5'- hexachloro-3-biphenylol
	4-OH-PCB 146	4'-OH-2,2',3,4',5,5'- hexachloro-4-biphenylol
	3-OH-PCB 138	3'-OH-2,2',3',4,4',5- hexachloro-3-biphenylol
	4-OH-PCB 130	4'-OH-2,2',3,3',4',5-hexachloro-4-biphenylol
	4-OH-PCB 163	4'-OH-2,3,3',4',5,6-hexachloro-4-biphenylol
	4-OH-PCB 187	4'-OH-2,2',3,4',5,5',6-heptachloro-4-biphenylol
	4-OH-PCB 172	4'-OH-2,2',3,3',4',5,5'-heptachloro-4-biphenylol
	4'-OH-PCB 193	4'-OH-2,3,3',4',5,5',6-heptachloro-4-biphenylol
	13C-PCP	13C- Pentachlorophenol
	13C 4-OH-PCB 107	13C-2,3,3',4',5-pentachloro-4-biphenylol
	13C 3-OH-PCB 153	13C-2,2',4,4',5,5'- hexachloro-3-biphenylol
	13C 3-OH-PCB 146	13C-2,2',3,4',5,5'- hexachloro-4-biphenylol
	13C 3-OH-PCB 138	13C-2,2',3',4,4',5- hexachloro-3-biphenylol
	13C 4-OH-PCB 163	13C-2,3,3',4',5,6-hexachloro-4-biphenylol
	13C 4-OH-PCB 187	13C-2,2',3,4',5,5',6-heptachloro-4-biphenylol
	13C 4-OH-PCB 172	13C-2,2',3,3',4',5,5'-heptachloro-4-biphenylol
	13C 4'-OH-PCB 193	13C-2,3,3',4',5,5',6-heptachloro-4-biphenylol
$MeSO_2$	3MeSOPCB52	3-Methylsulfonyl-2,2',5,5'-Tetrachlorobiphenyl
	3MeSOPCB49	3-Methylsulfonyl-2,2',4,5'-Tetrachlorobiphenyl
	4MeSOPCB52	4-Methylsulfonyl-2,2',5,5'-Tetrachlorobiphenyl
	4MeSOPCB49	4-Methylsulfonyl-2,2',4,5'-Tetrachlorobiphenyl
	3MeSOPCB91	3-Methylsulfonyl-2,2',3,4',6-Pentachlorobiphenyl
	4MeSOPCB91	4-Methylsulfonyl-2,2',3,4',6-Pentachlorobiphenyl
	3MeSOPCB101	3-Methylsulfonyl-2,2',4,5,5'-Pentachlorobiphenyl
	4MeSOPCB101	4-Methylsulfonyl-2,2',4,5,5'-Pentachlorobiphenyl
	3MeSO2DDE	3-Methylsulfonyl-p,p,'-Dichloro-diphenyl-dichloroethylene
	3MeSOPCB87	3-Methylsulfonyl-2,2',3,4,5'-Pentachlorobiphenyl
	3MeSOPCB110	3-Methylsulfonyl-2,2′,4′,5,5′-pentachlorobiphenyl
	4MeSOPCB110	4-Methylsulfonyl-2,2′,4′,5,5′-pentachlorobiphenyl
	3MeSOPCB149	3-Methylsulfonyl-2,2′,4′,5,5′,6-hexachlorobiphenyl
	4MeSOPCB149	4-Methylsulfonyl-2,2′,3,4′,5′,6-hexachlorobiphenyl
	3MeSOPCB132	3-Methylsulfonyl-2,2′,3′,4′,5,6-hexachlorobiphenyl
	4MeSOPCB132	4-Methylsulfonyl-2,2′,3,3′,4′,6-hexachlorobiphenyl
	3MeSOPCB141	3-Methylsulfonyl-2,5,2',3',4',5'-hexachlorobiphenyl
	4MeSOPCB141	4-Methylsulfonyl-2,5,2',3',4',5'-hexachlorobiphenyl
	3MeSOPCB174	3-Methylsulfonyl-2,2′,3′,4′,5,5′,6-heptachlorobiphenyl
	4MeSOPCB174	4-Methylsulfonyl-2,2′,3,3′,4′,5′,6-heptachlorobiphenyl

Table A.4: Detection frequency (DF) and limit of detection (LOD; ng/g ww) for all 77 compounds analysed in glaucous gull (GG) and common eider (CE) sampled in Kongsfjorden, Svalbard during the breeding season of 2015. Compounds detected in less than 60 % (DF<0.6) in both species are marked with a star (*) and were removed for further analysis.

Compound	GG DF	CE DF	GG LOD	CE LOD
HCB	1	1	0.31	0.59
a-HCH *	ND	ND	46.2	51.1
β-HCH	1	ND	68.0	72.0
g-HCH*	ND	ND	14.0	14.2
Heptachlor *	ND	ND	46.4	34.5
oxy-chlordane	1	1	25.2	24.0
t-chlordane*	ND	ND	3.20	2.42
c-chlordane	0.93	0.30	4.76	3.57
t-Nonachlor	1	1	3.76	2.57
c-Nonachlor	1	1	2.49	1.76
Mirex	1	0.65	24.3	20.3
o,p'-DDT*	ND	ND	238	226
p,p'-DDT*	0.07	ND	337	328
o,p'-DDD*	ND	ND	76.2	71.2
p,p'-DDD*	ND	ND	68.2	65.1
o,p'-DDE*	0.07	ND	46.8	52.8
p,p'-DDE	0.93	1	65.5	74.0
PCB 28	1	ND	16.1	17.0
PCB 52	0.86	ND	21.2	20.7
PCB 99	1	1	16.7	19.5
PCB 101	0.79	1	19.4	22.6
PCB 105	1	0.65	19.6	22.2
PCB 118	1	0.95	18.5	20.0
PCB 138	1	0.95	60.5	62.1
PCB 153	1	1	24.1	26.3
PCB 180	1	0.15	56.0	63.4
PCB 183	1	0.05	43.8	49.8
PCB 187	1	0.45	51.1	57.2
PCB 194	1	ND	209	235
PBDE 28	0.86	ND	2.98	2.19
PBDE 47	1	ND	46.2	6.73
PBDE 99	1	ND	68.0	72.0
PBDE 100	1	ND	3.05	14.2
PBDE 153	1	ND	19.05	31.95
PBDE 154	1	ND	24.6	18.9
PBDE 183*	0.20	ND	142	123.0
PCP*	ND	ND	78.7	78.7
4-OH-HpCS	1	1	0.4	0.4
4-OH-PCB 120*	ND	ND	N/A	N/A
4-OH-PCB 107	1	1	9.0	9.0
3-OH-PCB 153	0.93	0.6	2.4	2.4
4-OH-PCB 146	1	1	2.6	2.6
3-OH-PCB 138*	0.21	ND	14.1	14.1
4-OH-PCB 130	0.5	1	5.6	5.6
4-OH-PCB 163	1	1	2.4	2.4
4-OH-PCB 187	1	1	7.1	7.1
4-OH-PCB 172	0.71	0.15	13.6	13.6
4'-OH-PCB 193	0.93	0.10	3.5	3.5
13C-PCP	1	1		
13C 4-OH-PCB 107	1	1		
13C 3-OH-PCB 153	1	1		
13C 3-OH-PCB 146	1	1		

Compound	GG DF	CE DF	GG LOD	CE LOD
13C 3-OH-PCB 138	1	1		
13C 4-OH-PCB 163	1	1		
13C 4-OH-PCB 187	1	1		
13C 4-OH-PCB 172	1	1		
13C 4'-OH-PCB 193	1	1		
3MeSOPCB52*	ND	ND	5.6	5.6
3MeSOPCB49*	ND	ND	33.6	33.6
4MeSOPCB52*	ND	ND	3.8	3.8
4MeSOPCB49*	ND	ND	3.9	3.9
3MeSOPCB91*	ND	ND	3.0	3.0
4MeSOPCB91*	ND	ND	4.8	4.8
3MeSOPCB101	1	ND	6.0	6.0
4MeSOPCB101	1	ND	3,1	3,1
3MeSO2 DDE*	0.08	ND	13.9	13.9
3MeSOPCB87*	0.15	ND	6.1	6.1
3MeSOPCB110*	ND	ND	5.4	5.4
4MeSOPCB110	1	ND	3.6	3.6
3MeSOPCB149*	ND	ND	5.2	5.2
4MeSOPCB149	0.85	ND	5.1	5.1
3MeSOPCB132*	ND	ND	3.0	3.0
4MeSOPCB132*	ND	ND	5.2	5.2
3MeSOPCB141*	0.08	ND	4.3	4.3
4MeSOPCB141*	ND	ND	4.2	4.2
3MeSOPCB174*	ND	ND	4.0	4.0
4MeSOPCB174*	ND	ND	4.8	4.8

APPENDIX B- RESULTS

Table B1: Biometric estimates (weight (g), head (mm), tars (mm), wing (mm), and BCI) for glaucous gull (n=14), common eider (n=12), and kittiwake (n=19) sampled in Kongsfjorden, Svalbard during the breeding season 2015. Estimates labelled ND =not detected. Sex are labelled by male=♂ and female=♀.

Species	ID	Weight	Head	Tars	Wing	BCI
Glaucous gull ♂	GG01-15	1730	155	74.7	477	-0.87
Glaucous gull ♂	GG03-15	1840	152	75.0	484	1.36
Glaucous gull ♂	GG04-15	1850	153	78.0	489	-0.46
Glaucous gull ♂	GG10-15	1530	146	66.7	480	-0.33
Glaucous gull♀	GG07-15	1390	134	68.0	471	-0.18
Glaucous gull♀	GG09-15	1360	139	67.9	468	-0.92
Glaucous gull♀	GG12-15	1410	138	68.0	461	0.24
Glaucous gull♀	GG02-15	1380	134	69.0	450	-0.56
Glaucous gull♀	GG05-15	1470	139	70.5	458	1.56
Glaucous gull♀	GG06-15	1360	137	73.1	447	-1.75
Glaucous gull♀	GG08-15	1370	137	67.2	463	-0.68
Glaucous gull♀	GG11-15	1480	136	ND	481	1.78
Glaucous gull♀	GG14-15	1420	138	66.8	451	0.46
Glaucous gull♀	GG15-15	1370	138	66.8	455	-0.68
Common Eider ♀	CE01-15	1930	116	51.0	286	0.80
Common Eider ♀	CE02-15	2340	120	53.0	290	1.81
Common Eider ♀	CE05-15	1980	120	49.0	282	0.41
Common Eider ♀	CE09-15	1730	118	51.0	287	-0.26
Common Eider ♀	CE10-15	ND	124	52.0	283	ND
Common Eider ♀	CE11-15	1780	119	54.0	285	-0.22
Common Eider ♀	CE12-15	1740	118	48.0	296	-0.22
Common Eider ♀	CE14-15	1920	117	51.0	282	0.61
Common Eider ♀	CE16-15	1970	116	49.3	283	0.96
Common Eider ♀	CE17-15	1500	118	51.0	280	-1.15
Common Eider ♀	CE19-15	1440	114	50.3	275	-0.83
Common Eider ♀	CE20-15	1850	123	51.4	289	-0.56
Kittiwake♀	KW01-15	405	88.8	33.7	323	0.73
Kittiwake♀	KW02-15	400	87.8	35.0	301	0.73
Kittiwake♀	KW03-15	385	85.2	34.1	304	0.67
Kittiwake♀	KW04-15	455	93.4	35.3	320	1.65
Kittiwake♀	KW05-15	400	87.5	34.7	314	0.79
Kittiwake♀	KW06-15	445	92.2	37.0	303	1.52
Kittiwake♀	KW07-15	395	95.6	35.4	326	-0.86
Kittiwake♀	KW08-15	445	86.9	32.6	305	2.47
Kittiwake♀	KW09-15	365	88.9	34.3	305	-0.81
Kittiwake♀	KW10-15	425	93.4	34.7	324	0.64
Kittiwake♀	KW11-15	360	85.4	32.8	321	-0.37
Kittiwake♀	KW14-15	415	94.4	35.5	319	0.11
Kittiwake♀	KW15-15	395	92.1	36.2	320	-0.22
Kittiwake♀	KW17-15	345	88.8	32.0	311	-1.61
Kittiwake♀	KW18-15	425	90.6	35.5	323	1.11
Kittiwake♀	KW19-15	425	85.0	32.8	310	2.20
Kittiwake♀	KW20-15	365	85.8	33.7	300	-0.24
Kittiwake♀	KW21-15	425	97.9	36.3	326	-0.14
Kittiwake♀	KW22-15	395	94.7	36.6	321	-0.69

Table B.2: DNA measurements given as mean DNA-FTM (%) and MML (kpb) for glaucous gull, common eider, and kittiwake sampled in Kongsfjorden, Svaldbard during the breeding season of 2015. Standard derivation (SD) and coefficient of variation (CV) are presented for each sample triplicate ran in each gel.

Species	ID	gel	MML (kpb)	SD MML	CV MML	DNA-FTM (%)	SD DNA-FTM	CV DNA-FTM
Glaucous gull	GG01	1	311.50	16.80	5.39	38.74	2.50	6.44
Glaucous gull	GG01	2	268.78	17.09	6.36	41.27	5.36	12.99
Glaucous gull	GG02	1	334.06	8.08	2.42	18.76	1.39	7.41
Glaucous gull	GG02	2	353.31	5.07	1.44	11.90	1.19	10.01
Glaucous gull	GG03	1	390.46	4.89	1.25	6.43	0.18	2.81
Glaucous gull	GG03	2	393.80	16.22	4.12	5.94	0.84	14.22
Glaucous gull	GG03	3	361.34	1.75	0.48	6.03	0.74	12.27
Glaucous gull	GG04	1	405.58	1.70	0.42	5.14	0.63	12.31
Glaucous gull	GG04	2	370.51	11.82	3.19	11.63	1.12	9.63
Glaucous gull	GG04	3	365.37	15.46	4.23	11.63	1.58	13.58
Glaucous gull	GG04	4	356.18	3.48	0.98	11.49	1.40	12.15
Glaucous gull	GG04	5	342.61	1.71	0.50	4.48	0.10	2.29
Glaucous gull	GG04	6	345.37	10.21	2.96	4.30	0.46	10.81
Glaucous gull	GG05	1	343.71	1.83	0.53	9.31	0.60	6.46
Glaucous gull	GG05	2	398.84	11.79	2.96	11.01	1.96	17.82
Glaucous gull	GG05	3	354.84	12.42	3.50	10.53	1.39	13.17
Glaucous gull	GG05	4	366.94	5.56	1.51	8.81	0.87	9.89
Glaucous gull	GG05	5	355.18	1.85	0.52	9.27	1.50	16.20
Glaucous gull	GG06	1	323.79	5.93	1.83	9.46	0.77	8.14
Glaucous gull	GG06	2	381.99	3.51	0.92	9.22	0.99	10.72
Glaucous gull	GG07	1	309.41	22.05	7.13	4.99	0.63	12.53
Glaucous gull	GG07	2	329.79	3.51	1.07	4.26	0.41	9.53
Glaucous gull	GG08	1	343.03	8.14	2.37	4.51	0.28	6.22
Glaucous gull	GG08	2	362.25	5.33	1.47	4.06	0.43	10.51
Glaucous gull	GG08	3	383.47	3.76	0.98	4.02	0.12	3.10
Glaucous gull	GG09	1	333.60	9.14	2.74	25.10	0.34	1.34
Glaucous gull	GG09	2	319.74	7.41	2.32	22.94	4.50	19.62
Glaucous gull	GG10	1	343.43	6.05	1.76	4.78	0.53	11.13
Glaucous gull	GG10	2	338.88	8.78	2.59	3.74	0.14	3.72
Glaucous gull	GG11	1	327.60	3.40	1.04	11.38	0.39	3.45
Glaucous gull	GG11	2	335.73	2.93	0.87	4.69	0.37	7.81
Glaucous gull	GG11	3	351.50	4.98	1.42	4.63	0.07	1.54
Glaucous gull	GG12	1	386.74	7.45	1.93	5.01	0.19	3.69
Glaucous gull	GG12	2	330.52	5.11	1.55	5.21	0.45	8.64
Glaucous gull	GG12	3	372.28	1.85	0.50	4.77	0.58	12.10
Glaucous gull	GG12	4	360.52	5.56	1.54	4.42	0.19	4.32
Glaucous gull	GG14	1	371.55	15.75	4.24	7.49	0.50	6.63
Glaucous gull	GG14	2	341.34	8.12	2.38	8.00	0.71	8.83
Glaucous gull	GG15	1	365.00	14.06	3.85	9.42	1.02	10.83

Species	ID	gel	MML (kpb)	SD MML	CV MML	DNA-FTM (%)	SD DNA-FTM	CV DNA-FTM
Glaucous gull	GG15	2	357.31	12.00	3.36	7.58	1.05	13.87
Glaucous gull	GG15	3	338.30	10.75	3.18	6.90	0.79	11.41
Common eider	CE01	1	334.61	10.59	3.16	4.25	0.92	21.76
Common eider	CE01	2	303.92	13.19	3.36	3.52	0.59	16.87
Common eider	CE02	1	347.43	16.78	4.69	14.18	4.02	28.37
Common eider	CE02	2	364.35	8.24	2.26	16.52	3.28	19.86
Common eider	CE3-2	1	263.66	12.06	4.57	11.71	0.51	4.34
Common eider	CE3-2	2	292.29	25.87	8.85	8.32	0.95	11.43
Common eider	CE05	1	291.72	31.19	10.69	9.79	0.66	6.79
Common eider	CE05	2	310.50	23.23	7.48	5.45	1.71	31.38
Common eider	CE05	3	259.38	26.07	10.05	7.19	1.24	17.25
Common eider	CE05	4	324.17	3.03	0.93	4.65	1.31	28.30
Common eider	CE09	1	276.87	14.65	5.29	3.42	0.63	18.53
Common eider	CE09	2	340.03	6.00	1.76	9.72	1.73	17.80
Common eider	CE09	3	354.58	3.78	1.07	11.92	2.55	21.42
Common eider	CE10	1	350.17	12.63	3.61	14.55	1.77	12.18
Common eider	CE10	2	328.98	14.35	4.36	9.07	1.97	21.76
Common eider	CE11	1	387.06	18.36	4.74	14.10	1.95	13.83
Common eider	CE11	2	340.47	5.97	1.75	14.04	0.55	3.89
Common eider	CE12	1	396.25	13.70	3.46	29.85	7.26	24.32
Common eider	CE12	2	317.49	17.69	5.57	27.04	2.49	9.19
Common eider	CE14	1	376.87	5.68	1.51	19.56	3.11	15.92
Common eider	CE14	2	264.63	20.68	7.82	16.95	1.09	6.41
Common eider	CE15	1	380.64	13.89	3.65	31.26	2.45	7.84
Common eider	CE15	2	388.48	14.62	3.76	30.41	4.81	15.82
Common eider	CE16	1	283.08	19.04	6.73	25.14	2.90	11.54
Common eider	CE16	2	283.17	20.93	7.39	17.62	0.52	2.94
Common eider	CE17	1	332.25	15.37	4.63	22.91	1.46	6.37
Common eider	CE17	2	332.13	4.59	1.38	25.63	1.55	6.04
Common eider	CE17	3	372.05	6.99	1.88	15.13	1.34	8.87
Common eider	CE19	1	301.38	14.43	4.79	15.00	1.49	9.91
Common eider	CE19	2	328.44	9.59	2.92	12.91	1.42	10.98
Common eider	CE19	3	349.61	2.08	0.59	9.84	0.91	9.21
Common eider	CE20	1	214.80	26.15	12.17	7.77	3.59	46.24
Common eider	CE20	2	272.82	15.37	5.64	4.97	0.79	15.91
Common eider	CE72	1	364.37	21.44	5.88	7.46	1.00	13.44
Common eider	CE72	2	254.60	25.87	10.16	4.74	0.17	3.65
Common eider	CE78	1	341.48	5.24	1.54	9.00	0.78	8.64
Common eider	CE78	2	360.00	23.99	7.84	6.89	1.10	16.01
Common eider	CE82	1	316.30	12.38	3.91	16.85	0.65	3.86
Common eider	CE82	2	361.26	7.79	2.16	17.69	2.22	12.56

Species	ID	gel	MML (kpb)	SD MML	CV MML	DNA-FTM (%)	SD DNA-FTM	CV DNA-FTM
Common eider	CE83	1	340.12	5.01	1.47	4.12	0.58	14.07
Common eider	CE83	2	354.74	18.39	5.18	5.07	0.87	17.07
Common eider	CE91	1	323.75	10.59	3.27	4.09	0.69	16.90
Common eider	CE91	2	362.68	5.52	1.52	6.44	1.27	19.69
Common eider	CE91	3	369.03	4.62	1.25	3.25	0.10	3.02
Common eider	CE92	1	346.75	7.64	2.20	3.53	0.23	6.47
Common eider	CE92	2	321.40	13.31	4.14	2.18	0.07	3.33
Kittiwake	KW01	1	325.56	35.09	10.78	89.09	1.39	1.56
Kittiwake	KW01	2	473.10	45.11	9.53	90.58	1.67	1.85
Kittiwake	KW02	1	439.32	18.94	4.31	50.69	4.36	8.60
Kittiwake	KW02	2	568.11	14.30	2.52	52.66	1.82	3.45
Kittiwake	KW03	1	505.07	15.85	3.14	73.60	1.87	2.54
Kittiwake	KW03	2	441.02	18.33	4.16	73.50	2.89	3.93
Kittiwake	KW04	1	561.24	22.70	4.04	76.76	2.43	3.17
Kittiwake	KW04	2	349.30	10.88	3.12	49.30	6.51	13.21
Kittiwake	KW05	1	531.35	18.50	3.48	64.44	3.93	6.10
Kittiwake	KW05	2	380.23	6.96	1.83	62.00	3.02	4.88
Kittiwake	KW06	1	359.53	22.94	6.38	90.01	1.65	1.84
Kittiwake	KW06	2	297.49	20.52	6.90	73.70	6.61	8.97
Kittiwake	KW07	1	393.62	16.83	4.28	73.09	4.33	5.93
Kittiwake	KW07	2	289.01	15.71	5.44	71.14	4.69	6.59
Kittiwake	KW08	1	432.17	20.29	4.70	77.42	3.48	4.49
Kittiwake	KW08	2	316.31	33.37	10.55	67.59	3.02	4.47
Kittiwake	KW09	1	390.86	52.13	13.34	84.28	4.45	5.28
Kittiwake	KW09	2	335.38	4.64	1.38	61.29	7.70	12.56
Kittiwake	KW10	1	477.49	12.36	2.59	49.54	2.39	4.82
Kittiwake	KW10	2	346.64	13.78	3.98	48.27	4.35	9.01
Kittiwake	KW11	1	374.90	21.27	5.67	66.88	2.92	4.37
Kittiwake	KW11	2	284.82	9.14	3.21	75.26	2.89	3.84
Kittiwake	KW14	1	320.43	10.20	3.18	91.05	0.32	0.35
Kittiwake	KW14	2	141.05	24.82	17.60	87.14	5.09	5.84
Kittiwake	KW15	1	475.99	31.54	6.63	46.39	6.15	13.25
Kittiwake	KW15	2	399.81	39.72	9.94	37.28	7.00	18.78
Kittiwake	KW17	1	453.99	14.43	3.18	40.55	3.59	8.85
Kittiwake	KW17	2	258.70	12.29	4.75	46.07	5.68	12.33
Kittiwake	KW18	1	428.94	18.38	4.29	61.57	7.04	11.43
Kittiwake	KW18	2	378.51	9.10	2.41	57.39	3.50	6.09
Kittiwake	KW19	1	460.89	21.27	4.61	67.33	4.52	6.72
Kittiwake	KW19	2	330.67	24.43	7.39	37.24	4.92	13.21
Kittiwake	KW20	1	420.28	16.38	3.90	41.93	1.83	4.35
Kittiwake	KW20	2	385.67	5.68	1.47	32.04	3.30	10.30

Species	ID	gel	MML (kpb)	SD MML	CV MML	DNA-FTM (%)	SD DNA-FTM	CV DNA-FTM
Kittiwake	KW21	1	338.94	23.38	6.90	45.60	1.33	2.91
Kittiwake	KW21	2	351.33	6.79	1.93	46.60	5.54	11.90
Kittiwake	KW22	1	361.82	11.17	3.09	40.05	2.77	6.91
Kittiwake	KW22	2	339.85	10.29	3.03	41.54	2.67	6.42

Table B3: Contaminants given as mean concentration (ng/g ww) and standard error (±SE) for all compounds with DF>0.6 in glaucous gull (GG) and common eider (CE). The total concentration of OCP, PCB, PBDE, HPC, and $MeSO_2$ in each individual were calculated and are presented as the mean (±SE) of the sum (å) of each contaminant group. ND stand for not detected (in 60 % or more of the samples).

Contaminant (ng/g ww)	**GG (n=14)**		**GG (n=14)**				**CE (n=20)**	
			Male (n=4)		**Female (n=10)**			
	mean	**±SE**	**mean**	**±SE**	**mean**	**±SE**	**mean**	**±SE**
HCB	4.96725	±0.29	6.13	0.56	4.4	0.22	0.58598	±0.07
β-HCH	0.45933	±0.08	0.80	0.18	0.32	0.02	ND	
oxy-chlordane	9.99559	±2.54	19.63	6.34	6.14	1.37	0.19199	±0.01
c-chlordane	0.01124	±0.002	0.01	0.0006	0.01	0.002	ND	
t-Nonachlor	0.67552	±0.07	0.77	0.16	0.64	0.09	0.07304	±0.01
c-Nonachlor	0.69779	±0.07	0.74	0.22	0.68	0.07	0.04240	±0.007
Mirex	4.44611	±1.07	8.80	2.41	2.71	0.59	0.02915	±0.004
p,p'-DDE	49.43133	±11.21	93.05	29.24	31.98	4.66	0.51585	±0.08
∑OCPs	70.68416	±14.82393	129.93		46.98		1.079091	±0.1479155
PCB 28	0.52986	±0.07	0.83	0.16	0.41	0.03	ND	
PCB 52	0,21809	±0.04	0.19	0.14	0.23	0.03	ND	
PCB 99	7.26386	±1.64	14.09	4.08	4.53	0.58	0.09	±0.007
PCB 101	0.44.00	±0.12	0.39	0.39	0.46	0.08	0.04804	±0.005
PCB 105	3.25837	±0.65	5.59	1.74	2.33	0.33	0.0860	±0.01
PCB 118	12.92878	±2.90	24.05	7.37	8.48	1.45	0.23535	±0.02
PCB 138	30.26169	±7.31	60.22	18.15	18.28	2.89	0.24294	±0.03
PCB 153	56.09325	±14.54	113.52	36.03	33.12	7.02	0.38354	±0.04
PCB 180	32.71896	±8.90	63.72	22.16	20.32	5.91	ND	
PCB 183	4.53323	±1.18	9.23	2.91	2.66	0.56	ND	
PCB 187	5.52160	±1.43	11.11	3.89	3.29	0.42	ND	
PCB 194	4.56327	±1.26	7.89	2.71	3.23	1.24	ND	
∑PCBs	158.333	±39.01683	310.83		97.34		0.9545455	±0.04838038
PBDE 28	0.02	$\pm 3.64e^{-03}$	0.03	0.007	0.01	0.003	ND	
PBDE 47	1.57	$\pm 2.44e^{-01}$	2.42	0.70	1.23	0.11	ND	
PBDE 99	0.26	$\pm 4.46e^{-02}$	0.34	0.08	0.23	0.05	ND	
PBDE 100	0.39	$\pm 7.23e^{-02}$	0.66	0.20	0.29	0.03	ND	
PBDE 153	0.50	$\pm 9.51e^{-02}$	0.55	0.07	0.49	0.13	ND	
PBDE 154	0.16	$\pm 2.46^{-02}$	0.24	0.07	0.13	0.01	ND	
∑PBDE	4.32	±0.0006	0.006		0.003		ND	
4-OH-HpCS	0.01249	±0.002	0.02	0.005	0.010	0.002	0.02751	±0.005
4-OH-PCB 107	0.19884	±0.04	0.35	0.08	0.14	0.01	0.16573	±0.03
3-OH-PCB 153	0.02297	±0.007	0.05	0.02	0.01	0.002	0.00576	±0.0009
4-OH-PCB 146	0.46471	±0.15	1.13	0.39	0.20	0.02	0.0582	±0.01
4-OH-PCB 130	ND		ND		ND		0.02514	±0.003
4-OH-PCB 163	0.01975	±0.006	0.04	0.014	0.010	0.001	0.04849	±0.01
4-OH-PCB 187	1.13267	±0.41	3.30	0.85	0.53	0.07	0.12829	±0.03
4-OH-PCB 172	0.02790	±0.008	0.07	0.02	0.01	0.001	ND	
4'-OH-PCB 193	0.01041	±0.003	0.02	0.007	0.005	0.0007	ND	
13C-PCP	0.07422	±0.001	0.07	0.001	0.07	0.002	0.05540	±0.004

Contaminant (ng/g ww)	GG (n=14)		GG (n=14)				CE (n=20)	
			Male (n=4)		Female (n=10)			
	mean	±SE	mean	±SE	mean	±SE	mean	±SE
13C 4-OH-PCB 107	0.06620	±0.001	0.06	0.002	0.07	0.002	0.05305	±0.004
13C 3-OH-PCB 153	0.03677	±0.0008	0.04	0.001	0.04	0.001	0.02842	±0.002
13C 3-OH-PCB 146	0.06426	±0.002	0.06	0.002	0.07	0.002	0.05556	±0.004
13C 3-OH-PCB 138	0.04983	±0.001	0.05	0.002	0.05	0001	0.03944	±0.003
13C 4-OH-PCB 163	0.06116	±0.002	0.06	0.003	0.06	0.002	0.05152	±0.004
13C 4-OH-PCB 187	0.06552	±0.001	0.06	0.002	0.07	0.002	0.04997	±0.004
13C 4-OH-PCB 172	0.06213	±0.002	0.06	0.003	0.06	0.06	0.04726	±0.003
13C 4'-OH-PCB 193	0.06317	±0.002	0.06	0.004	0.06	0.002	0.04913	±0.004
∑HPC	2.6218	±0.6168	5.50		1.47		0.7390909	±0.06159183
3MeSOPCB101	0.08140	±0.02	0.18	0.05	0.04	0.007	ND	
4MeSOPCB101	0.05389	±0.01	0.12	0.02	0.03	0.005	ND	
4MeSOPCB110	0.01870	±0.003	0.03	0.005	0.01	0.001	ND	
4MeSOPCB149	0.01446	±0.002	0.02	0.007	0.01	0.002	ND	
åMeSO$_2$-PCBs	0.1544807	±0.0356	0.30		0.10		ND	
∑OHCs	236.13		265.68		95.54		2.77	

Table B4: Contaminants given as median concentration (ng/g ww) and range for all compounds with DF>0.6 in glaucous gull (GG) and eider (CE). The total concentration of OCP, PCB, PBDE, HPC, and MeSo2 in each individual were calculated and are presented as the median and range of the sum (å) of each contaminant group. ND stand for not detected (in 60 % or more of the samples).

Contaminant (ng/g ww)	GG (n=14)		GG (n=14)				CE (n=20)	
			male		female			
	Median	Range	Median	Range	Median	Range	Median	Range
HCB	4.78	3.69 – 7.58	5.89	5.16-7.58	4.43	3.69-5.96	0.45	0.285 – 1.25
β-HCH	0.33	0.22 – 1.11	0.87	0.36-1.11	0.32	0.22-0.46	ND	
oxy-chlordane	5.79	3.03 – 36.78	16.48	8.78-36.78	4.35	3.03-16.09	0.17	0.13 – 0.31
c-chlordane	0.01	0.004 – 0.03	0.01	0.0097-0.013	0.01	0.004-0.03	ND	
t-Nonachlor	0.66	0.29 – 1.24	0.89	0.29-1.00	0.54	0.37-1.24	0.05	0.02 – 0.22
c-Nonachlor	0.75	0.13 – 1.15	0.85	0.13-1.15	0.69	0.27-1.00	0.03	0.02 – 0.12
Mirex	2.75	1.13 – 15.06	8.07	3.99-15.06	1.99	1.13-6.23	0.03	0.004 – 0.07
p,p'-DDE	36.31	13.37 – 151.31	93.72	33.46-151.31	29.31	13.37-51.68	0.35	0.18 – 1.31
∑OCPs	55.31	22.67-196.70	132.93		43.57		0.89	0.64- 2.40
PCB 28	0.45	0.28 – 1.22	0.81	0.46-1.22	0.40	0.28-0.60	ND	
PCB 52	0.19	0.014 – 0.61	0.07	0.014-0.61	0.21	0.07-0.40	ND	
PCB 99	4.97	2.45 – 21.18	14.64	5.90-21.18	3.99	2.45-7.70	0.08	0.05 – 0.17
PCB 101	0.49	0.001 – 1.55	0.008	0.001-1.55	0.52	0.08-0.79	0.04	0.02 -0.08
PCB 105	2.34	1.32 – 10.57	4.55	2.69-10.57	1.99	1.32-4.18	0.11	0.006 – 0.16
PCB 118	8.26	4.56 – 44.66	19.82	11.91-44.66	6.87	4-56-17.29	0.23	0.04 – 0.38
PCB 138	18.72	8.30 – 94.75	60.39	25.34-94.75	14.89	8.31-36.10	0.22	0.03 – 0.53

Contaminant (ng/g ww)	GG (n=14)		GG (n=14)				CE (n=20)	
			male		female			
	Median	Range	Median	Range	Median	Range	Median	Range
PCB 153	32.87	13.65 – 198.17	101.58	52.75-198.17	23.05	13.65-79.40	0.31	0.19 – 0.90
PCB 180	17.10	6.72 – 121.39	55.30	22.88-121.39	11.32	6.72-62-15	ND	
PCB 183	2.61	1.08 – 15.93	8.52	3.95-15.93	1.91	1.08-6.22	ND	
PCB 187	3.82	1.36 – 21.94	9.28	3.96-21.94	2.90	1.36-6.08	ND	
PCB 194	2.11	0.82 – 15.42	6.73	2.70-15.42	1.47	0.82-12.78	ND	
∑PCBs	94.44	41.32 - 536.35	278.83		71.03		0.87	0.77- 1.18
PBDE 28	0.00002	0.000002 - 0.00005	0.03	0.02-0.05	0.02	0.002-0.04	ND	
PBDE 47	0.00130	0.0007 – 0.0041	2.40	0.75-4.13	1.23	0.66-1.73	ND	
PBDE 99	0.00022	0.00007 - 0.0005	0.37	0.12-0.50	0.18	0.07-0.50	ND	
PBDE 100	0.00030	0.00016 – 0.00119	0.62	0.21-1.19	0.27	0.16-0.46	ND	
PBDE 153	0.00045	0.00010 – 0.00132	0.61	0.34-0.63	0.29	0.10-1.32	ND	
PBDE 154	0.00014	0.00007 - 0.00037	0.25	0.08-0.37	0.13	0.07-0.18	ND	
∑PBDEs	0.003705	0.00172-0.01061	0.006		0.003		ND	
4-OH-HpCS	0.00980	0.004 -0.03	0.02	0.01-0.03	0.008	0.004-0.03	0.01905	0.0067 – 0.0945
4-OH-PCB 107	0.14995	0.07 – 0.50	0.39	0.11-0.50	0.14	0.07-0.19	0.12485	0.0421 – 0.4933
3-OH-PCB 153	0.01120	0.001 – 0.0898	0.05	0.018-0.09	0.009	0.001-0.03	0.0071	0.00034 – 0.0124
4-OH-PCB 146	0.2356	0.12 – 1.84	1.17	0.30-1.85	0.19	0.12-0.31	0.0373	0.0098 – 0.2201
4-OH-PCB 130	ND		ND	-	ND	-	0.0211	0.0102 – 0.0719
4-OH-PCB 163	0.00965	0.0065 – 0.07770	0.04	0.02-0.08	0.008	0.007-0.02	0.0323	0.0094 – 0.1645
4-OH-PCB 187	0.53610	0.31 – 4.85	3.49	1.35-4.85	0.47	0.31-1.10	0.07745	0.0304 – 0.05562
4-OH-PCB 172	0.01490	0.003 – 0.12	0.06	0.03-0.12	0.014	0.003-0.02	ND	
4'-OH-PCB 193	0.00530	0.003 – 0.043	0.02	0.01-0.04	0.005	0.003-0.01	ND	
13C-PCP	0.07460	0.065 -0.083	0.07	0.07-0.08	0.07	0.07-0.08	0.0626	0.0117 – 0.0733
13C 4-OH-PCB 107	0.06860	0.053 – 0.072	0.06	0.06-0.07	0.07	0.05-0.07	0.0598	0.0118 – 0.0693
13C 3-OH-PCB 153	0.03755	0.029-0.0404	0.04	0.03-0.04	0.04	0.03-0.04	0.0322	0.0066 – 0.0374
13C 3-OH-PCB 146	0.0638	0.0503 – 0.073	0.06	0.06-0.07	0.07	0.05-0.07	0.0622	0.0124 – 0.0755
13C 3-OH-PCB 138	0.05090	0.0402 – 0.056	0.05	0.05-0.05	0.05	0.04-0.06	0.0445	0.0091 – 0.0524
13C 4-OH-PCB 163	0.06095	0.0499 – 0.0723	0.06	0.05-0.06	0.06	0.05-0.07	0.05805	0.0115 - 0.0667
13C 4-OH-PCB 187	0.06515	0.0543 – 0.0735	0.06	0.06-0.07	0.07	0.05-0.07	0.05685	0.0118 – 0.0642

Contaminant (ng/g ww)	GG (n=14)		GG (n=14)				CE (n=20)	
			male		female			
	Median	Range	Median	Range	Median	Range	Median	Range
13C 4-OH-PCB 172	0.06140	0.0517 – 0.0734	0.06	0.06-0.07	0.06	0.05-0.07	0.0534	0.0117 – 0.0599
13C 4'-OH-PCB 193	0.06370	0.0492 – 0.0774	0.06	0.05-0.06	0.07	0.05-0.08	0.0558	0.012 – 0.0619
∑HPCs	1.50425	1.1066-7.8056	5.91		1.40		0.855	0.33- 2.01
3MeSOPCB101	0.04500	0.0202 – 0.2517	0.21	0.05-0.25	0.03	0.02-0.01	ND	
4MeSOPCB101	0.02905	0.0182 – 0.1532	0.13	0.05-0.15	0.02	0.02-0.07	ND	
4MeSOPCB110	0.01598	0.009 – 0.0437	0.03	0.02-0.04	0.01	0.009-0.02	ND	
4MeSOPCB149	0.01475	0.002 – 0.0382	0.02	0.005-0.04	0.01	0.002-0.02	ND	
∑$MeSO_2$-PCBs	0.10244	0.0623-0.47326	0.29		0.08		ND	
∑OHC	147.52		271.20		88.52		2.99	

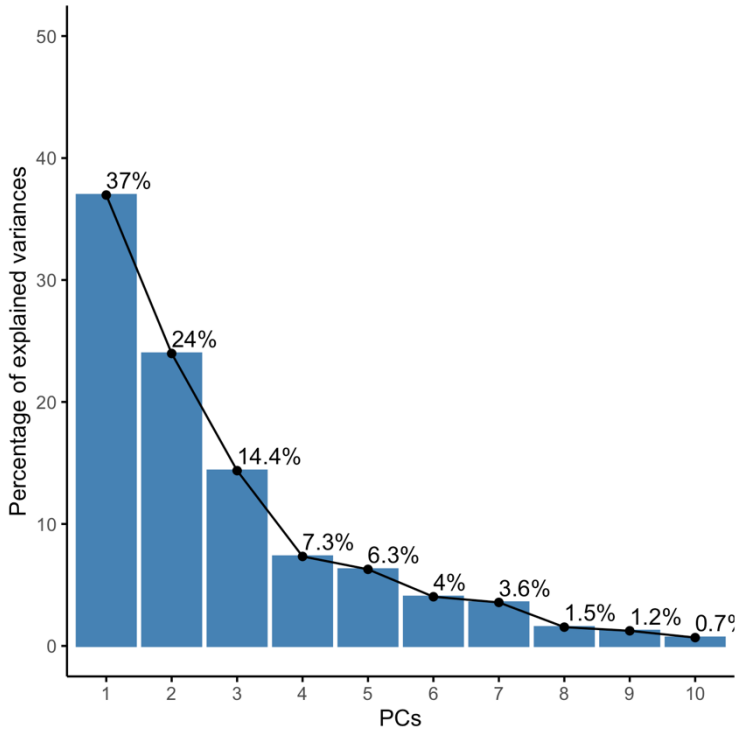


Figure B.1: Percentage of explained variances (%) of each PC to the total variance in the dataset including all predictor variables for common eider (n=11).

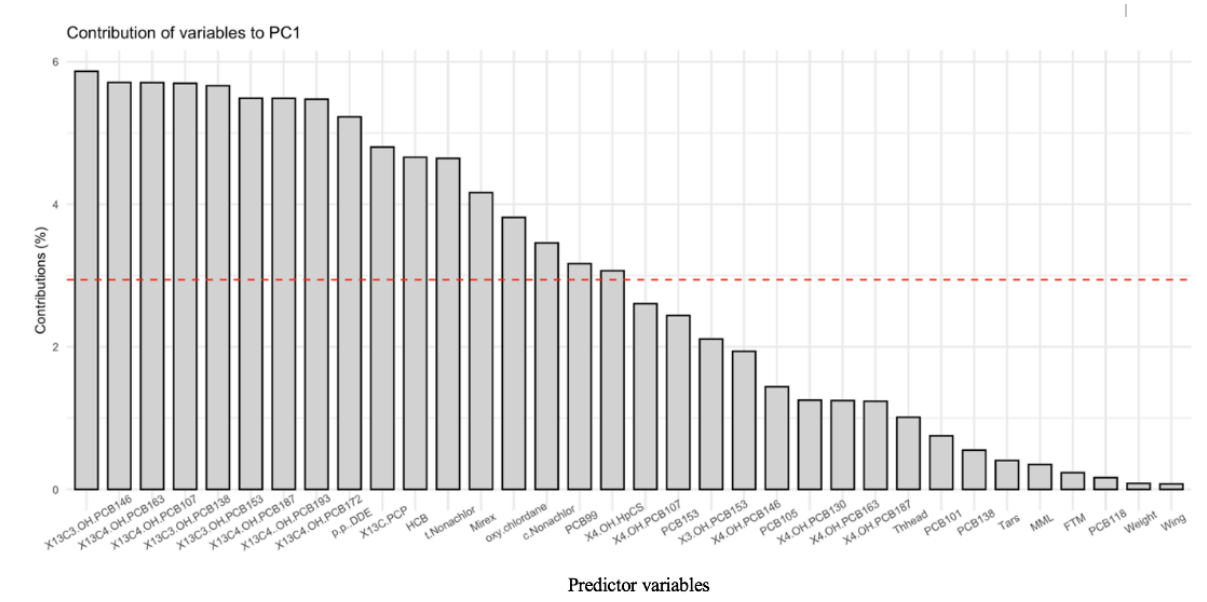


Figure B.2: Contribution (%) of each predictor variables in the PCA for common eider (n=11). The red dotted line represents a reference line corresponding to the expected value if the contributions were equal.

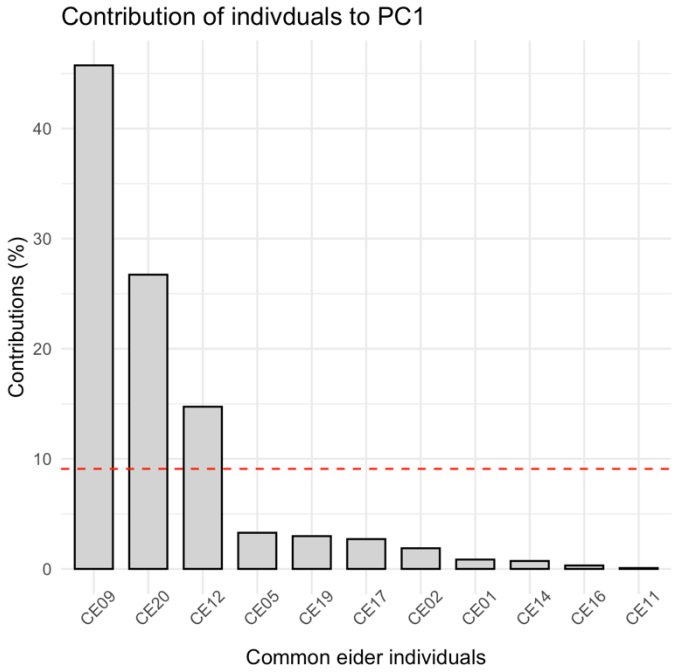


Figure B.3: Contribution (%) of each individual in the PCA for common eider (n=11). The red dotted line represents a reference line corresponding to the expected value if the contributions were equal.

Table B.5: Correlation table for contaminant groups, DNA-measurements and body condition index (BCI) for eider . Significant correlation is marked in bold.

	OCPs	PCBs	HPCs	MML	DNA-FTM	BCI
OCPs	1	0.17	**0.69**	-0.33	-0.31	-0.18
PCBs		1	0.36	0.28	0.47	-0.06
HPCs			1	-0.04	0.33	-0.21
MML				1	0.37	0.24
FTM					1	0.07
BCI						1

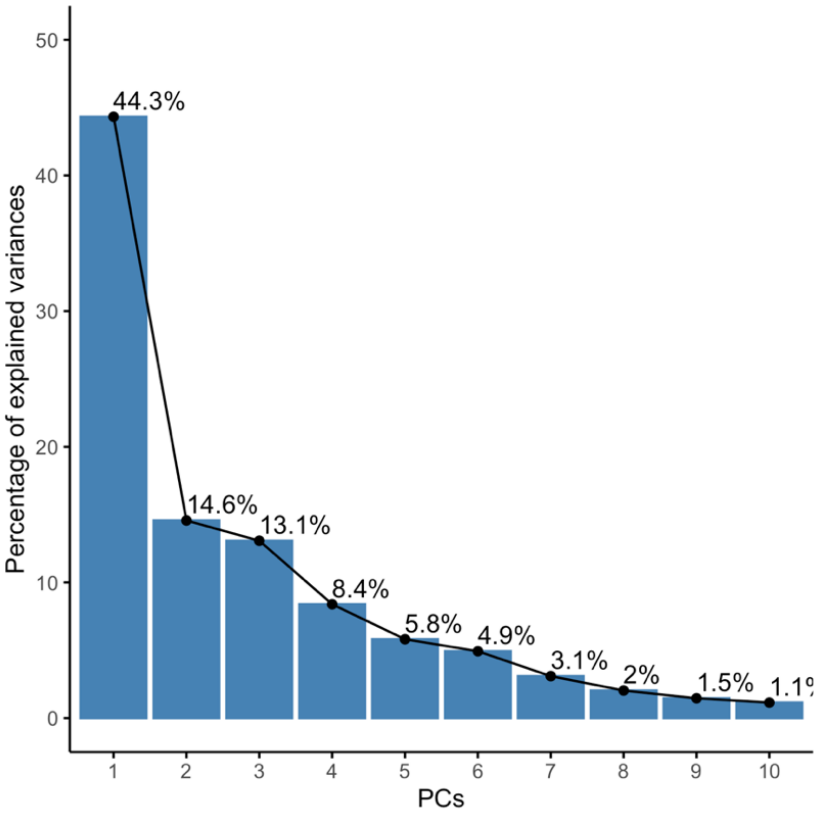


Figure B.4: Percentage of explained variances (%) of each PC to the total variance in the dataset including all predictor variables for glaucous gull, both sexes pooled together (n=13).

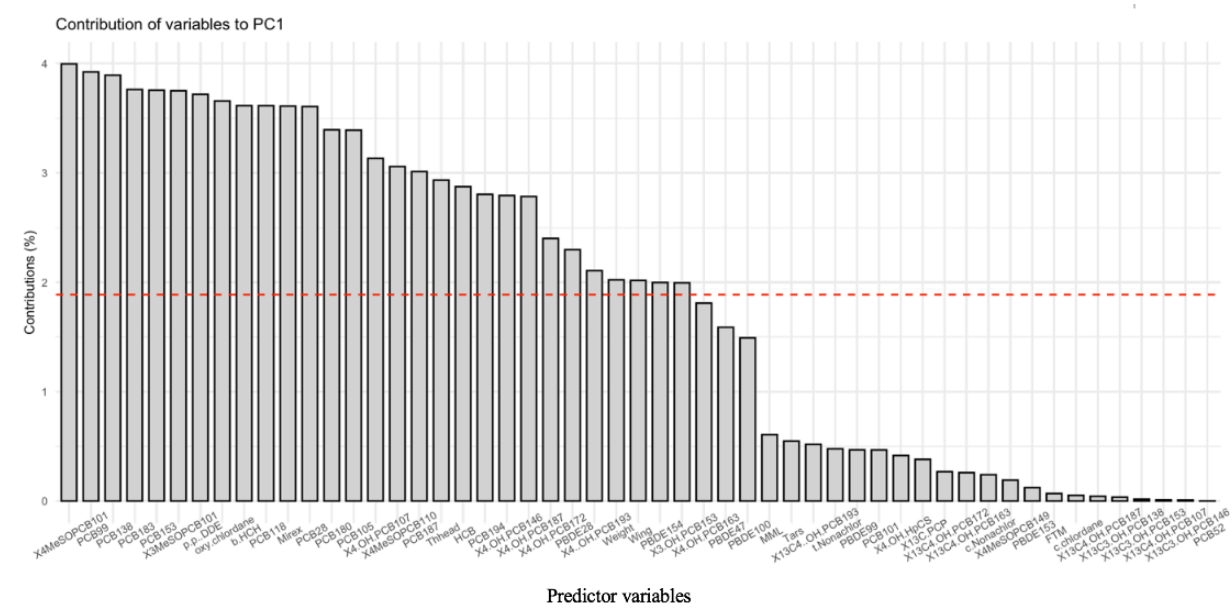


Figure B.5: Contribution (%) of each predictor variables in the PCA for glaucous gull (n=13). The red dotted line represents a reference line corresponding to the expected value if the contributions were equal.

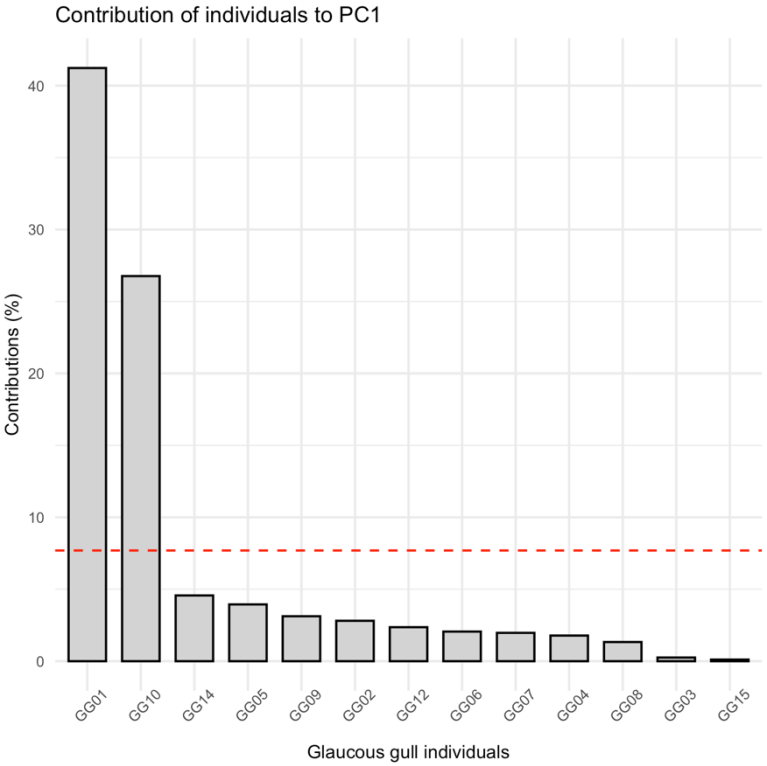


Figure B.6: Contribution (%) of each individual in the PCA for glaucous gull (n=13). The red dotted line represents a reference line corresponding to the expected value if the contributions were equal.

Table B.6: Correlation table for contaminant groups, DNA-measurements, and body condition index (BCI) for glaucous gull. Both sexes are pooled together. Significant correlation is marked in bold.

	OCPs	PCBs	PBDEs	HPCs	$MeSO_2$	MML	DNA-FTM	BCI
OCPs	1	**0.96**	0.43	**0.76**	**0.80**	-0.26	0.19	-0.32
PCBs		1	0.36	**0.78**	**0.82**	-0.26	0.07	-0.38
PBDEs			1	0.15	0.41	-0.41	0.09	-0.12
HPCs				1	**0.65**	0.37	-0.09	-0.23
$MeSO_2$					1	-0.20	-0.14	-0.38
MML						1	**-0.60**	0.35
FTM							1	-0.30
BCI								1

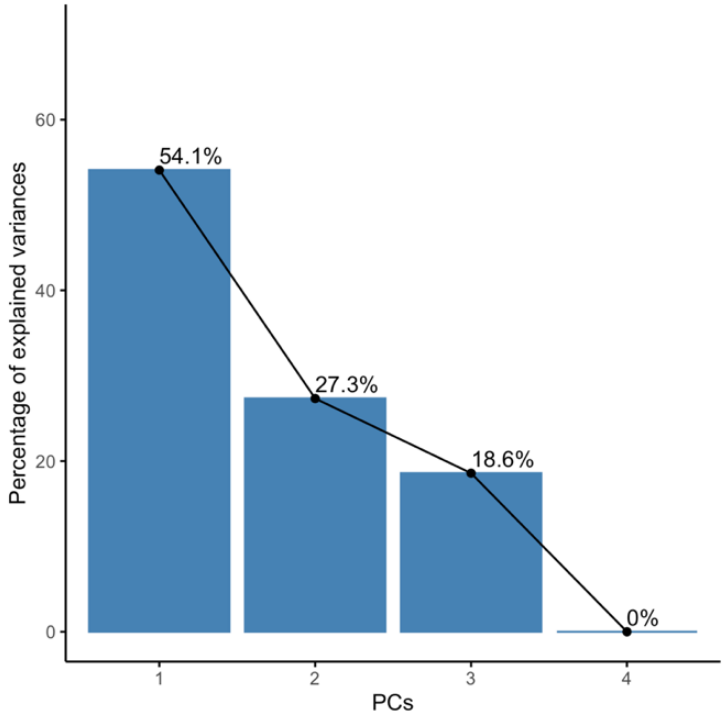


Figure B.7: Percentage of explained variances (%) of each PC to the total variance in the dataset including all predictor variables for glaucous gull males (n=4).

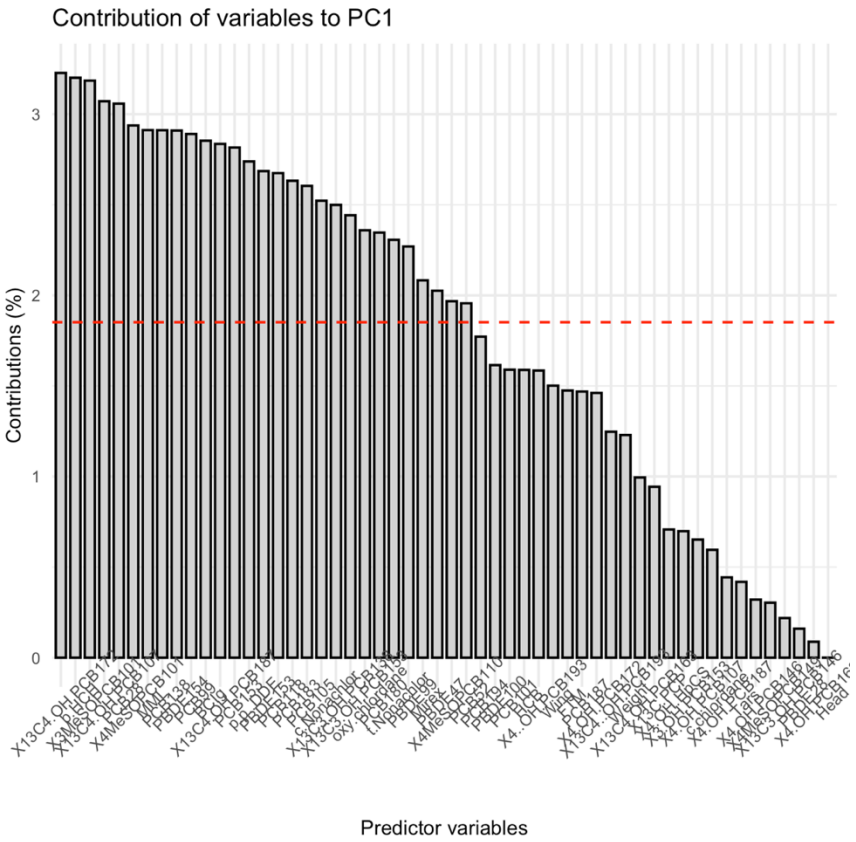


Figure B.8: Contribution (%) of each predictor variables in the PCA for glaucous gull males (n=4). The red dotted line represents a reference line corresponding to the expected value if the contributions were equal**.**

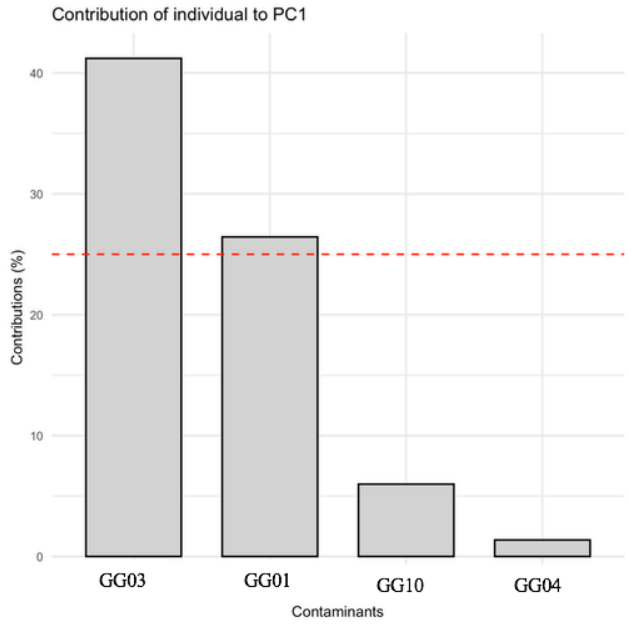


Figure B.9: Contribution (%) of each individual in the PCA for glaucous gull males (n=4). The red dotted line represents a reference line corresponding to the expected value if the contributions were equal.

Table B.7: Correlation table for contaminant groups, DNA-measurements, and body condition index (BCI) for glaucous gull males. Significant correlation is marked in bold.

	OCPs	PCBs	PBDEs	HPCs	$MeSO_2$	MML	FTM	BCI
OCPs	1	**0.96**	0.80	0.08	0.87	-0.86	0.40	-0.69
PCBs		1	0.62	0.01	0.81	**-0.95**	0.60	-0.66
PBDEs			1	0.006	0.84	-0.49	-0.08	-0.72
HPCs				1	-0.39	0.29	-0.54	0.63
$MeSO_2$					1	-0.84	0.46	-0.65
MML						1	-0.81	0.77
FTM							1	-0.52
BCI								1

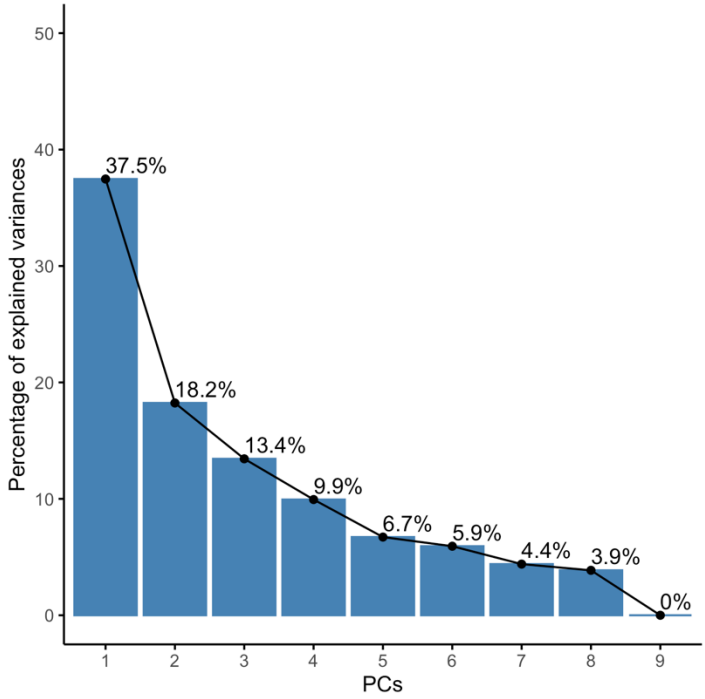


Figure B.10: Percentage of explained variances (%) of each PC to the total variance in the dataset including all predictor variables for glaucous gull females (n=9).

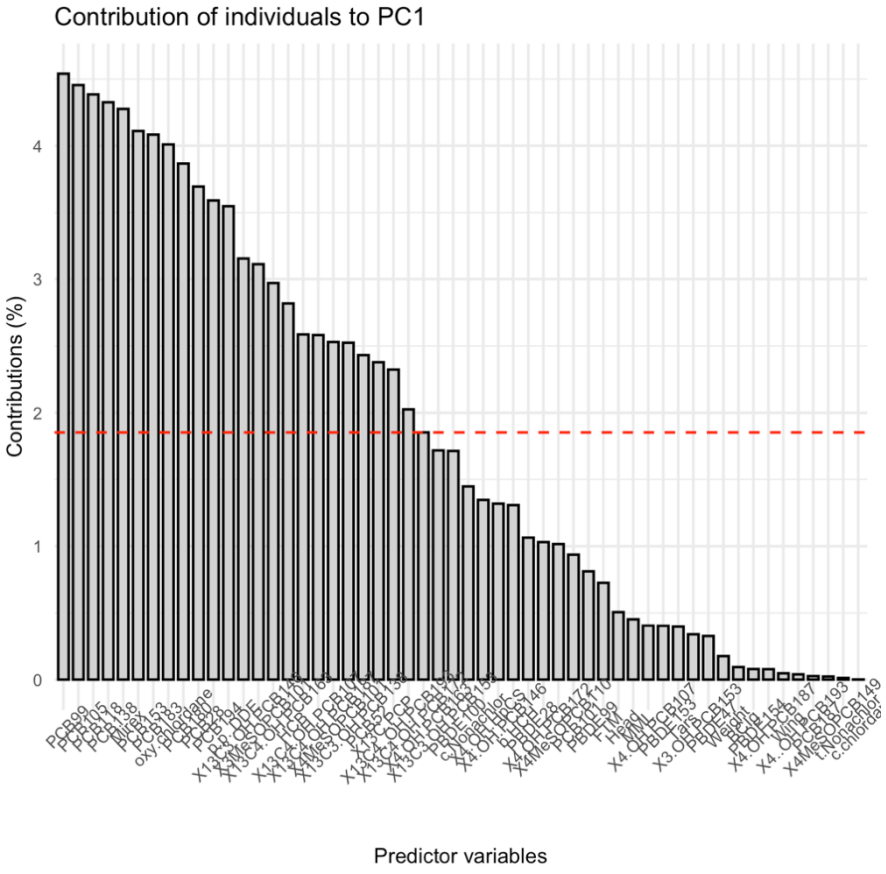


Figure B.11: Contribution (%) of each predictor variables in the PCA for glaucous gull females (n=9). The red dotted line represents a reference line corresponding to the expected value if the contributions were equal**.**

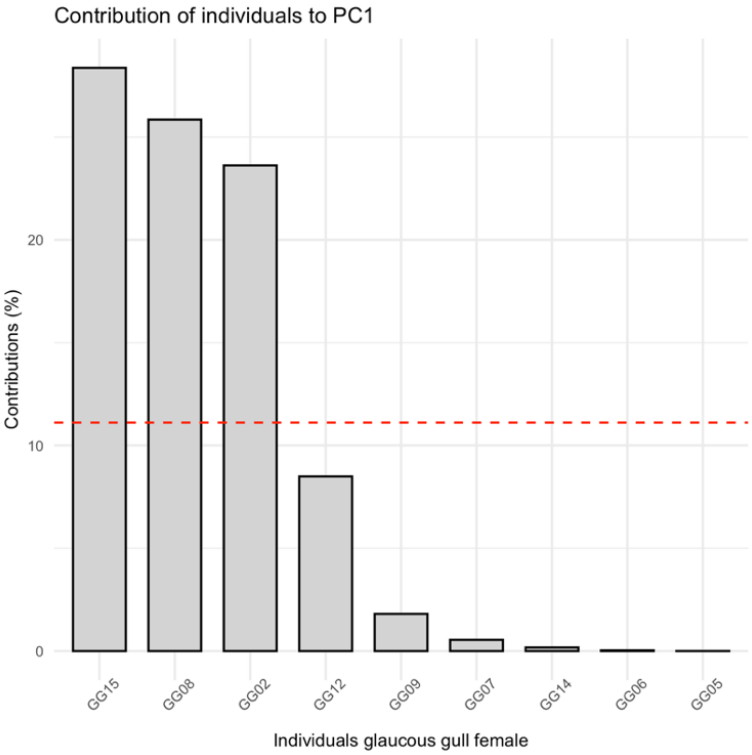


Figure B.12: Contribution (%) of each individual in the PCA for glaucous gull females (n=9). The red dotted line represents a reference line corresponding to the expected value if the contributions were equal.

Table B.8: Correlation table for contaminant groups, DNA-measurements, and body condition index (BCI) for glaucous gull females. Significant correlation is marked in bold.

	OCPs	PCBs	PBDEs	HPCs	$MeSO_2$	MML	FTM	BCI
OCPs	1	**0.92**	-0.54	0.50	**0.67**	0.39	-0.33	-0.29
PCBs		1	-0.48	0.50	**0.74**	0.31	-0.40	-0.49
PBDEs			1	-0.30	-0.21	-0.22	0.07	0.19
HPCs				1	0.30	0.46	0.04	**-0.67**
$MeSO_2$					1	-0.17	-0.15	-0.48
MML						1	-0.29	0.13
FTM							1	-0.22
BCI								1

Marit Vågenes Michelsen

NTNU
Norwegian University of
Science and Technology