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Levels and Potential Genotoxic Effects of Organohalogenated Contaminants in Seabird Eggs from Svalbard

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Abstract

Organohalogenated contaminants (OHCs) have been detected in relative high levels in arctic seabirds and eggs and are of great concern due to their potential toxic effects. OHCs are incorporated into the eggs during egg formation and the avian embryo get exposed when it absorbs these contaminants together with albumen and yolk during development. Critical developmental processes occur at the same time, which makes the embryo extra sensitive and vulnerable to toxic effects. Assessing levels and toxic effects in eggs during avian embryo development is therefore important. The objective of the present study is to investigate the potential for OHC induced genotoxic effect in form of DNA double strand breaks (DNA DSB) in eggs from glaucous gull (*Larus hyperboreus*), kittiwake (*Rissa tridactyla*) and common eider (*Somateria mollissima*). 33 eggs from glaucous gull (n = 5), kittiwake (n = 11) and common eider (n = 17) were collected around the Ny-Ålesund archipelago. The blastodisc was analysed for DNA DSB by agarose gel electrophoresis where the migrated fraction of the total DNA (DNA-FTM) and median molecular length (MML) were quantified. Yolk and albumen were homogenized and analysed for 82 OHCs. Eggs from glaucous gull had the highest OHCs levels (Σ_{48}OHC 726.98 ng/g ww) followed by eggs from kittiwake (Σ_{50}OHC 608.61 ng/g ww) and common eider (Σ_{34}OHC 45.07 ng/g ww). The pattern was similar in the species and consistent with previous studies. There were no significant differences in DNA-FTM or MML levels in eggs from the three species. For kittiwake and common eider eggs, no relationship was observed between egg volume and lipid content with OHCs, except for lipid with polychlorinated biphenyls (PCBs) in kittiwake. However, developmental stage showed a positive relationship with organochlorine pesticides (OCPs) and PCBs in common eider eggs, but not in kittiwake. None of the biological variables showed a relationship with DNA-FTM or MML. Furthermore, a positive relationship was found between MML and OCPs and MML and Polybrominated diphenyl ethers (PBDEs) in kittiwake eggs. Hence, a negative association between OHCs and DNA DSB. A possible explanation could be low OHCs levels in the blastodisc and an upregulation of the antioxidant defence system and repair systems. However, the mechanisms behind this negative relationship are unclear and repair systems, antioxidant defence, apoptosis and OHC levels in the embryo early in development should be further investigated.

Sammendrag

Organohalogenerte miljøgifter (OHCs) har blitt detektert I relativt høye nivåer i arktisk sjøfugl og egg, og er av stor bekymring grunnet deres potensiale for toksiske effekter. OHCs blir inkorporert i egg og embryoet blir eksponert når det absorberer disse miljøgiftene sammen med eggehvite og eggeplomme under utvikling. Kritiske utviklingsprosesser skjer samtidig, noe som gjør embryoet ekstra sensitivt og sårbart for toksiske effekter. Vurderinger av nivåer og toksiske effekter under embryoets utvikling er derfor viktig. Målet med denne studien er å undersøke potensialet for OHC indusert genotoksisk effekt i form av DNA dobbelt trådbrudd (DNA DSB) i egg fra polarmåke (Larus hyperboreus), krykkje (Rissa tridactyla) og ærfugl (Somateria mollissima). 33 egg fra polarmåle (n = 5), krykkje (n = 11) og ærfugl (n =17) ble samlet inn fra øyene rundt Ny-Ålesund. Blastocysten ble analysert for DNA DSB med agarose gel elektroforese hvor den migrerte fraksjonen av det totale DNA (DNA-FTM) og median molekylær lengde (MML) ble kvantifisert. Eggehvite og eggeplomme ble homogenisert og analysert for 82 organohalogenerte miljøgifter. Egg fra polarmåke hadde de høyeste nivåene (Σ_{48}OHC 726.98 ng/g ww) etterfulgt av egg fra krykkje (Σ_{50}OHC 608.61 ng/g ww) og ærfugl (Σ_{34}OHC 45.07 ng/g ww). Mønsteret var tilnærmet likt mellom artene, og i samsvar med tidligere studier. Det var ingen signifikante forskjeller av DNA-FTM eller MML nivåer mellom de tre artene. For Ingen relasjon mellom eggvolum eller fettinnhold og OHCs ble observert i krykkjeegg og ærfuglegg, bortsett fra fettinnhold og polyklorerte bifenyler (PCBs) i krykkjeegg. Utviklingsstadiet viste derimot en positiv relasjon med organiske klorerte pesticider (OCPs) og PCBs i ærfuglegg men ikke i krykkjeegg. Ingen av de biologiske variablene viste en relasjon med DNA-FTM eller MML. En positiv relasjon ble detektert mellom MML og OCPs og MML og polybromerte difenyl-etere (PBDEs) i krykkjeegg. Altså, en negativ relasjon mellom OHCs og DNA DSB. En mulig forklaring kan være lave OHC nivåer i blastocysten og en oppregulering av antioksidantforsvarssystemet og reparasjonssystemet. Mekanismen bak den negative relasjonen er uklart og reparasjonssystemer, antioksidantforsvar, apoptose og OHCs nivåer i embryo under tidlig utvikling må undersøkes nærmere.

Abbreviations

ΔAIC/c	Change in AIC/c to lowest AIC
AIC	Akaike information criterion
AICc	AIC corrected for small sample size
ANOVA	Analysis of variance
BFR	Brominated flame retardant
CE	Common eider
CV	Coefficient of variation
DCM	Dichloromethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DF	Detection frequency
DNA DSB	DNA double-strand breaks
DNA-FTM	DNA fraction of total DNA, that migrated into the gel
EOM	Extracted organic material (lipid content %)
GC	Gas chromatography
GG	Glaucous gull
GPC	Gel permeation chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HPC	Halogenated phenolic compound
ISTD	Internal standards
HR	Homologous recombination
Kbp	Kilo basepair
KW	Black-legged kittiwake
LOD	Limit of detection
LOEL	Lowest observed effect level
LOQ	Limit of quantification
$MeSO_2$-PCB	Methylsulfone-polychlorinated biphenyl
MML	Median molecular length
MS	Mass spectrometry

NHEJ	Non-homologous end-joining
NILU	Norwegian Institute for Air Research
nl	Natural logarithm
NPI	Norwegian Polar Institute
NTNU	Norwegian University of Science and Technology
OCP	Organochlorine pesticides
OHC	Organohalogenated contaminant
OH-PCB	Hydroxy-polychlorinated biphenyl
p	Probability of rejecting the hypothesis
PBDE	Polybrominated diphenyl ether
PC	Principal components
PCA	Principal component analysis
PCB	Polychlorinated biphenyls
PFAS	Per- and polyfluoroalkyl substance
POP	Persistent organic pollutant
PP	Polypropylene
QQ	Quantile-quantile
R2	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)
SIM	Single ion monitoring
SRM	Standard reference material
TCN	Tetrachloronaphtalene
UHPLC	Ultra-high pressure liquid chromatography
VIF	Variance inflation factor
WW	Wet weight

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1 Introduction

1.1 Organohalogenated contaminants

Organohalogenated contaminants (OHCs) or persistent organic pollutants (POPs) comprise of a wide range of anthropogenic organic compounds, their degradation and metabolites (AMAP, 2004). The main sources of OHCs are industrial production, by-products from industrial processes and pesticides (UNEP, 2008a). The Stockholm convention is a global treaty that aims to protect human health and environment from anthropogenic organic pollutants by evaluating, regulating and phase out POPs. The criteria for compounds to be categorized as POPs under the Stockholm Convention are that they are persistent in the environment, have potential for long-range transport, bioaccumulate in species and cause adverse effects (UNEP, 2008b).

Twelve POPs (referred to as legacy POPs) were initially marked as banned or restricted in 2004. This included, among others, polychlorinated biphenyls (PCBs), and chlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT), chlordane, heptachlor, hexachlorobenzene (HCB) and mirex (UNEP, 2008a). PCBs were produced for use in many industrial purposes and are lipophilic and extremely persistent (de Wit et al., 2010). There are 209 different PCB congeners, and their properties and biological activity depends on the positions and number of chlorines on the biphenyl rings (Gabrielsen, 2007, de Wit et al., 2010). Some PCB congeners are metabolized in vertebrates to methylsulfone-PCBs ($MeSO_2$-PCBs) and halogenated phenolic compounds (HPCs) which have been detected and shown a toxic potential in arctic animals (Letcher et al., 2000, Verreault et al., 2005b). Chlorinated pesticides are produced for use in agriculture and have toxic properties by design (Walker et al., 2006). DDT and chlordane are of particular concern because their toxic metabolites p,p'-dichlorodiphenyldichloroethylene (DDE) and oxychlordane are found in high levels in top predators (AMAP, 2004). The ban and strict regulations of PCBs and chlorinated pesticides have resulted in decreasing levels in the arctic environment (Hung et al., 2016, Rigét et al., 2018). Despite of this PCBs and pesticides are still the most prominent contaminants in arctic seabirds (Letcher et al., 2010, Verreault et al., 2010).

The list of POPs is constantly updated and several “emerging contaminants” have been regulated and added to the list (UNEP, 2017). Among them are several per- and polyfluoroalkyl substances (PFASs) and brominated flame retardants (BFRs). PFASs are fluorinated compounds with different length of their carbon chains and functional groups. They bind to proteins and are associated with protein rich tissue (Armitage et al., 2013, AMAP, 2017). PFASs have been widely used in surfactants and polymers since the 1950s (Kissa, 2001), due to their character as water and oil repellent (Kelly et al., 2009). Polybrominated diphenyl ethers (PBDEs), a subgroup under BFRs, and congeners have similar structure to PCBs with different number of bromine atoms (AMAP, 2017). They were commercially produced as additive flame retardants in a variety of products, which means that they easily migrate out of the final product in contrast to covalently bonded flame retardants (Sellström et al., 1998). Both PFASs and PBDEs have been increasing in the environment, but more recent reports are now showing a declining trend (AMAP, 2017, Rigét et al., 2018).

OHCs are of great concern in the Arctic because of their uptake and potential effects on wildlife (Gabrielsen, 2007, Letcher et al., 2010). There are few local sources of OHCs in the Arctic region, however, these chemicals are still found in relative high levels in top predators in the marine food chain (Letcher et al., 2010). Many OHCs are volatile and reach the Arctic mainly due to long-range atmospheric transport and to a less extent via ocean currents (Hop et al., 2002). Due to their properties these compounds are not easily broken down, and will, to a various degree, persist in the environment for a long time (Braune et al., 2001).

In addition to being persistent, most OHCs are also lipophilic and will be accumulated in lipid-rich tissue over time due to the resistance to biodegradation and elimination (Furness and Camphuysen, 1997, Hop et al., 2002). This bioaccumulation will result in higher concentration in animals compared to their food source, causing the concentration of OHCs to magnify up the food chain and accumulate high levels in top predators (Furness, 1993, Borgå et al., 2001). Arctic species live in a harsh environment and store lipids as

an energy source (Gabrielsen, 2009). Thus, the high lipid reserves and utilization of lipid as an energy source will redistribute accumulated OHCs and make arctic species extra vulnerable for potential toxic effects (Letcher et al., 2010).

Animals in wildlife are exposed to a mixture of contaminants. These contaminants may together have a synergistic, antagonistic or additive effect (Eaton and Gilbert, 2013) which complicates the evaluation of contaminants in field studies. In addition to OHCs exposure, wildlife animals are also exposed to multiple stressors such as climate change, food limitations and parasites. These multiple stressors can have synergistic or additive effects on survival and reproduction (Kimberly and Salice, 2015, Bårdsen et al., 2018). Thus, lower concentrations of contaminants may also cause adverse effects when they co-occur with multiple stressors (Bårdsen et al., 2018). Extrapolation of effects and threshold levels from laboratory studies is difficult because the contaminant composition in wildlife varies between sites and is different from the technical produced mixtures and single compounds (Sagerup et al., 2009). In addition, the hazardous threshold levels of contaminants derived from laboratory studies might be to high due to controlled laboratory conditions that do not take multiple stressors into account (Bårdsen et al., 2018). Some compounds may be more harmful and are more important as causative agents than others. However, it is likely that various OHCs are producing adverse effect (Bustnes, 2006). Hence, it is important to evaluate the effects of natural mixtures of contaminants found in wildlife.

1.2 Maternal transfer of OHCs and exposure during avian development

1.2.1 Maternal transfer

During egg formation contaminants are transported from the mother and incorporated into the egg along with essential lipids and proteins. This mechanism is known as maternal transfer. The concentration and composition of contaminants in the egg will reflect the female plasma at the time off egg laying (Bargar et al., 2001, Drouillard and Norstrom, 2001, Verreault et al., 2006). Maternal transfer is influenced by several biological factors and the physicochemical properties of the contaminants (Drouillard and Norstrom, 2001, Verreault et al., 2006). The biological factors include clutch size, egg mass, body

condition, maternal fat stores and yolk content. The physicochemical factors of the contaminant are halogenation, molecular structure, lipophilicity, induction of metabolizing enzymes, rate of metabolism and affinity to macromolecules (Drouillard and Norstrom, 2001).

1.2.2 Exposure during embryogenesis

The germinal disc (hereafter referred to as the blastodisc), is the embryo forming part of the egg (Perry et al., 1978). It is a disc-shaped white spot visible on the surface of the yolk (Figure 1). The disc is placed on the top of a white yolk column extending to the core of white yolk in the centre of the yellow yolk, named latebra, (Perry et al., 1978).

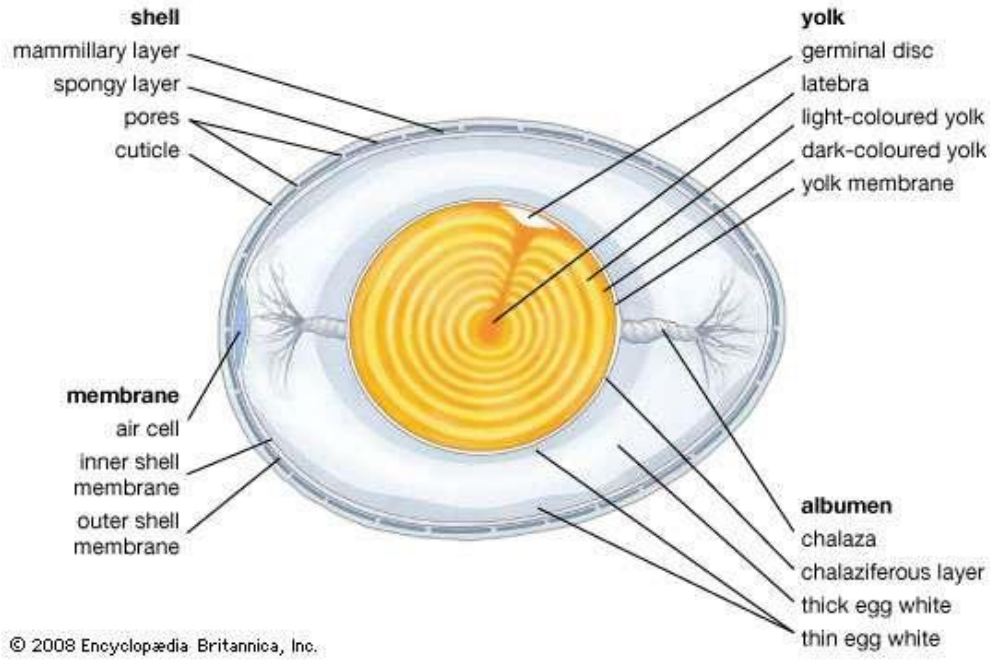


Figure 1. The structure of an egg with the germinal disc (Encyclopædia Britannica, 2008).

The embryo utilizes yolk and albumen during development, and at the same time get exposed to contaminants due to absorptions of contaminants together with yolk and albumen (Bargar et al., 2001). Lipophilic compounds are more present in the yolk as it is more lipid rich compared to the amino acid rich albumen (Dean et al., 2018). Lipid is the major source over the whole embryogenesis, accounting for 84-98 % of the total material oxidized. However, different components are digested at different times and the main energy source in the early embryonic stages is carbohydrates in form of glucose (Murray,

1925, Romanoff and Romanoff, 1967). Contaminants gradually increases in the embryo from the first day of development (Dean et al., 2018). Due to the higher uptake of lipids in the later stages of development (after organ development), the embryo will be more exposed to OHCs during these stages (Bargar et al., 2001, de Roode and van den Brink, 2002, Maervoet et al., 2005). When hatching occurs, a main share of the chemicals remains in the yolk sack, which the newly hatched chick will utilize during the first days of growth (Bargar et al., 2001, Maervoet et al., 2005).

Programmed cell death and apoptosis are central during avian embryo development and regulates several developmental scenarios such as deletion of autoreactive cells during development of the immune system, elimination of aberrant or excess cells, morphogenesis of the embryonic heart, formation of digits during the development of the limb bud and during re-modelling of the embryonic brain (Jacobson et al., 1997). These processes are essential for the development of the embryo and are strictly regulated pathways that involve both morphological and biochemical events. Radiation, temperature shock and chemical exposure can also result in unscheduled apoptosis and can result in abnormal development (Muscarella et al., 1998).

1.3 Genetic toxicology

The field genetic toxicology assesses the effect of chemicals and physical agents on DNA and the genetic processes of living cells (Preston and Hoffmann, 2013). DNA damage (Figure 2) includes DNA adducts (chemical addition to DNA bases), DNA strand breaks and cross-links, and lay the basis for generic alterations and genotoxicity in general (Preston and Hoffmann, 2013).

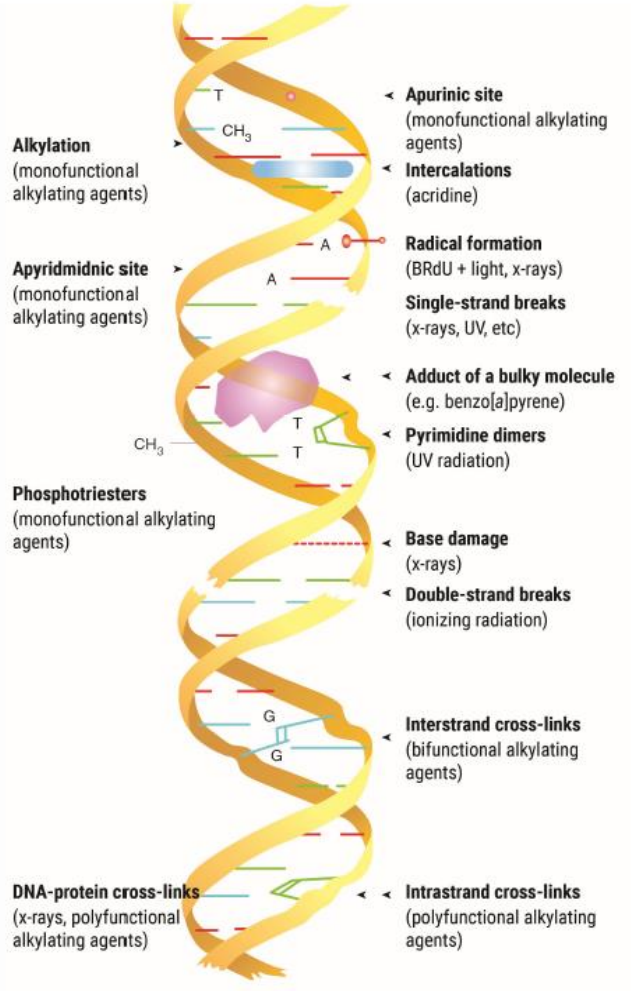


Figure 2: Types of DNA damage induced by chemical and physical agents (Preston and Hoffmann, 2013).

DNA breaks can be single-stranded or double-stranded (Preston and Hoffmann, 2013), where double-strand breaks (DNA DSB) are the result of simultaneously breakage of the phosphate backbone of two complementary strands in close sites to one another causing the two DNA ends to dissociate (Jackson, 2002). DNA DSB are considered the most fatal DNA damage because this disruption of the integrity of the DNA template cause a problem for cellular mechanisms such as repair, replication and transcription (Pfeiffer, 1998).

Production of DNA DSB are a common event as it is the result of common endogenous processes in the cell including replication, transcription, recombination and during DNA-repair (Vamvakas et al., 1997). DNA DSB are also induced during active cell death, such as apoptosis, when cells are damaged or no longer required (Vamvakas et al., 1997). DNA DSB are rapidly repaired under normal conditions. However, exogenous agents, such as irradiation and genotoxic chemicals, also induced DNA DSB and changes in DNA integrity (Vamvakas et al., 1997). *In vitro* and *in vivo* studies have shown that several OHCs and their metabolites have genotoxic potential and may induce DNA DSB (Srinivasan et al., 2001, Binelli et al., 2008). The genotoxic chemicals can interact and damage DNA directly in their parent form, through electrophilic xenobiotic metabolites or formation of reactive oxygen species (ROS) (Klaunig, 2013) (Figure 3). The accumulation of ROS and DNA DSB activates signalling cascades which induces apoptosis and cell death. At the same time DNA repair and antioxidant defences will also be induced to cancel out the negative effects from reactive species (Mrema et al., 2013).

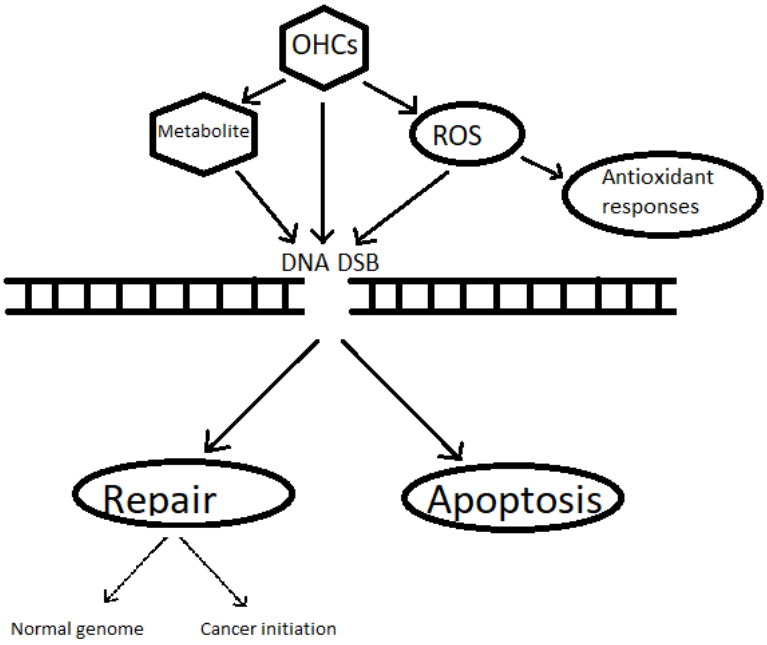


Figure 3. Induction of DNA DSB by genotoxic chemicals in the parent form (OHC), their metabolites or through ROS formation. Induction of DNA DSB causes signalling cascades that induces repair systems and apoptosis. ROS induce antioxidant responses directly and can also induce the repair systems and apoptosis directly (not shown).

There are two repair pathways for DNA DSB; homologous recombination (HR) and non-homologous end-joining (NHEJ). They work in complementary ways to efficiently reduce DNA DSB (Preston and Hoffmann, 2013). If the DNA repair system fails to repair, it can result in apoptosis, mutations, chromosomal rearrangement and carcinogenesis (Pfeiffer, 1998, Jackson, 2002). Thus, genotoxic compounds represent a major challenge because the damage may be transmitted to offsprings (DeRosa et al., 1998). Population bottlenecks, mutations and selection caused by genotoxic compounds may alter the genetic varibility and allele frequency of populations (Bickham et al., 2000). Such long-term effects could possibly alter dynamics at population and community level which may have a great ecological impact (Wurgler and Kramers, 1992, Bickham et al., 2000). Studies on pollutant-induced DNA DSB are important because DNA DSB can act as an early warning biomarker and indicate severe genotoxic effect.

1.4 Study species

Several arctic seabirds have been used in monitoring to assess levels and effects of OHCs. The three species chosen for the present study represents the different trophic positions; glaucous gull as a top predator, kittiwake as an intermediate and common eider at a low trophic level.

Figure 4. Study species: a) glaucous gull, b) black-legged kittiwake and c) common eider female. (Photo: glaucous gull: Geir Wing Gabrielsen, kittiwake and common eider: private)

Glaucous gull (*Larus hyperboreus*)

Glaucous gull (Figure 4a) is the most numerous avian predator and one of the largest gulls in the Arctic (Anker-Nilssen et al., 2000). It is a top predator and scavenger in the marine food web (Borgå et al., 2001, Hop et al., 2002), and feeds opportunistically on a wide range of species, mainly egg and chicken from seabirds, fish, carrion, crustaceans, seal pups and other marine organism (Haftorn, 1971, Lydersen and Smith, 1989, Erikstad, 1990, Borgå et al., 2001). Bioaccumulation of contaminants through the marine food web and a diet consisting of eggs and chicks of other seabirds and carcasses causes high OHC levels in the top predator glaucous gull and its eggs (Helgason et al., 2011).

Glaucous gull is a migrating bird and spends the winter in the northern part of the Atlantic Ocean before it migrates north to Svalbard during the breeding season (Løvenskiold, 1964). Depending on the climate change condition, the egg laying season usually starts at the end of May and last until middle of June. The incubation period lasts for 28 to 30 days, and the number of eggs varies from two to three (Løvenskiold, 1964, Haftorn, 1971).

Glaucous gull has the status as near threatened at Svalbard, and the population is estimated to 4250 pairs (3600 at Spitsbergen) (Fauchald et al., 2015). A decreasing population trend is seen in the biggest colony located at Bjørnøya, and is related to high levels of OHCs (Strøm, 2007). Monitoring of the population of glaucous gulls located at Spitsbergen (including Kongsfjorden) indicates an increasing population trend (14 %) over the last ten years (Anker-Nilssen et al., 2018). OHC levels are generally lower in glaucous gull and eggs from Kongsfjorden compared to Bjørnøya, and differences in diet have been suggested to explain these differences in OHC levels (MOSJ, 2018b).

One of the most extensively studied arctic avian species is the glaucous gull (Verreault et al., 2010). The main reproductive effects of OHCs seen in glaucous gulls are found at the embryonic stage as dead embryos or reduced fitness (Bustnes et al., 2003). The occurrence of nonviable eggs and poor body condition of hatchling has been related to high levels of OHCs in glaucous gulls at Bjørnøya (Bustnes et al., 2003, Erikstad et al., 2011). Genotoxic effects have been associated with OHCs in Svalbard glaucous gull

chicks fed with contaminated eggs. Levels of DNA adducts were significantly higher in the exposed chicks compared to control (Østby et al., 2005), also chromosome aberrations and DNA DSB were higher in the exposed chicks compared to control, although not significantly (Krøkje et al., 2006).

Black-legged kittiwake (*Rissa tridactyla*)

Black-legged kittiwake (hereafter "kittiwake") (Figure 4b) is a medium sized gull and occupies an intermediate trophic level in the marine food chain. It is a pelagic feeder and consumes mainly fish and marine invertebrates (Mehlum and Gabrielsen, 1993). This diet causes lower OHC levels in kittiwake eggs compared to glaucous gull eggs (Helgason et al., 2011). Kittiwake breed in the circumpolar zone (Mehlum and Gabrielsen, 1993) and build their nests on cliffs in colonies (Haftorn, 1971). The incubation period lasts for 27 to 28 days and usually two eggs are laid (Haftorn, 1971).

The kittiwake population at Svalbard is classified as near threatened and is estimated at 283 689 pairs (153 689 at Spitsbergen) (Fauchald et al., 2015). The population on Spitsbergen declined with 40 % in the period from 1988-2013 (Fauchald et al., 2015). However, more recent data indicates that the population in Kongsfjorden, Spitsbergen is slightly increasing (2 %) during the last ten years (Anker-Nilssen et al., 2018).

Studies on reproductive parameters show that Svalbard kittiwakes with higher levels of OHC lay their eggs earlier (Tartu et al., 2014) and have reduced probability of breeding the following year (Goutte et al., 2015). Moreover, a study of genotoxicity indicates a reduction of telomere length in relation to increasing levels of OHCs (Blévin et al., 2016).

Common eider (*Somateria mollissima*)

The sea duck common eider (Figure 4c) feeds at a low trophic level in the marine food chain on benthic invertebrates (Dahl et al., 2003). Eggs from common eider generally have lower OHC levels because the bird feed at a lower trophic level compared to top predatory and intermediate level birds such as glaucous gulls and kittiwake (Huber et al., 2015). Common eider breeds in the circumpolar zone along the coast in Arctic and Europe. The breeding season in Kongsfjorden depends on the ice melting and vary from

year to year (Mehlum, 1991). They breed 24-26 days from the first egg is laid. After the first egg is laid they will lay one egg each day till they reach a clutch size of three to six eggs (Swennen et al., 1993, Watson et al., 1993, Hanssen et al., 2005).

The common eider population on Svalbard decreased in the 1900s due to egg and dawn harvesting (Prestrud and Melum, 1991). The population, with an estimated population of 17 000 pairs at Spitsbergen, has remained stable in the last decades, with the status least concern at Svalbard (Fauchald et al., 2015, MOSJ, 2018a). However, more recent data from 2012-2017 shows a decline (27 %) in the breeding population at Kongsfjorden (MOSJ, 2018a).

The female common eider incubates the eggs alone. They do not feed during the incubation period and experience an extreme weight loss up to 46 % of their body mass (Gabrielsen et al., 1991). (Fenstad et al., 2014) showed that OHC levels increases with decreasing body mass during incubation and decreasing body mass was related to increasing DNA DSB in Svalbard common eiders. Moreover, eiders in poor condition have also shown to lay smaller final eggs which may result in reduced size and growth of the ducklings, although this was not measured (Hanssen et al., 2002).

1.5 Objective

Embryos undergo critical developmental processes during early life stages and are more sensitive to effects from chemicals (Zheng et al., 2014), and therefore exhibit higher toxicological sensitivity compared to adults (Barron et al., 1995). The main reproductive effects of OHCs seen in the declining population of glaucous gulls from Bjørnøya are found at the embryonic stage as dead embryos or reduced fitness of hatchlings (Bustnes et al., 2003). Moreover, the mortality is greater in the early developmental stages (2-6 days, prior to organ development) and in the last trimester (Carlson and Duby, 1973, Christensen, 2001). OHCs may cause genotoxic effects in this sensitive period which may lead to mortality. Assessing levels and toxic effects during avian embryo development is therefore important.

The objective of the present study is to assess levels of OHCs and DNA DSB in eggs from glaucous gull, kittiwake and common eider and investigate the potential OHC induced genotoxic effect in form of DNA DSB.

It is hypothesized that levels of OHCs will be higher in eggs from glaucous gull and kittiwake compared to eggs from common eider due to biomagnification and trophic levels. It is also hypothesized that the pollutants will affect the levels of DNA DSB, and that DNA DSB will increase with increasing contaminant levels.

2 Material and Methods

2.1 Study area and sampling material

Fieldwork was conducted in the fjords around Ny-Ålesund, Svalbard (78°´N, 12°´E) during the breeding season in June 2015 (Figure 5). Collecting eggs is easy and considered a non-destructive method, as collecting one egg from each nest does only have minor impact on the population level (Furness, 1993). Several species such as gulls and common eiders also have the ability to lay an extra egg if the conditions are good (Løvenskiold, 1964, Waldeck et al., 2011). The fieldwork was co-ordinated with other projects on seabirds to minimize the disturbance of the seabird colonies. The time spent at each location, and the handling time were kept at a minimum. The fieldwork was registered in the database Research in Svalbard (RiS) with the RiS-ID: 10186. The egg sampling was approved by the Governor of Svalbard (application reference number: 2014/00489-4), and the sampling was in accordance with the current regulation of the Norwegian Animal Welfare Act.

Figure 5. Sampling site. Glaucous gull eggs (blue dots) were collected from Krossfjorden between Kapp Guissez and Fjortende Julibukta. Common eider eggs (grey dots) were collected from the islands; Prins Heinrish, Breøyene and Storholmen in Kongsfjorden. The kittiwake eggs (orange dot) were collected from Krykkjefjellet in Kongsfjorden. The sampling was conducted during the breeding period in 2015.

Glaucous gull eggs were collected from Krossfjorden between Kapp Guissez and Fjortende Julibukta. Common eider eggs were collected from the islands; Prins Heinrish, Breøyene and Storholmen in Kongsfjorden. The kittiwake eggs were collected from Krykkjefjellet in Kongsfjorden. The nests were located by visual observation. One egg was collected from each nest containing two or more eggs. A total of 66 eggs were collected from glaucous gull (n = 13), kittiwake (n = 20) and common eider (n = 33).

Identification number, clutch size, date, GPS-position and nest location were noted for each egg. The eggs were transported back to the field station at Ny-Ålesund within 6-8 hours after capturing and kept at 4 °C. The length and width were measured before the eggs were opened, germinal disc isolated and yolk and albumen were homogenised (described in section 2.2.2). The samples were stored at -80 °C until transportation to the mainland.

The samples for DNA double strand break analysis were transported in a dryshipper (- 70 °C) from Ny-Ålesund to Trondheim, and immediately transferred to a freezer (-80 °C), at the Department of Biology, Norwegian University of Science and Technology (NTNU). The samples for chemical analyses were transported in a freezer (-20 °C) to the laboratory at the Norwegian Institute for Air Research (NILU) in Tromsø and kept in a freezer (-20 °C).

2.2 DNA double-strand breaks analysis

The analysis for DNA DSB was conducted on 33 egg samples (5 glaucous gulls, 11 kittiwakes, 17 common eiders) by agarose gel electrophoresis at the Department of Biology, NTNU. The use of gel electrophoresis is a well-established method to detect DSB and has the advantages of being a relatively rapid, inexpensive, sensitive method with high reproducibility and 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 for analysing fish samples by Theodorakis et al. (1994). The method has later been modified for avian blood (Krøkje et al., 2006, Fenstad et al., 2014). In the present study, the method was modified to analyse egg material. A list of chemicals and equipment used for this analysis is found in Appendix A.1.

2.2.1 Principles of DNA Double-Strand Break analysis

The principle of this procedure is to embed the nucleated cells in agarose gel plugs to protect the DNA from procedural damage. Lysis and enzymatic digestion of the cells removes nucleases and DNA-associated proteins leaving the DNA as a nucleoid in the gel plug (Theodorakis et al., 1994, Shaposhnikov et al., 2008). Neutral pH conditions during digestion and electrophoresis hinder unwinding of the DNA strands. Thus, maintaining the supercoiled and duplex structure of the DNA (Collins et al., 2008, Shaposhnikov et al., 2008). The presence of DNA DSB will relax the supercoiling, and the more breaks the more relaxed structure (Collins et al., 2008, Shaposhnikov et al., 2008).

During electrophoresis, the DNA, which is negatively charged, will migrate towards the positively charged anode. DNA fragments will be separated based on size, as shorter fragments will migrate further into the gel than larger ones. The factor determining the amount and migrating distance of DNA is the fragmentation of the structure produced by DNA DSB (Theodorakis et al., 1994, Collins et al., 2008). As the frequency of DNA DSB increases, more fragments will be released and migrate during electrophoresis.

The amount of DNA DSB can be quantified with the relative measures; migrated DNA-fraction of total DNA (DNA-FTM) and median molecular length (MML). DNA-FTM is the fraction of the total DNA loaded into the well that migrates into 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 increasing frequency of DNA DSB will result in more fragmented DNA causing DNA-FTM to increase and the MML value to decrease. Hence, an increase in DNA DSB is positively related to DNA-FTM and negatively related to MML.

2.2.2 Modification of the DNA double-strand breaks analysis to egg material

The initial development of the method from blood to egg material was done on gull (*Laurus*) eggs bought at Ravnkloa fish marked in Trondheim. Different techniques for opening the egg and isolating the germinal disc with the embryonic DNA were carried out. Only the best successful technique, incorporated in the final protocol, is described here. The top of the eggs was carefully opened with a scalpel. The albumen was transferred to

a glass beaker and the yolk was transferred to a petri dish for visual scoring of the developmental stage on the germinal disc. The developmental stage was visually scored from 0 (infertile) to 20 (fully developed) by morphological characters according to Appendix B. The germinal disc was isolated with a glass pasteur pipet and transferred to a cryo tube (1.5 mL, Nalgene). The albumen and yolk were homogenized together using Ultra Turrax disperser (Ultra Turrax T18 basic, IKA, Germany), transferred to a vial (15 mL, Qorpark). All samples were immediately frozen and stored at -80 °C.

Electrophoresis of the germinal disc samples was carried out according to the method described by Fenstad et al. (2014). After the electrophoresis only four out of eight eggs gave results in form of bands in the gel, and the intensity from the fluorescence was varying. The most developed eggs (observed as expanded germinal disc with visible blood vessels) showed strong fluorescence. The eggs that were less developed (expanded disc without blood vessels) showed weak fluorescence and the infertile (no expansion of the germinal disc) showed weak fluorescence in the well and had no band in the gel. Based on this initial testing, it was proposed that eggs should be developed with visible blood vessels to get enough amount of DNA for a successful analysis.

To further evaluate if the developmental stage was a factor for isolating enough DNA, speckled sussex chicken eggs (*Gallus Gallus Domesticus*) were delivered from a local farm at Jonsvatnet in Trondheim. The hen eggs were incubated at 37.8 °C with 70 % humidity for three days to get eggs with an expanded germinal disc with visible blood vessels. The incubation was successful and results from electrophoresis showed strong visible DNA bands indicating that a lot of DNA was isolated. However, there was a lot of streaking present in the bands.

The strong fluorescence from the DNA bands in the gel together with the streaking could indicate that the plugs contained too much DNA. In attempt to lower the amount of DNA the plugs were split into smaller pieces. This did not improve the results, probably because the density of DNA in the plugs was still the same. Therefore, a series of dilutions (described in section 2.2.3) were made for each sample to ensure that at least one dilution

contained the appropriate amount of DNA for gel electrophoresis. This was successful and was incorporated into the protocol.

An egg flotation test of all the eggs was performed, according to Kilpi and Lindström (1997), to investigate if it was possible to score the developmental stage without opening the eggs. The test was not able to separate the developmental stages from each other during the first week of incubation because all eggs sank and lied flat on the bottom.

2.2.3 Applied method

Preparations of plugs

Agarose plugs for electrophoresis were prepared according to the procedure described by Fenstad et al. (2014). The egg was opened, and the germinal disc was isolated as described in section 2.2.2. The germinal disc was suspended in TE buffer (Tris 10 mM, EDTA 1 mM, pH 8, 250 µL, 37 °C) and mixed together with 1 % low melting point agarose (LMPA dissolved in TE, 250 µL, 37 °C) in a 1:1 relationship. The sample was spun to ~8000 rpm to homogenize and avoid particles in the mix. 300 µL of the supernatant was taken out to make plugs. The rest of the mixture was diluted by adding more TE buffer (100 µL) and LMPA (100 µL) before a new volume (300 µL) was taken out to make another batch of more diluted plugs. This step was repeated four times to make a series of plug dilutions. 0.5 % agarose plugs (50 µL) were casted in a BioRad gel plug mould and cooled to 4 °C (30 min). The cells in the plugs were lysed and enzymatic digested at 55 °C for 16 hours in Theodorakis lysis buffer (NaCl 100 mM, Tris 10 mM, EDTA 25 mM, 0.5 % SDS, pH 8) with freshly made proteinase K (1 mg/mL) added.

Gel electrophoresis

The plugs were cooled to room temperature, inserted into the wells of the agarose gel (0.6 % agarose in TBE buffer (Tris-borate 45 mM, EDTA 1 mM, pH 8, 23 °C)) and sealed with 1% LMPA. Whole linearized lambda phage DNA (37 µL, 11 µg) and Hind III digested lambda phage DNA (100 µL, 50 µg) in TE buffer (63 µL) were used as ladder and positive control (base pair sizes of ladder in Appendix A.2). Loading dye (15 µL) was added to the ladder mix to indicate the progress of the run. The gel was run in TBE buffer at 2.3 V/cm for 14 hours before the gel was stained in ethidium bromide (0.2 µg/mL TBE) for 1 hour.

The gel was washed thoroughly several times with tap water to remove excess ethidium bromide.

To minimize the effect of potential changes in conditions between runs, each egg sample was run on electrophoresis two or more times at different days. In addition, two gels with the same setup were run in parallel. The individual samples were in triplicates in each gel with a total of four different egg samples on each gel in addition to three lanes with ladder as illustrated in Figure 6. Hence, each egg sample was loaded in triplicates on four different gels run at two different times.

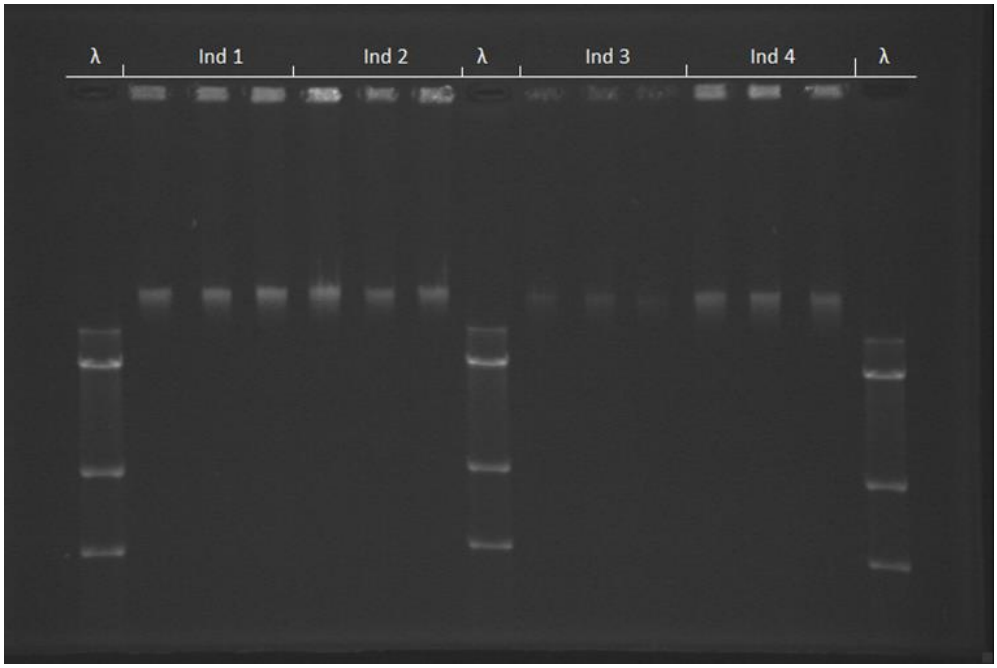


Figure 6. Demonstration of the gel setup. Each individual sample (Ind) was loaded onto the gel in triplicates. The lambda ladder (λ) was loaded on each side and in the middle of the well.

To counter for possible variation during the time of analysis all samples were chosen randomly. There was no available internal control sample for this method due to low amount of egg material. Instead each individual sample was run several times to account for variation between the electrophoresis runs and to detect eventual outliers. The coefficient of variation (CV % = SD/mean*100) was calculated for every individual between all the runs to evaluate the reproducibility. The CV value for DNA-FTM ranged

from 0.1 % to 18.9 %, except for one individual (KW16) with a high CV of 50.5 %. The CV value for MML results ranged from 0.1 % to 29.1 %, with the exception of two samples with relative high CV of 41.7 % (CE91) and 42.3 % (KW07) (Appendix K). DNA-FTM results were more reproducible compared to MML, but overall, the precision of the relative measures of DNA DSB were high. Thus, the results were highly reproducible.

Quantification of DNA double-strand breaks

The gel was imaged under UV-light where the fluorescence was emitted from the ethidium bromide stained DNA. The gel image data for densiometric quantification analysis was acquired on the documentation system BioRad Gel Doc 2000. Three horizontal DNA intensity staining curves were made for each lane in the gel. Hence, nine curves were made for each sample. The intensity curves (Figure 7) were used for calculating the two relative measures of DSB; DNA-FTM and MML.

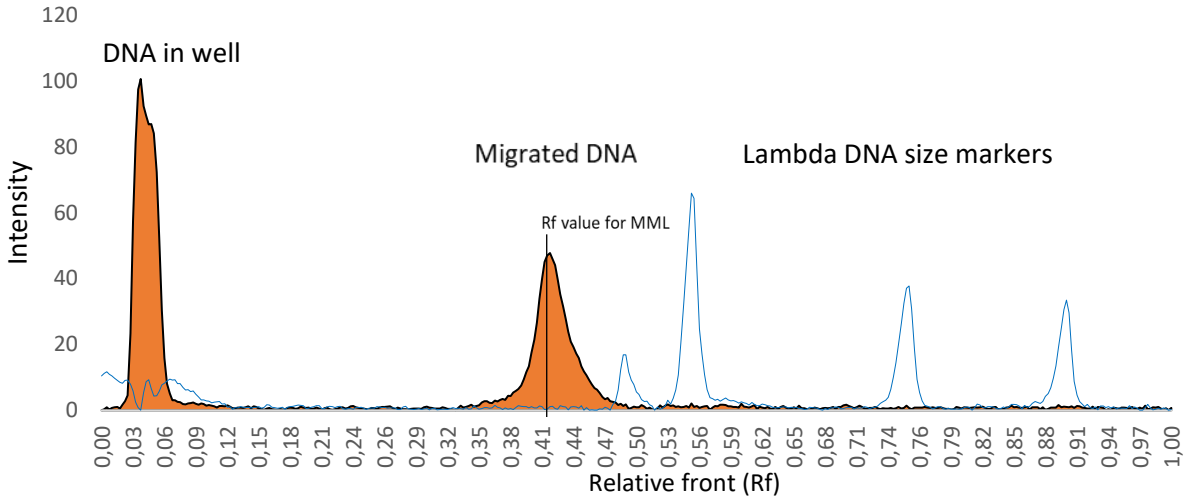


Figure 7. DNA intensity staining curve after electrophoresis. Intensity of the fluorescence from the DNA against the electrophoretic migration distance of the DNA (relative front rf). The area under the two intensity curves (coloured orange) represent the DNA in the well and the migrated DNA in the gel respectively. Rf-value of the MML acquired from the area corresponding to 50 % of the second peak of the intensity curve. Hence, the peak of the migrated DNA. The blue curve peaks represent the lambda DNA size markers 483337, 23130, 9416, 6557 bp.

The DNA in the well and the migrated DNA (DNA in gel) were determined from the area under the respective peaks from the intensity curves, and the DNA-FTM was calculated using equation 1.

$$DNA - FTM\ (\%) = \frac{DNAgel}{DNAgel + DNAwell}\ x\ 100 \qquad \text{Equation 1}$$

The migrating distance (rf-value) to the median of the area under the second peak in the intensity curve was acquired. The MML (kbp) corresponding to this rf-value was extrapolated from a standard curve obtained from the known relationship between the size (kbp) and corresponding rf-values to the Lambda DNA size markers (Appendix C.1). An extra point (400 kbp) was added to the standard curve (Appendix C.2) to extend its range because the size of available DNA size markers was lower than the MML value of the egg samples. The extra point was positioned at a set distance from the size markers to achieve consistency between the standard curves from different gels and comparable MML values.

2.3 Chemical analyses of OHCs

Chemical analyses of OHCs in the homogenized egg samples were conducted in the laboratories at NILU in Tromsø in autumn 2015. The analyses were funded by the Norwegian Polar Institute (NPI). All solvents applied were purchased from Merck (Darmstadt, Germany), and the internal and labelled standards provided by NILU were purchased from Wellington laboratories.

33 egg samples (5 glaucous gulls, 11 kittiwakes, 17 common eiders) were analysed for 82 contaminants including 12 PCB congeners, 7 PBDE congeners, 17 chlorinated pesticides, 20 $MeSO_2$-PCBs/DDE, 11 HPCs and 15 PFAS listed in Appendix D.

2.3.1 Chemical analyses of chlorinated and brominated compounds

Preparation

The homogenized egg samples were thawed in room temperature and mixed with a Vortex Mixer. The sample (2 g) was weighed out and added to Na_2SO_4 (burned at 600 °C for 8 hours) in relation 1:20 to remove water from the egg. The samples were stirred with a spatula to a homogenous solution, covered with aluminium foil and kept in the freezer (-20 °C) over night. The dried samples were thawed in room temperature, and big pieces were crushed with a spatula and transferred to an extraction column.

Cold column extraction

The purpose of the extraction step is to release the lipids and the lipid soluble compounds from the sample by letting suitable solvents run through the sample in the column.

Each sample was spiked with ^{13}C Internal standards (ISTD: POP I 20 µL, PBDE I 20 µL, HPC I 20 µL, $MeSO_2$-PCB 50 µL) prior to the extraction for later quantification of the concentrations for the analysed compounds. The samples were extracted three times with cyclohexane:acetone (1:1, 50 mL, 1 hour). The columns were covered with aluminium foil during the extraction to prevent contamination. The extract (150 mL) was concentrated to 0.5 mL with a turbovap evaporator (TurboVap 500, Zymark, 30 °C water bath). An empty vial (4 mL) was weighed before the extract was transferred. The turbovap glasses were rinsed once with hexane (1 mL) and once with dichloromethane (DCM, 1 mL) which were also transferred to the vial. The vial with extract was weighted and stored at 4 °C. The turbovap was cleaned with acetone between every sample to avoid cross contamination.

Lipid removal with gel permeation chromatography

Gel Permeation Chromatography (GPC) was used to remove lipids from the extract. The machine separates molecules by size. The largest molecules, such as lipids, will be released before smaller molecules, such as OHCs, thereby allowing removal of lipids before collecting the sample with the desired compounds.

The flow of the GPC (Waters 515 HPLC pump equipped with Waters Envirogel GPC columns) was set to 5 ml/min with DCM as eluent. A GPC standard was run to determine

the fraction collection time set for the samples in Waters Fraction Collector II. The extract (~1.0 mL) was injected to the GPC by using a syringe. Isooctane (~50 µL) was added as a keeper solvent to the lipid free extract prior to concentration to 0.5 mL using Turbovap. The extract was transferred to a test tube. Turbovap glasses was rinsed with hexane (~1.0 mL) and added to the extract. The blank samples were run first and the standard reference material samples (SRMs) were run last to assure no contamination of blanks from carryover from the samples.

Clean up with florisil

Florisil is a powder used for removal of remaining lipids and other matrix from the extract.

Fractionation

The program “testflo2.spe” was run using RapidTrace (Zymark) two times to remove air bubbles in the instrument. Test tubes for the different fractions were rinsed with cyclohexane and labelled before the fractionation. Columns were washed with cyclohexane and packed with florisil (1g ± 0.05 g, burned at 400 °C for 8 hours,) and frits (rinsed with DCM). The program “floall.spe” was used to clean up and fractionate three fractions:

Fraction 1 contains PCBs, DDTs, PBDEs and other chlorinated pesticides.
Fraction 2 contains $MeSO_2$-PCBs/DDE and some chlorinated pesticides.
Fraction 3 contains HPCs/Hydroxy-PCBs (OH-PCBs)

Isooctane (~20 µL) was added as a keeper solvent to each test tube after the fractionation. The test tubes were stored in the fridge covered with aluminium foil overnight.

Fraction 1:

The fraction 1 samples were evaporated to 0.2 mL with RapidVap (Labconco) and transferred to a GC vial with insert. The test tubes were rinsed with n-hexane which was added to the vials. The extract volume was reduced to ~30 µL using an N_2-evaporation unit. ^{13}C PCB 159 (200 pg/µL) recovery standard was added to each sample. The samples were stored in the fridge until quantification.

Fraction 2:

The extract volume was reduced to 0.5 mL with Rapid Vap. The Rapid Trace was checked for air by running the program “tubetest.spe” twice. The extracts were cleaned with columns packed with acidic silica (0.1 g Na_2SO_4, 0.7 g acidic silica, 0.2 g activated silica burned at 600 °C for 8 hours) and run with the program “sil_meso.spe” on the Rapid Trace. Isooctane (~20 µL) was added to each test tube before the volume was reduced to 0.2 mL using Rapid Vap. The extracts were transferred to GC-vials with insert. The test tubes were rinsed with hexane and added to the extracts before the extracts were reduced to ~30 µL using the N_2-evaporation unit. Tetrachloronaphtalene (TCN) (200 pg/µL) was added as a recovery standard and the vials were stored in the fridge until quantification.

Fraction 3:

In the first step, derivatization, the hydrogen atom on the hydroxyl groups on the HPC molecule was replaced with a methyl group. The deviation standard HPCI internal standard (100 µL) was used for quality control of the process. The standard was added to an empty test tube and treated the same way as the samples.

The extracts were evaporated to dryness using the Rapid Vap before isooctane was added and the samples were mixed by vortexing. Diazomethane (~1 mL) was added to each sample as a derivatization agent. The samples were kept in the fume hood with cap on and covered with foil for three hours. A yellow colour indicated that the derivatization was successful. The caps were kept open until the colour disappeared.

In step two the volume was reduced to 0.5 mL with the Rapid Vap and cleaned up with columns packed with acidic silica (0.1 g Na_2SO_4, 0.7 g acidic silica, 0.2 g activated silica burned at 600 °C for 8 hours). The samples were run with the program “sil_meo.spe” on the Rapid Trace, before the extract volume was reduced to ~30 µL using the N_2-evaporation unit. ^{13}C PCB 159 (200 pg/µL) recovery standard was added to each sample. The samples were stored in the fridge until quantification.

Instrumental analyses of chlorinated and brominated compounds

Detection and quantification of PCBs, DDTs, PBDEs and $MeSO_2$-PCBs/DDE were conducted one by one using an Agilent 7890 gas chromatograph (GC) equipped with a triple-quadrupole mass-spectrometer, Quattro Micro GC (Waters Corporation, Manchester UK) in electron ionization (EI) mode. Non-DDT organochlorine pesticides (OCPs) were analysed using an Agilent 7890A gas chromatograph equipped with a 5975C mass spectrometer (Agilent Technology, Boblingen, Germany) in negative chemical ionization (NCI) mode. Analysis of the derivatized HPCs was performed on an Agilent 7890A GC with a 5975C mass spectrometer (MS) (Agilent Technology, Boblingen, Germany) with the mass spectrometer operated in negative chemical ionization using methane (5.0 quality; Yara Praxair AS, Porsgrunn, Norway) and run in single ion monitoring (SIM) mode.

A DB-5MS column (length 30 m, 0.25 µm film thickness, 0.25 mm inner diameter; J&W Scientific, Folsom, CA, USA) was used for separation with a split/splitless injector heated at 250 °C for OH-PCBs, 280 °C for PCBs, $MeSO_2$-PCBs/DDE, OCPs and PBDEs and 220 °C for DDTs. A sample volume (1 uL) was injected using splitless mode with helium (6.0 quality; Yara Praxair AS, Porsgrunn, Norway) as a carrier gas with flow rate of 1 mL/min under constant flow. The temperature program for separation was set to 70 °C for 3 min (2 min for OCPs), followed by a temperature ramp of 15 °C/min to 180 °C followed by a final temperature ramp of 5 °C/min to 280 °C where the temperature was held for 6 min (10 min for OCPs and 5 min for OH-PCBs).

2.3.2 Chemical analysis of perfluorinated compounds (PFASs)

Preparation and extraction

The frozen homogenized egg samples were thawed in room temperature and mixed by vortexing before 1 g was weighed out and transferred to a polypropylene (PP) centrifuge tube (50 mL). The samples were spiked with ^{13}C labelled internal standard (allPFCs, 0.5 ng/µL, 20 µL). Acetonitrile (8 mL) was added to each sample and mixed by vortexing. The samples were sonicated in an ultrasonic bath (10 min) for extraction of contaminants. The sonication in ultrasonic bath disrupts the cell membrane which facilitates extraction.

The sonication step was repeated three times and mixed by vortexing between each sonication. The tube was centrifuged (2000 rpm, 5 min) for sedimentation. The extract was transferred to a PP-centrifuge tube (10 mL) and concentrated to 1 mL using the RapidVap.

Clean up

Activated carbon (ENVI-Carb, 120/400, Supelco 57210-U, Bellefonte, PA, USA) was used to remove proteins and matrix from the extract. ENVI-Carb (25 mg ± 1 mg) and glacial acetic acid (50 µL) were prepared in an Eppendorf centrifuge tube (1.7 mL) before the supernatant (0.8 mL) was transferred. The sample was mixed by vortexing thoroughly and centrifuged (10 000 rpm, 10 min). The supernatant (0.5 mL) was transferred to an autoinjector vial and recovery standard (0.1 ng/µl, 3.7 brPFDcA in methanol 20 µL) was added. The extract (100 µL) was transferred to LC-vials with insert and NH_4Oac in HLB-water (100 µL, 2 mM) was added.

Instrumental analysis of PFASs

Detection and quantification of PFASs were conducted using an ultra-high pressure liquid chromatography triple-quadrupole mass-spectrometry (UHPLC-MS/MS). PFASs were analysed on a Thermo Scientific quaternary Accela 1250 pump (Thermo Fisher Scientific Inc., Waltham, MA, USA) together with a PAL Sample Manager (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a Thermo Scientific Vantage MS/MS (Thermo Fisher Scientific Inc., Waltham, MA, USA). A sample volume (10 µL) was injected onto a Waters Acquity UPLC HSS 3 T column (2.1 x 100 mm, 1.8 µm, Waters Corporation, Milford, MA, USA) equipped with Waters Van guard HSS T3 guard column (2.1 x 5 mm, 1.8 µm, Waters Corporation, Milford, MA, USA). Compounds were separated using NH_4OAc (2 mM) in 90:10 methanol:water and NH4OAc (2 mM) in methanol as the mobile phases. Further details on the instrumental analysis is described in Hanssen et al. (2013).

2.3.3 Quantification

Quantification of concentrations of the individual compounds was conducted by using internal standard method. The ISTDs contains known concentrations of ^{13}C labelled equivalents to the compounds analysed and were used to quantify the amount of compounds in the samples. The ratio of the areas of the unlabelled standard versus the area of the internal standard produced a standard curve which was used to calculate the response factor (Rf) which again is used to calculate the concentrations of the compounds in the samples as shown in Equation 2.

$$C_{sample} = Rf \frac{C_{Istd} \text{ x } Area_{sample}}{Area_{Istd}} \qquad \text{Equation 2}$$

C_{sample} is the unknown concentration of the compound in the sample. C_{Istd} is the known concentration of the internal standard added to the sample. $Area_{sample}$ and $Area_{Istd}$ are the peak areas found from the chromatogram for the sample and the internal standard.

2.3.4 Quality assurance

Recovery standards were added to the samples prior to the instrumental analyses to calculate the recovery of ISTDs to assess the quality of the method. The recovery was within limit (50 %) for all samples except for the HPCs where the recovery was low (10 – 25 %), resulting in a larger uncertainty for the acquired results. SRMs (contaminated fish, EDF2525, Lot R543 Cambridge Isotope Laboratories for POP analyses and AM-S-Y1504, human serum, Program AMAP for PFAS samples) and blank samples were analysed for every tenth sample. The SRM was used to validate the accuracy repeatability of the extraction method. The SRM samples were within the limits of accuracy of ± 20 % for PCBs, HCB and hexachlorocyclohexanes (HCHs) and ± 50 % for pesticides. Blank samples were used for monitoring potential contamination that originates from handling the samples during the procedure. The SRMs and blanks were treated the same way as the egg samples. Limit of detection (LOD) was set to three times the background signal. In case of contamination in the blank samples the LOD was set to the sum of the average of the blank concentrations and three times the standard deviation of the blanks. LOD values for all compounds are listed in Appendix G.

2.4 Extracted organic material

The extracted organic material (EOM) also referred to as lipid content, was quantified gravimetrically by evaporating an aliquot of the extract from the cold column extraction step. The extract (~0.5 mL) was transferred to a Crimp vial (1.5mL) and covered with aluminium foil until the solvent had evaporated. The vial was weighed empty, with extract prior to evaporation and with the dried extract after evaporation. Percentage lipid content was calculated using equation 3.

$$\text{Lipid content } (\%) = \frac{\text{vial with dried extract – empty vial}}{\text{vial with extract – empty vial}}\ x\ 100 \qquad \text{Equation 3}$$

2.5 Egg volume

The egg volume was calculated by using Hoyt`s equation (Equation 4) (Hoyt, 1979).

$$\text{Egg volume } (\text{cm}^3) = \text{Width}^2 \text{ x Length x } 0.51 \qquad \text{Equation 4}$$

Where the width is the maximum diameter of the egg and 0.51 is the volume coefficient. This coefficient is applicable to egg from all species except for very pointed eggs (Hoyt, 1979).

2.6 Statistical analyses

From the 66 eggs collected, 33 eggs (5 glaucous gulls, 11 kittiwakes, 17 common eiders) were in developmental stage between day one and four, and were chosen for DNA DSB analysis and chemical analyses. Excel (2016) was used for calculations of MML, DNA-FTM, lipid content and egg volume and to make tables and simple graphs. All statistical analyses were conducted using R (version 3.4.0). Level of significance was set at 0.05. Individual concentrations and values for all contaminants, biological measures, DNA-FTM and MML are found in Appendix K.

2.6.1 Data treatment of samples below limit of detection

The detection frequency (DF) for all contaminants are listed in Appendix G. Compounds detected in less than 60 % of the samples for each species (DF < 0.6) were considered poorly represented due to a high proportion of non-detected values. On this background, 29 compounds were excluded from further statistical analyses (Appendix G). 21 of these compounds were not detected in any of the samples from the three species. 53 compounds had an DF ≥ 0.6 for least one of the species and were included in the dataset. To avoid missing values for compounds with a DF ≥ 0.6, individual samples under LOD were given a random number between 0 and the compound-specific LOD using the RANDBETWEEN function in excel. Seven, 20 and 30 substitutions were made for glaucous gull, kittiwake and common eider respectively, representing 4.1 % of the total dataset (2.9 % for glaucous gull, 3.8 % for kittiwake and 5.0 % for common eider). Individual samples measured between LOD and limit of quantification (LOQ) were treated equal to samples above LOQ. Contaminant data from $MeSO_2$-PCBs/DDE were missing for kittiwake individual 20. This individual was therefore given the median kittiwake concentrations (due to non-normality of data) for $MeSO_2$-PCBs/DDE.

2.6.2 Distribution of variables

Contaminants, biological measurements and measures of DNA DSB were checked for normal distribution ($p > 0.05$) with Shapiro-Wilk tests and quantile-quantile (QQ) plots. Non-normally distributed variables were transformed with natural logarithm (ln) and retested for normal distribution. Pearson correlation test was performed on normal distributed variables. Spearman rank correlation test was used on untransformed non-normally distributed variables.

Lipid normalizing data can be conducted to account for the effect of variation in lipid content. There was no significant correlation between lipid content and the contaminant concentrations (except for lipid and PCBs in kittiwake), and the data in the present study was therefore not lipid normalized.

2.6.3 Comparison between species

Differences in concentrations, biological parameters and DNA DSB between species were tested with analysis of variance (ANOVA). The assumption for the test; normal distribution of residuals and homogeneity of variance were tested with diagnostic plots (QQ-plot, Residual vs Fitted, Scale-location and Residuals vs Leverage). Kruskal-Wallis analysis was performed on untransformed variables in the cases where assumptions for ANOVA were not met. Tukey`s Post hoc comparison was performed on significant results from ANOVA and Kruskal-Wallis analysis.

2.6.4 Principal components analysis

Principal component analysis (PCA) is a multivariate procedure to reduce response variables to new uncorrelated variables named principal components (PCs). The first PC, PC1, contains most of the variability in the data, the following PCs account for the remaining variability in the dataset (Jolliffe, 2011). A PCA including all three species, was conducted for visualization of all variables together to explore relationships between the different variables (OHCs, biological variables and DSB variables). Contaminants and biological variables were transformed with natural logarithm due to non-normality and skewness. All variables were mean centred (subtracted the arithmetic mean) and scaled (divided by the standard deviation).

Comparison of variables between species showed that most variables were significantly different. Moreover, clustering of individuals according to their respective species was seen in PCA conducted on species together. Therefore, PCA with all variables was also conducted for each species separately to avoid masking any significant result. This was only performed for common eider and kittiwake due to the low number of glaucous gull eggs. Hence, glaucous gull samples were only used for investigating contaminant levels and for comparisons between species and will not be further explored with multivariate statistics. An additional PCA with only contaminants was performed for common eider and kittiwake. The scores from PC1, PC2 and PC3 were extracted and run in linear regression with MML and FTM as response variables to assess the effect of contaminants on DNA DSB.

2.6.5 Grouping of contaminants

PCA and correlation tests indicated correlations among the contaminants. To reduce the number of variables, contaminants were grouped based on the compounds position in PCA plots, correlation tests and physiochemical properties. DDTs, DDT metabolites and other chlorinated pesticides were grouped together as OCPs. Fluorinated compounds were grouped together as PFASs and all the PCB congeners were grouped together as PCBs. PCB metabolites were grouped into HPCs and $MeSO_2$-PCBs/DDE. The brominated compounds, detected only in glaucous gull and kittiwake were grouped as PBDEs. Hence, 53 contaminants were placed into six groups according to Appendix D, and the number of variables for the multivariate analysis was reduced.

2.6.6 Associations between contaminants and biological variables

Correlation tests were conducted for each species to explore possible associations between contaminant groups, biological variables (developmental stage, lipid and volume), and MML and DNA-FTM seen in the PCA plots. Significant correlations were further investigated with linear regression with contaminant groups as response variables and biological variables as explanatory variables to assess effects of developmental stage, lipid and volume on contaminant levels. Model residuals were checked for normal distribution, independence from response variable and equal variance with diagnostic plots (QQ-plot, Residual vs Fitted, Scale-location and Residuals vs Leverage) to ensure that assumptions for the models were met. Transformation of variables with natural logarithm was performed if the assumptions were not met. The transformed models were checked with diagnostic plots to avoid violations of model assumptions.

2.6.7 Associations between DNA DSB and contaminants

Multiple linear regression models were used to examine the potential effects of contaminant levels on DNA DSB (MML and DNA-FTM) for kittiwake and common eider. Candidate models were made based on prior knowledge, expectations and associations from PCA and correlation analyses. MML and FTM were set as response variables and the six contaminant groups as explanatory variables. The contaminants were transformed with natural logarithm to meet the assumptions of the model. Developmental stage and lipid were also included as explanatory variables in the models to assess potential effects

of these variables on DNA DSB. Opening time and volume were not included in the models. Volume was not expected to affect levels of DNA DSB and opening time was unfortunately measured in days (not hours) and is not accurate enough to detect potential effects from opening time as it was the same for almost every individual. The different candidate models (Appendix I.2) included the effect of each contaminant group alone, in combination with each other and in combination with lipid and developmental stage. Models including lipid and developmental stage together and alone, without contaminants, were also included.

Model selection was based on an information-theoretic approach using second-order Akaike information criterion (AICc) adjusted for small sample size. AIC estimates the relative quality of a collection of statistical models of the data. AICc, ΔAICc and weight was calculated using the package AICcmodavg in R. The best model is the model with lowest AICc score and have a ΔAICc = 0. All other models are ranked and given an ΔAICc value according to the best model. Models with ΔAICc ≤ 2.00 was considered to be good models with substantial support and was further explored (Burnham and Anderson, 2004). Further exploration of parameters estimates, standard error (SE), p-values and adjusted R^2 of the best models (ΔAICc ≤ 2.00) were conducted with multiple linear regression. All models were checked with diagnostic plot, as described above, to ensure that assumptions were met.

Multicollinearity occur when two or more of the explanatory variables are correlated. Multicollinearity in multiple regression arise a problem because it affects the variance of the parameter estimates causing significant parameters to be insignificant (Kerns, 2010). To assess correlations among the explanatory variables the variance inflation factor (VIF, usdm package R) was calculated (Appendix I.1). All predictors for kittiwake had acceptable VIF values below 10 (O'brien, 2007). Common eider showed higher VIF values for OCP, and $MeSO_2$-PCBs/DDE. Models including more than one explanatory variable might give an incorrect insignificant result when multicollinearity is present. Hence, results from common eider may yield wrong results.

3 Results

3.1 Biological variables

Volume was significantly different in the three species ($p < 0.05$) with glaucous gull having eggs with the largest volume (117.65 ± 2.79 cm^3) followed by common eider (97.65 ± 1.51 cm^3) and kittiwake (47.23 ± 1.88 cm^3) (Table 1). Common eider had significant higher lipid content (20.82 ± 0.9 %) compared to the other species ($p < 0.05$). No significant differences in lipid content were detected between kittiwake (7.39 ± 1.12 %) and glaucous gull (10.46 ± 1.67 %). The developmental stage (age), was similar among the species and ranged from 1 - 4 days for all species.

Table 1. Volume (cm^3), lipid content (%) and developmental stage (age in days) in eggs from glaucous gull, kittiwake and common eider collected at Svalbard 2015. Reported as mean ± SE and range. Significant differences ($p < 0.05$) are noted with different letters a, b and c.

	Glaucous gull (n=5)	**Kittiwake (n=11)**	**Common eider (n=17)**
Volume (cm^3)	117.65 ± 2.79 a 105.49 - 131.86	47.23 ± 1.88 b 39.99 - 53.11	97.65 ± 1.51 c 82.04 - 110.34
Lipid (%)	10.46 ± 1.67 a 8.97 – 11.78	7.39 ± 1.12 a 2.42-10.92	20.82 ± 0.90 b 13.64 - 33.87
Age (days)	2.60 ± 0.47 1 - 4	2.73 ± 0.32 1 - 4	2.12 ± 0.25 1 - 4

3.2 DNA DSB

The MML and DNA-FTM values for the three species were very similar, and not significantly different from each other (Figure 8). Mean DNA-FTM and MML with SE, median and range for all species are listed in Appendix F.1

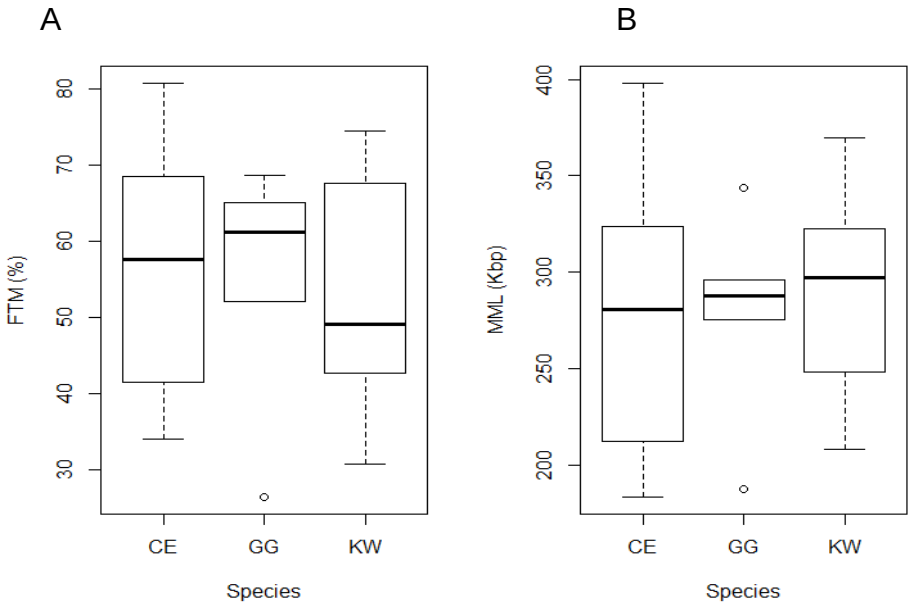


Figure 8. A) DNA-fraction of total DNA that migrated (DNA-FTM (%)) and B) median molecular length (MML (Kbp)) in eggs from glaucous gull (GG, n = 5), kittiwake (KW, n = 11) and common eider (CE, n = 17) collected at Svalbard 2015. The box plots represent median value (horizontal bold line) with the bottom and top line of the boxes representing the first and third quartiles respectively. Highest and lowest values are represented by whiskers extending from the box, and potential outliers are marked as circles.

The mean percentage migrated fraction (DNA-FTM) was highest for common eider (56.57 ± 4.17 %) followed by glaucous gull (54.72 ± 7.58 %) and kittiwake (53.09 ± 4.50 %). However, the differences in DNA-FTM values were not significant (p = 0.8601). Kittiwake had the highest MML values (280.42 ± 16.17 Kbp) followed by glaucous gull (277.86 ± 25.39 Kbp) and common eider (280.42 ± 16.95 Kbp), although the differences were not significant (p = 0.924).

MML and DNA-FTM are both measures of DNA DSB, and as expected, they showed a negative correlation with each other for species separately (glaucous gull: r = 0.80, p = 0. 108, kittiwake r = - 0.58, p = 0.061, common eider: r = - 0.65, p = 0.005) and all three species pooled together (r = - 0.65, p = 0,000044).

3.3 Contaminant levels and patterns

53 out of 82 analysed compounds had a DF ≥ 0.6 and were included in statistical analyses. Mean concentrations with SE, median and range of these contaminants are listed in Appendix F.2. Eggs from glaucous gull had the highest overall contaminant concentration (Σ_{48}OHC 726.98 ng/g ww), followed by kittiwake egg (Σ_{50}OHC 608.61 ng/g ww). Eggs from kittiwake and glaucous gulls had 12.5 and 15 times higher levels compared to common eiders (Σ_{34}OHC 45.07 ng/g ww).

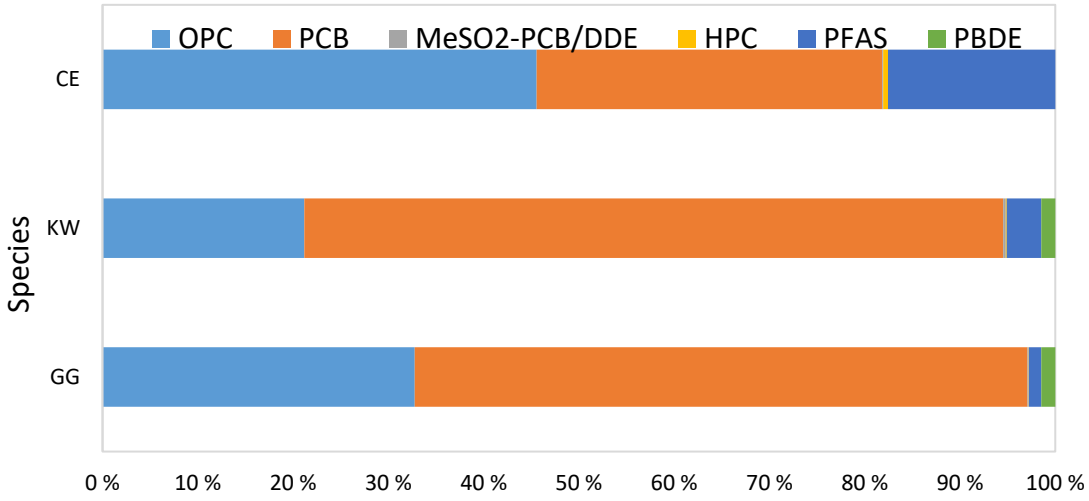


Figure 9. Contribution of contaminant groups OCPs, PCBs, $MeSO_2$-PCBs/DDE, HPCs, PFASs and PBDEs to the total contaminant burden in common eider (CE), kittiwake (KW) and glaucous Gull (GG) eggs from Ny-Ålesund archipelago 2015.

The contribution of each contaminant group to the total burden varied between the species (Figure 9 and Figure 10) In glaucous gull, the distribution was as followed: Σ_{12}PCB (64.32 %) > Σ_{9}OPC (32.78 %) > Σ_{5}PBDE (1.47 %) > Σ_{10}PFAS (1.32 %) > $\Sigma_{8}MeSO_2$-PCBs/DDE (0.09 %) > Σ_{4}HPC (0.03 %). For kittiwake: Σ_{11}PCB (73.35 %) > Σ_{9}OPC (21.17 %) > Σ_{9}PFAS (3.65 %) > Σ_{6}PBDE (1.46 %) > $\Sigma_{12}MeSO_2$-PCBs/DDE (0.28 %) > Σ_{3}HPC (0.09 %), and for common eider: Σ_{9}OPC (45.57 %) > Σ_{9}PCB (36.27 %) > Σ_{10}PFAS (17.56 %) > Σ_{3}HPC (0.49 %) > $\Sigma_{3}MeSO_2$-PCBs/DDE (0.11 %) > ΣPBDE (ND).

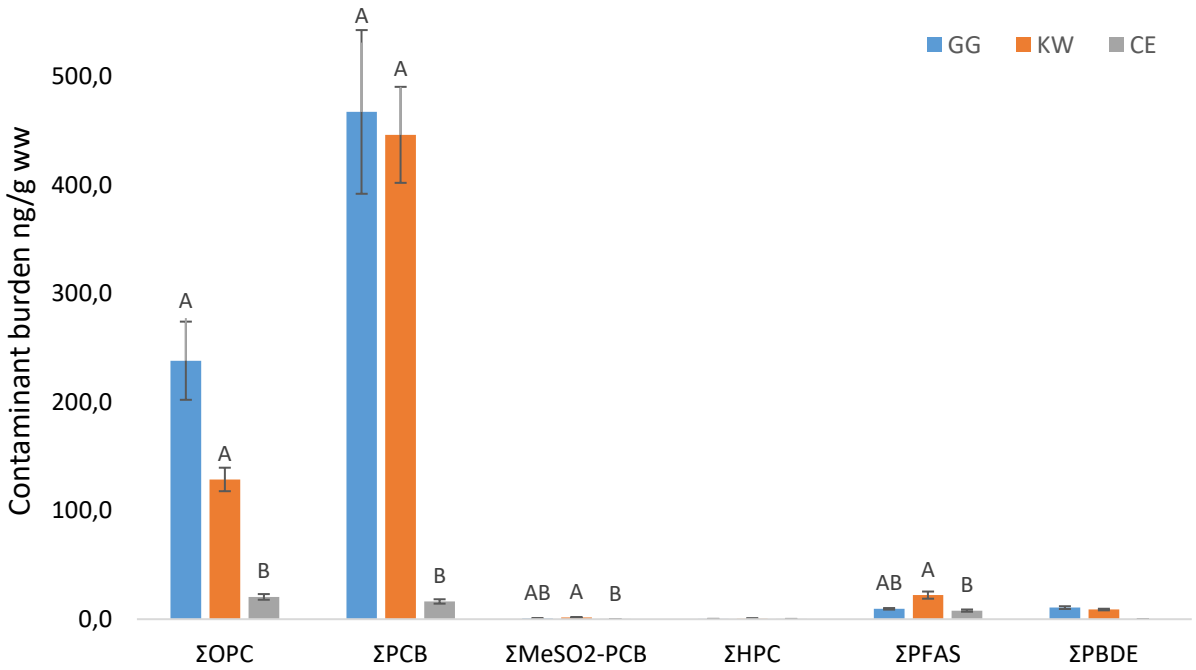


Figure 10. Levels for OPC, PCBs, $MeSO_2$-PCBs/DDE, HPC, PFASs and PBDEs measured in eggs from glaucous gull (GG, blue), kittiwake (CE, orange) and common eider (CE, grey) from Svalbard 2015. Presented as mean concentration ± SE (ng/g ww). The mean sum is based on all contaminants with DF ≥ 0.6 in the representative species. Significant differences ($p < 0.05$) are noted with different letters A and B.

The OPCs was the prominent contaminant group for common eider and the second major group for glaucous gull and kittiwake, representing respectively 45.57 %, 32.78 % and 21.17 % of the total contaminant load for each species. Comparison of mean ΣOPCs between species showed that glaucous gull had the highest levels (238.27 ng/g ww) followed by kittiwake (128.81 ng/g ww) and common eider (20.54 ng/g ww). However, the levels in glaucous gull and kittiwake were not significantly different. Levels in common eider were significantly lower compared to glaucous gull and kittiwake. p,p'-DDE was by far the most prominent OCP for all species (49-75 % of ΣOPCs) (Figure 11). The second most abundant compound for respectively glaucous gull and kittiwake was oxy-chlordane (12.19 % and 18.70 % of ΣOPCs) followed by HCB (4.05 % and 7.54 % of ΣOPCs). For common eider HCB (24.36 % of ΣOPCs) was more abundant than oxy-chlordane (10.27 % of ΣOPCs).

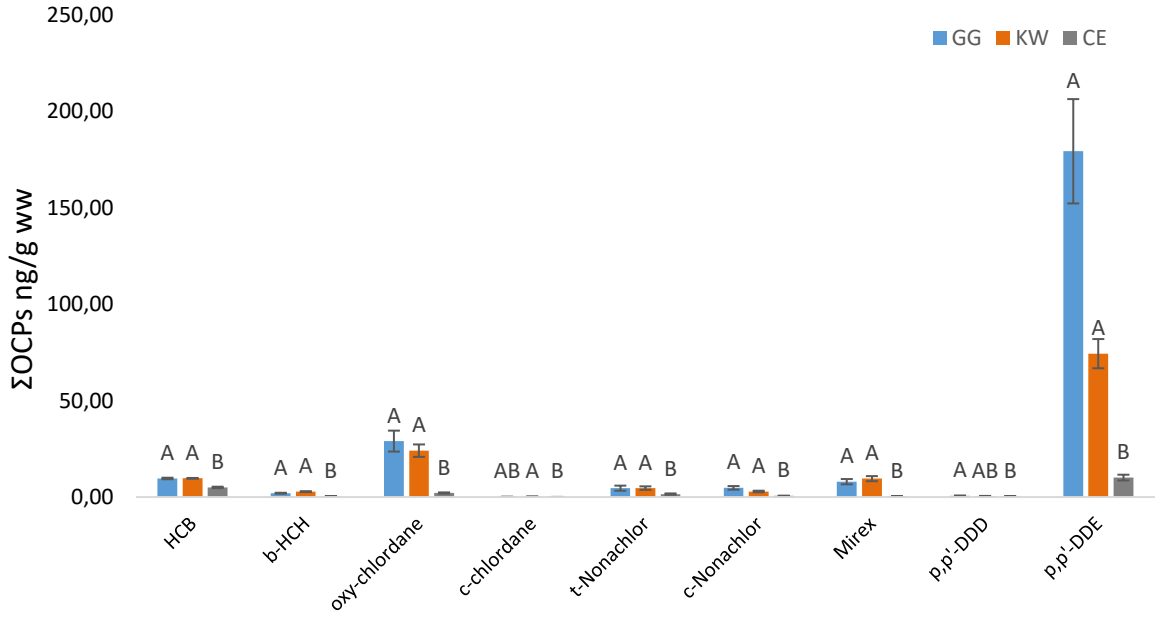


Figure 11. Levels of organochlorine pesticides (OCPs) in eggs from glaucous gull (GG, n = 5, blue), kittiwake (KW, n = 11, orange) and common eider (CE, n = 17, grey) from Svalbard 2015, presented as mean concentration (ng/g ww). Error bars represent the standard error (SE). Significant differences ($p < 0.05$) are noted with different letters A and B.

PCBs was the prominent contaminant group for glaucous gull and kittiwake and the second major group for common eider contributing 64.32 %, 73.35 % and 36.27 % of total contaminant burden. Glaucous gull (467.60 ng/g ww) and kittiwake (446.47 ng/g ww) had similar levels. Moreover, the mean ΣPCBs levels in glaucous gull and kittiwake were significantly higher compared to the levels in common eider (16.35 ng/g ww). The congener pattern was similar in the species (Figure 12), with PCB 153 (35.27 – 38.37 % of ΣPCBs) followed by PCB 138 (21.68 – 25.99 % of ΣPCBs) as the most prominent congeners for all three species.

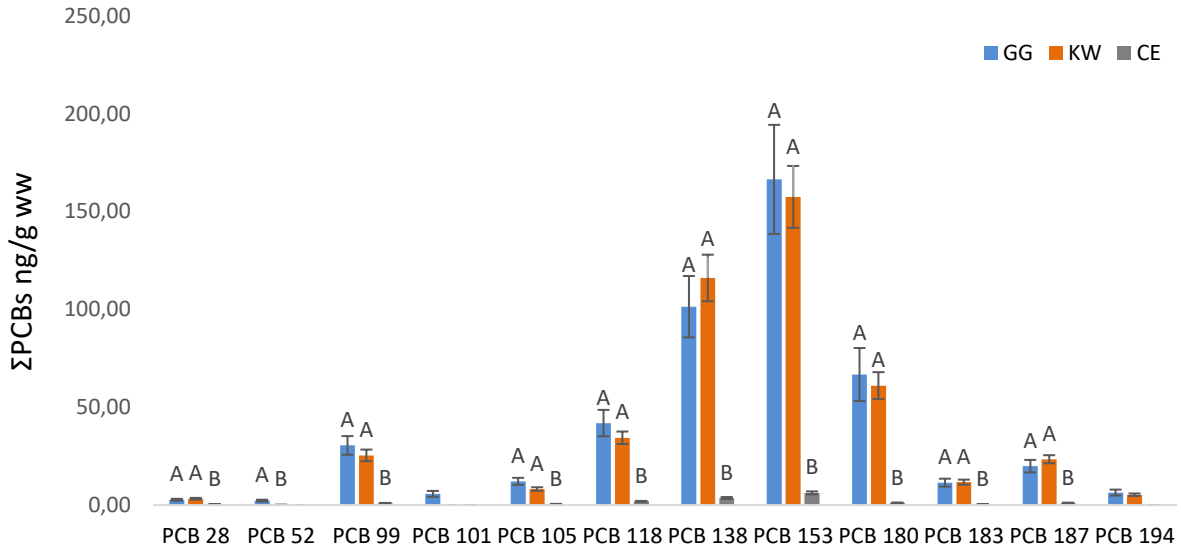


Figure 12. Levels of PCB congeners in eggs from glaucous gull (GG, n = 5, blue), kittiwake (KW, n = 11, orange) and common eider (CE, n = 17, grey) from Svalbard 2015, presented as mean concentration (ng/g ww). Error bars represent the standard error (SE). Significant differences ($p < 0.05$) are noted with different letters A and B.

The PCB metabolites were detected in relative low amounts for all three species. Σ$MeSO_2$-PCBs/DDE contributed with 0.09 - 0.28 % of the total contaminant load and ΣHPCs contributed with 0.03 - 0.49 % of the total contaminant load. The levels of Σ$MeSO_2$-PCBs/DDEs in kittiwake (1.67 ng/g ww) were not significantly different from glaucous gull (0.62 ng/g ww). However, levels in kittiwake were significantly higher compared to common eider (0.05 ng/g ww). The most abundant $MeSO_2$-PCBs/DDE was 3-$MeSO_2$-PCB 101 for glaucous gull (33.90 %) and common eider (33.58 %) and 3-$MeSO_2$-DDE for kittiwake (53.61 %) (Figure 13). Kittiwake had highest level of ΣHPCs

(0.57 ng/g ww) followed by glaucous gull (0.25 ng/g ww) and common eider (0.22 ng/g ww). The levels were similar and did not differ significantly between the species. The most abundant HPC was PCP (55.05 - 75.35 % of ΣHPC) followed by 4-OH-PCB 187 (12.52-39.27 %) for all three species (Figure 14).

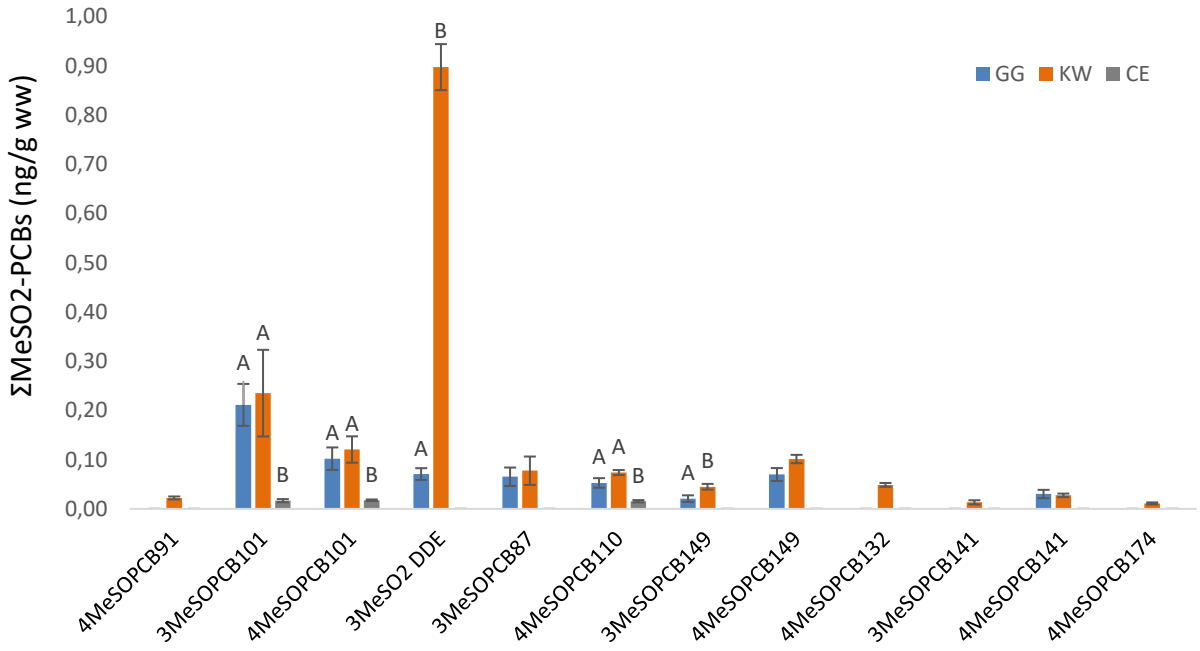


Figure 13. Levels of $MeSO_2$-PCBs/DDE in eggs from glaucous gull (GG, n = 5, blue), kittiwake (KW, n = 11, orange) and common eider (CE, n = 17, grey) from Svalbard 2015, presented as mean concentration (ng/g ww). Error bars represent the standard error (SE). Significant differences ($p < 0.05$) are noted with different letters A and B.

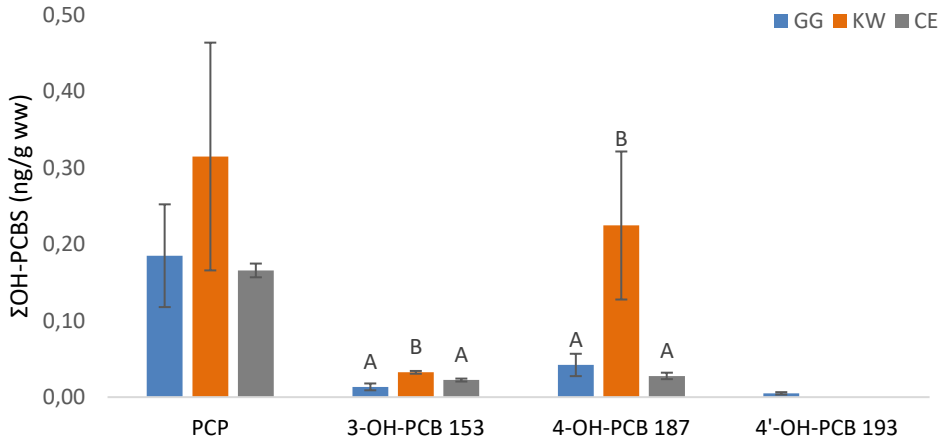


Figure 14. Levels of HPCs in eggs from glaucous gull (GG, n = 5, blue), kittiwake (KW, n = 11, orange) and common eider (CE, n = 17, grey) from Svalbard 2015, presented as mean concentration (ng/g ww). Error bars represent the standard error (SE). Significant differences ($p < 0.05$) are noted with different letters A and B.

PFASs was the third most prominent group for kittiwake and common eider and the fourth most prominent group for glaucous gull, contributing respectively 3.65 %, 17.56 % and 1.32 % to the total contaminant load. Mean ΣPFASs levels were highest for kittiwake (22.19 ng/g ww) followed by glaucous gull (9.57 ng/g ww) and common eider (7.91 ng/g ww). The most abundant PFASs were linPFOS (27.24 - 48.58 %), PFTriA (11.06 - 35.91 %) and PFUnA (8.14 - 16.65 %) for all three species (Figure 15).

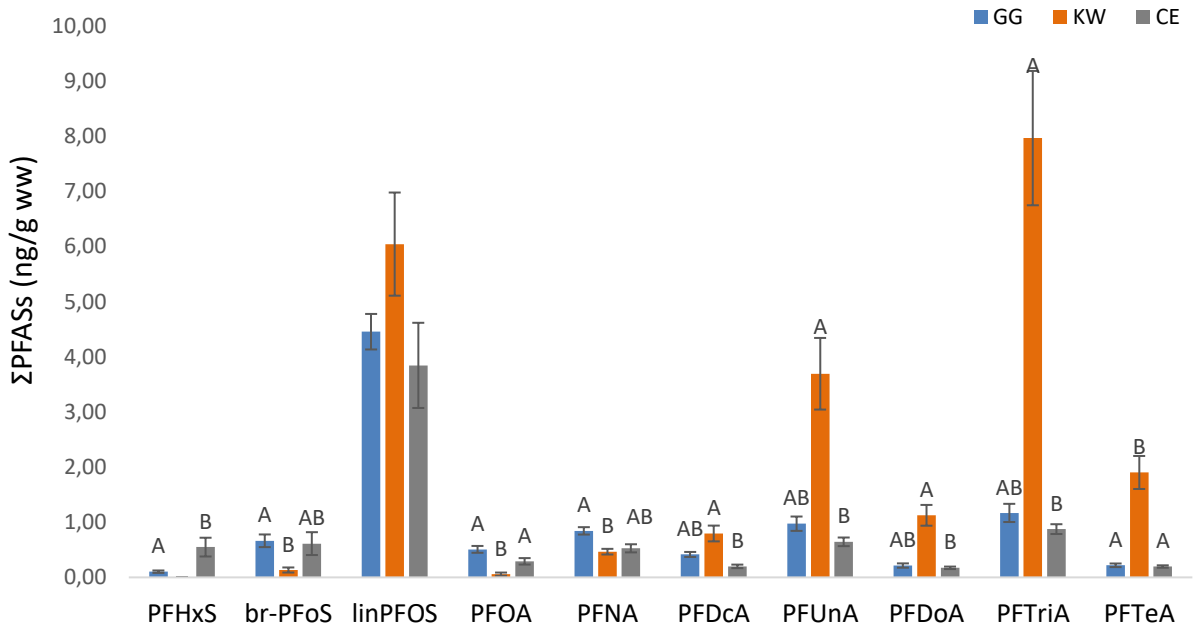


Figure 15. Levels of PFASs in eggs from glaucous gull (GG, n = 5, blue), kittiwake (KW, n = 11, orange) and common eider (CE, n = 17, grey) from Svalbard 2015, presented as mean concentration (ng/g ww). Error bars represent the standard error (SE). Significant differences ($p < 0.05$) are noted with different letters A and B.

PBDEs was the third most prominent group for glaucous gull and the fourth for kittiwake contributing 1.47 % and 1.46 % to the total contaminant load respectively. None of the PBDE congeners were above the DF of 0.6 for common eider. The mean ΣPBDEs levels were slightly higher, but not significant, for glaucous gull (10.67 ng/g ww) compared to kittiwake (8.89 ng/g ww). The most abundant congener for both species was PBDE 47 (69.21 %) followed by PBDE 100 (12.27 %) for glaucous gull, and PBDE 47 (61.66 %) followed by PBDE 99 (11.59 %) for kittiwake (Figure 16).

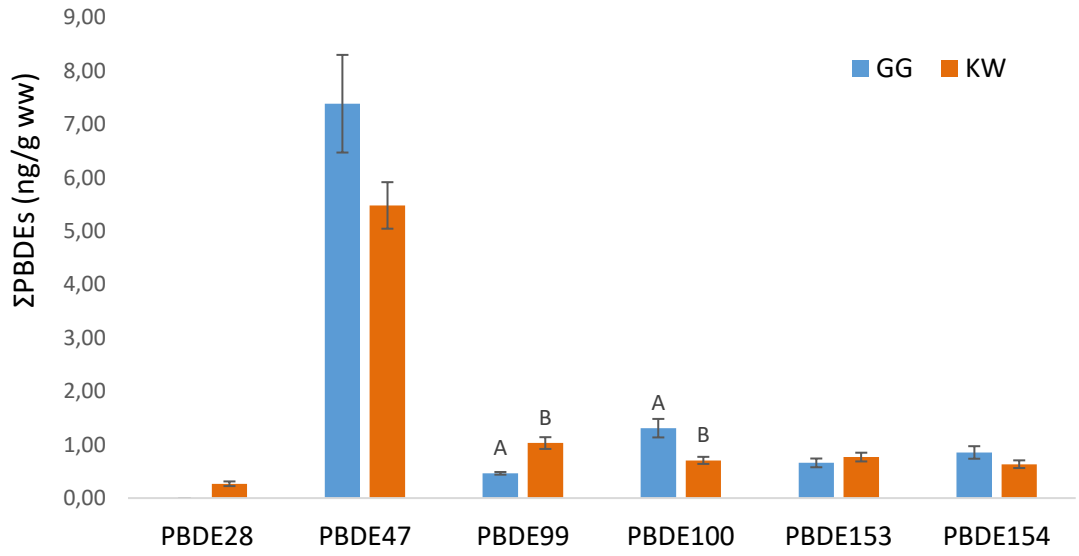


Figure 16. Levels of PBDE congeners in eggs from glaucous gull (GG, n = 5, blue), kittiwake (KW, n = 11, orange) and common eider (CE, n = 17, grey) from Svalbard 2015, presented as mean concentration (ng/g ww). Error bars represent the standard error (SE). Significant differences ($p < 0.05$) are noted with different letters A and B.

3.4 Associations between DNA DSB and contaminants

3.4.1 Principal component analysis

A PCA including all three species, was conducted for visualization of all variables together to explore relationships between the different variables (OHCs, biological variables and DSB variables). PC1 and PC2 accounted for respectively 57.8 % and 10.0 % of the total variation in the dataset (Figure 17).

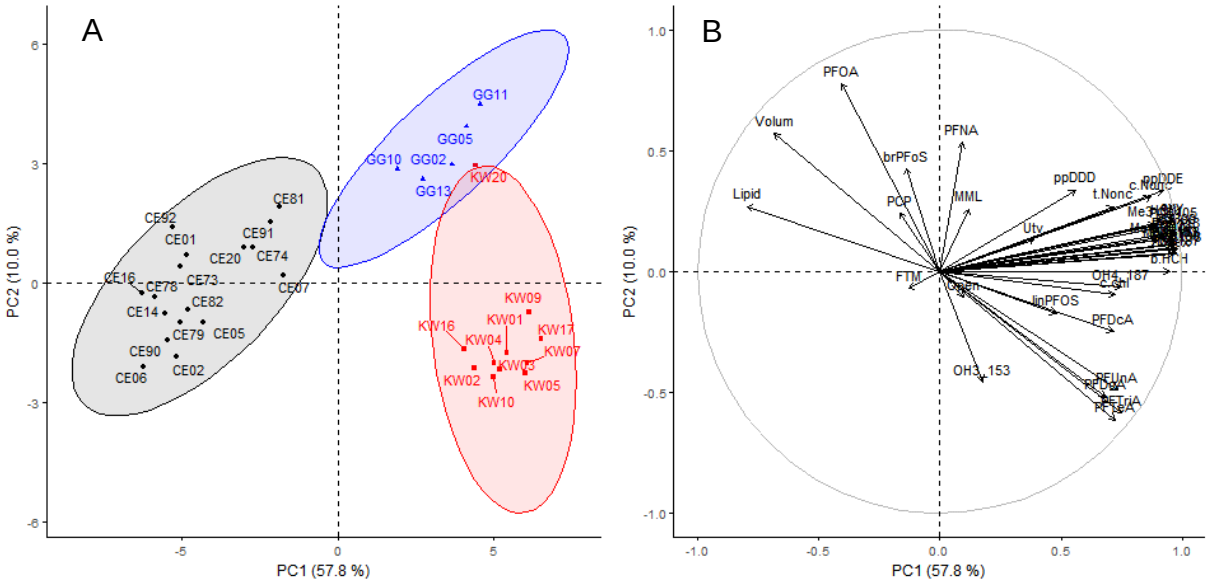


Figure 17. A) Score plot and B) loading plot from the PCA with all species. The different individuals are coloured according to species glaucous gull (GG, n = 5, blue), kittiwake (KW, n = 11, orange) and common eider (CE, n = 17, grey). PC1 and PC2 explained respectively 57.8 % and 10.0 % of the variation in the data set.

The different individuals, shown in the score plot (Figure 17a), were clearly grouped according to their respective species. As presented above, the major contaminant groups, volume and lipid were significantly different among the species and explained the grouping in the score plot. Moreover, the individuals within their respective species were similar to each other. Hence, they were grouped together in the plot. Kittiwake and glaucous gull were located on the same side as the big contaminant cluster in the loading plot along PC1 and was associated with high OHC levels. Common eider was separated from kittiwake and glaucous gull, as it was located on the opposite side, and was associated

with lower OHC values and a larger volume and lipid content. Kittiwake was separated from glaucous gull along PC2 and was located in the same direction as the PFAS cluster in the loading plot. Hence, kittiwake was associated with high PFASs levels compared to the other species. An exception was KW20 which was more associated with glaucous gull due to lower PFCAs levels.

The grouping of species in the score plot, and the differences in contaminant levels and biological variables between species (section 3.1) indicated species differences among most variables. To avoid masking any significant result or eliminate potential confounding factors due to species differences, a PCA was conducted for each species separately. Glaucous gull was not analysed with multivariate statistics due to low sample size (n=5).

PCA Kittiwake

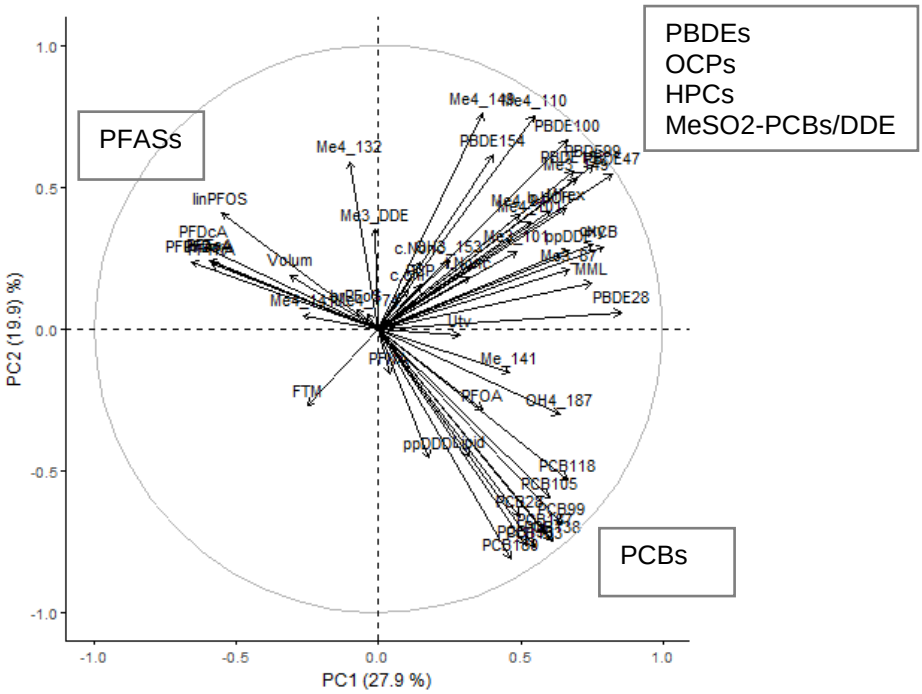


Figure 18. Loading plot visualizing the orientation of OHCs, biological variables, MML and DNA-FTM from the PCA for kittiwake (n = 11).

In the PCA for kittiwake PC1 and PC2 explained most of the variation in the dataset, respectively 27.9 % and 19.9 % and was plotted against each other in a loading plot (Figure 18). The contribution of each PC is listed in appendix H.1. The PCA indicated a

positive relationship among the PBDEs, OCPs, HPCs and $MeSO_2$-PCBs/DDE as most of the contaminants within these groups were located in the same area in the plot. MML was also located in the same area, which indicates that these contaminants are associated with each other and MML. DNA-FTM was located on the opposite side of the plot and may be negatively correlated to these contaminants and MML. Correlation tests confirmed a positive relationship between ΣOCP and ΣPBDE ($r = 0.77$, $p = 0.006$). Moreover, MML was significantly correlated with ΣOCP ($r = 0.61$, $p = 0.045$) and ΣPBDE ($r = 0.62$, $p = 0.042$). No significant correlations were detected between the other contaminant groups and MML or DNA-FTM.

PCBs were also located on the positive side of the PC1 axis but separated from the other OHCs along PC2 axis. All the PCB congeners were clustered together indicating a strong positive correlation among these contaminants. Lipid was also projected with the PCBs in the plot, indicating association between lipid and PCBs. Correlation tests between lipid and the contaminant groups showed a significant correlation with ΣPCB ($r = 0.62$, $p = 0.041$). Linear regression, with ΣPCBs as response variable and lipid as explanatory variable, confirmed a positive relationship between PCBs and lipid, showing that levels of PCBs increased with increasing lipid (estimate 34.89, $F_{1,9} = 5.671$, $p = 0.041$, $R^2 = 0.318$). No correlations were detected between MML or DNA-FTM and the biological variables. Moreover, no intercorrelations between biological variables (developmental stage (denoted Utv in the plot), lipid and volume) or between biological variables and contaminant groups were found for kittiwake, except for correlation with PCB and lipid.

PFASs were clustered together along the negative side of PC1 axis in the plot and were positively correlated with each other. Except for PFNA and PFOA which were located on the opposite side from the PFAS cluster. Hence, showed a negative association to the PFAS cluster. Volume was located in the same direction as PFASs but showed no significant correlation with ΣPFAS. The plot furthermore indicated a negative association between ΣPFAS (except PFNA and PFOA) and ΣPCBs as these contaminant groups were located in clusters opposite from each other. However, this association was not significant ($r = -0.37$, $p = 0.258$).

PCA Common eider

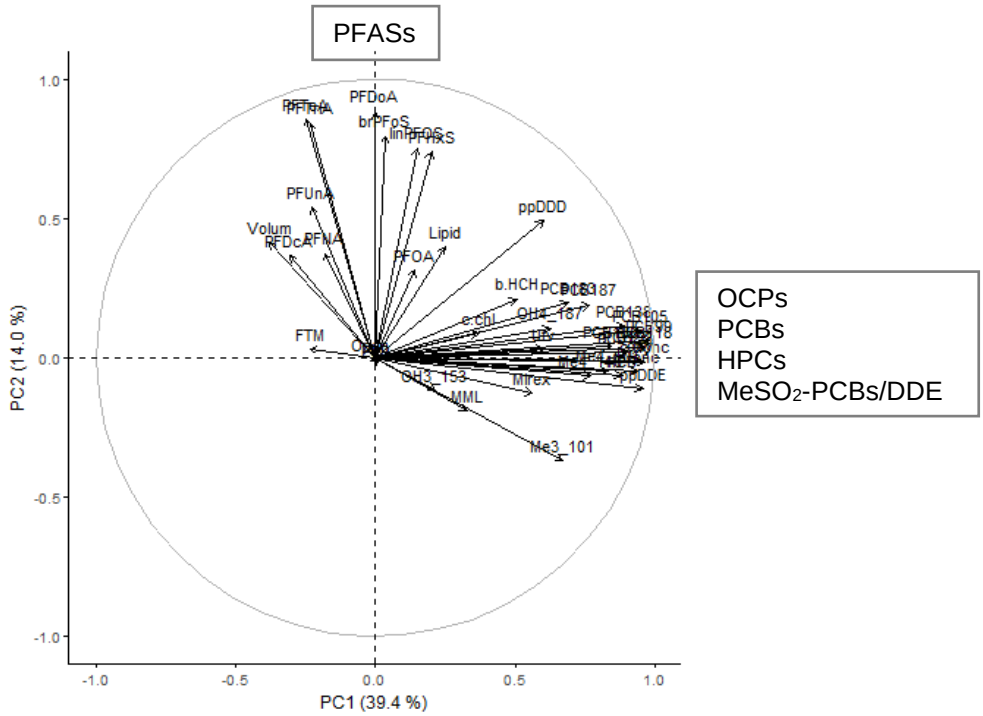


Figure 19. Loading plot visualizing the orientation of OHCs, biological variables, MML and DNA-FTM from the PCA for common eider (n = 17).

The PCA conducted for common eider showed a similar pattern to the PCA conducted for kittiwake. PC1 and PC2 accounted for respectively 39.4 % and 14.0 % of the total variation in the dataset and were plotted against each other in a loading plot (Figure 19). The contribution for each PC is listed in appendix H.2.

PCBs, HPCs, $MeSO_2$-PCBs/DDE and OCPs were located in a cluster on the positive side of PC1 axis and were positively correlated to each other and PC1. FTM and MML were poorly projected on the loading plot as they were relatively close to origin. However, MML was projected in the same direction as the contaminants along the positive side of PC1 axis, indicating a positive relationship. FTM was projected on the opposite side from the contaminant cluster, on the negative side of the PC1 axis, indicating a negative relationship with these contaminants. No significant correlations were detected between the contaminant groups and MML or between DNA-FTM and the contaminant groups.

PFASs were associated with PC2 and each other. The plot indicates that PFASs were uncorrelated with the other contaminants as they were located on the positive side of PC2 axis, perpendicular to the contaminant cluster.

The plot furthermore indicated an association between developmental stage (denoted Utv in the plot) and the big contaminant cluster. Exploration of this association with correlation test revealed correlations between developmental stage and ΣOCP ($r = 0.57$, $p = 0.018$), developmental stage and ΣPCB ($r = 0.52$, $p = 0.031$). Further investigation with linear regression showed increasing levels for these contaminant groups with increasing developmental stage (all $p < 0.05$). No intercorrelations between biological variables (developmental stage, lipid and volume) or between biological variables and contaminant groups (except for correlation with developmental stage as listed above) were detected. Moreover, no correlations between MML or DNA-FTM and biological variables were found for common eider.

Linear regression with PC scores

An extra PCA containing only the contaminants were conducted to investigate the association between DNA DSB and contaminants. The loading plots and contribution for of each PC for kittiwake and common eider are found in Appendix H.3. The PC1, PC2 and PC3 scores were extracted and used in linear regression against MML and FTM for kittiwake and common eider. Linear regression with MML as response variable showed a significant relationship with PC1 scores (estimate = 10.07, $F_{1,9} = 8.695$, $p = 0.016$, $R^2 = 0.43$, Figure 20) for kittiwake. The contaminants contributing most to PC1 were PBDE 28, PBDE 47, HCB, PBDE 99 and oxy-chlordane (Appendix H.4). This result indicated that increasing contaminant levels were associated with increasing MML. Thus, indicating that increasing levels of contaminants results in decreasing DNA DSB for kittiwake. No other significant associations were detected for the different PCs and MML and DNA-FTM in kittiwake, or for MML and DNA-FTM for common eider.

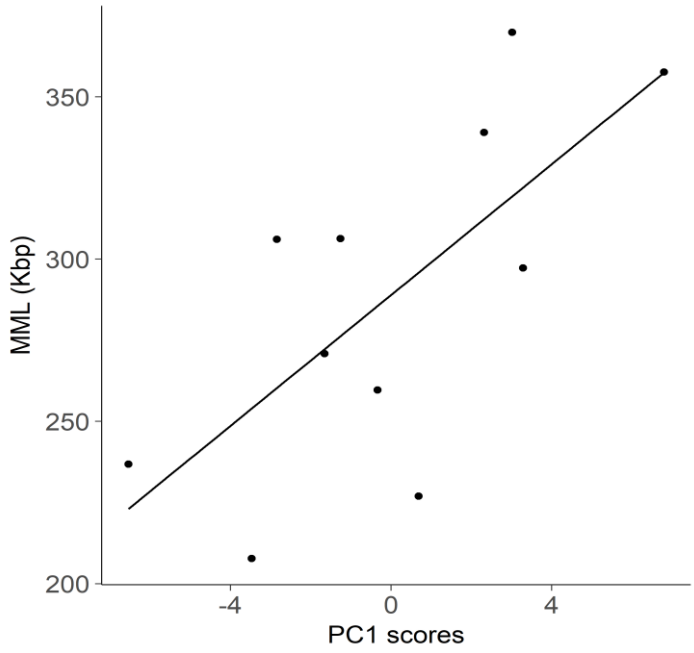


Figure 20. Linear regression with MML (Kbp) against PC1 scores for contaminants from the PCA (with only contaminants) for kittiwake. $R^2 = 0.43$, $p = 0.016$. R^2 denotes the adjusted R2 for the model.

3.4.2 Multiple regression

Model selection based on the second-order Akaike's Information Criterion was performed to investigate DNA DSB in relation to pollutants, developmental stage (age) and lipid content in kittiwake and common eider. The top ranked models predicting DNA DSB showed different results between species and between MML and FTM (Table 2). Only the top three models predicting MML for kittiwake were significant ($p < 0.05$).

Table 2. Top ranked models predicting DNA DSB (MML or DNA-FTM) in egg from kittiwake and common eider from Svalbard. Model selection is based on the second-order Akaike's Information Criterion (AIC), and only models with ΔAICc ≤ 2.00 are presented. Significant models ($p < 0.05$) are marked in bold.

Kittiwake	**Model**	**AICc**	**ΔAICc**	**AICc Weight**
1	**MML~ OCP**	121.33	0.00	0.19
2	**MML~ PBDE**	121.55	0.22	0.17
3	**MML~ PBDE** + Age	122.82	1.49	0.09
4	MML~$MeSO_2$-PCBs/DDE	123.24	1.90	0.07
1	FTM~$MeSO_2$-PCBs/DDE	97.78	0.00	0.13
2	FTM~ PBDE	98.09	0.31	0.11
3	FTM~OCP	98.35	0.57	0.10
4	FTM~PFAS	98.55	0.77	0.09
5	FTM~HPC	98.68	0.90	0.09
6	FTM~PCB	98.75	0.97	0.08
7	FTM~Age	99.00	1.22	0.07
8	FTM~Lipid	99.04	1.26	0.07
Common Eider				
1	MML~HPC	196.78	0.00	0.11
2	MML~ Age	196.80	0.02	0.11
3	MML~HPC + Age	197.17	0.39	0.09
4	MML~OCP	197.29	0.51	0.09
5	MML~ PCB	198.24	1.46	0.05
6	MML~$MeSO_2$-PCBs/DDE	198.42	1.64	0.05
7	MML~Lipid	198.65	1.87	0.04
8	MML~PFAS	198.78	2.00	0.04
1	FTM~PCB	149.94	0.00	0.13
2	FTM~Lipid	150.26	0.32	0.11
3	FTM~PFAS	151.18	1.24	0.07
4	FTM~OCP	151.28	1.33	0.06
5	FTM~Age	151.37	1.43	0.06
6	FTM~HPC	151.67	1.72	0.05
7	FTM~MeSO2-PCBs/DDE	151.79	1.85	0.05
8	FTM~PCB + $MeSO_2$-PCBs/DDE	151.88	1.93	0.05

Kittiwake

ΣPBDE, ΣOCP and Σ$MeSO_2$-PCBs/DDE were predictor variables in the best models predicting both MML and DNA-FTM for kittiwake. The top ranked models predicting MML for kittiwake included ΣOCP, ΣPBDE (alone and with developmental stage (age)) and Σ$MeSO_2$-PCB/DDE (Table 3). All parameter estimates were positive for the predictor variables indicating a positive effect on MML (Table 4, Figure 21). Hence, a negative effect on DNA DSB. All parameters were significant except for Σ$MeSO_2$-PCBs/DDE (p = 0.079). Developmental stage and lipid was not included in the best models, except for one model with developmental stage and PBDE. However, developmental stage was not significant. This suggest that these variables do not explain the variation in MML.

Table 3. Linear regression parameter estimates and model statistics for the top-ranked models based on the second-order Akaike`s Information Criterion, explaining variation in MML (Kbp) in kittiwake (n = 11). All contaminants were transformed by natural logarithm (ln ng/g ww) to meet the assumption for the model. Significant models and parameters are denoted with *.

Model 1	**MML ~ OCP**		**Adj R^2 = 0.35**	***p* = 0.033***
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	-281.39	226.64	-1.24	0.246
OCP	118.30	46.93	2.52	0.033*
Model 2	**MML ~ PBDE**		**Adj R^2 = 0.34**	***p* = 0.036***
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	22.37	109.08	0.20	0.842
PBDE	123.99	50.37	2.46	0.036*
Model 3	**MML~ PBDE + Age**		**Adj R^2 = 0.48**	***p* = 0.030***
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	-32.48	101.00	-0.32	0.756
PBDE	117.41	44.75	2.62	0.031*
Age	25.30	13.58	1.86	0.099
Model 4	**MML~ $MeSO_2$-PCBs/DDE**		**Adj R^2 = 0.23**	***p* = 0.079**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	235.19	30.68	7.66	0.000*
$MeSO_2$-PCBs/DDE	114.19	57.73	1.98	0.079

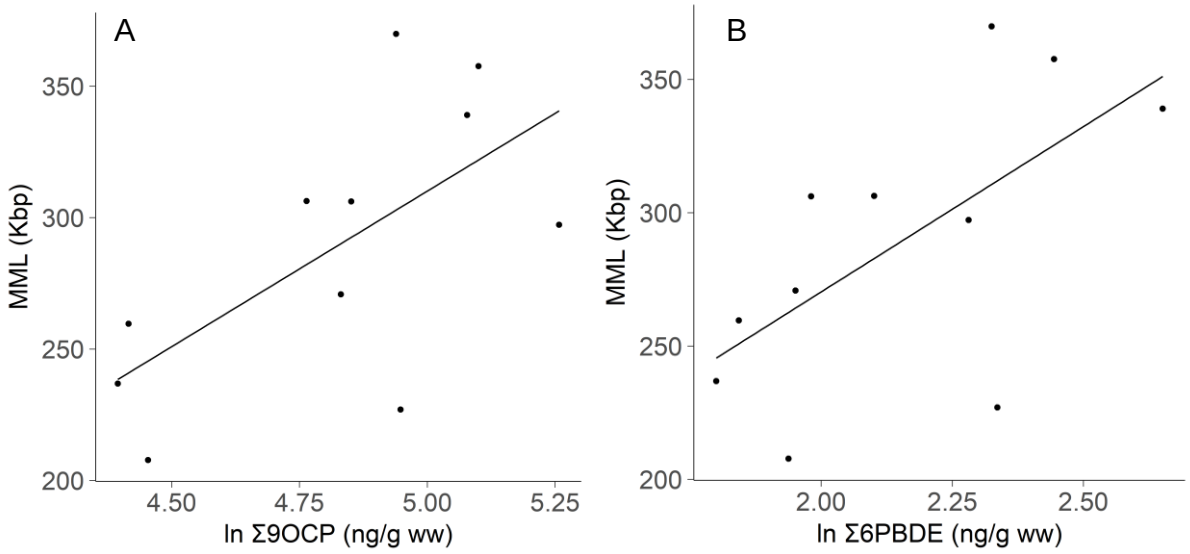


Figure 21. Linear regression for the two significant models from AIC explaining variation in MML (Kbp) in kittiwake, A) ΣOCP (R^2 = 0.35, *p* = 0.033), B) ΣPBDE (R^2 = 0.34, *p* = 0.036). R^2 denotes the adjusted R2 for the model.

The best models explaining the variation in DNA-FTM were Σ$MeSO_2$-PCBs/DDE, ΣPBDE and ΣOCP, which is in accordance with the best models explaining MML. The contaminant estimates were negative, except for ΣPFAS and ΣPCB, indicating a negative effect on DNA-FTM (Appendix J.1). Although, none of the FTM models were significant.

Common eider

ΣOCP, ΣHPC and developmental stage (age) were the best predictors explaining variations in MML for common eider. All contaminant estimates (except PFAS), lipid and age were positive, indicating positive associations between the OHCs and MML (Appendix J.2). However, none of the models were significant.

The best models explaining variations in DNA-FTM included ΣPCB, lipid and ΣPFAS as the only explanatory variable in the models. None of the models were significant in explaining the variation in DNA-FTM. All variables (except PFAS) had negative parameter estimates (Appendix J.3). Hence, OHCs showed negative associations with DNA-FTM.

4. Discussion

The present study reports OHC levels in eggs from glaucous gull, kittiwake and common eider and aimed to investigate the potential genotoxic effect of these contaminants on DNA DSB in the avian blastodisc during early development. Highest levels of contaminants were observed in glaucous gull eggs followed by kittiwake and common eider eggs and OCPs and PCBs were the most prominent groups for all three species. Similar levels of DNA DSB were detected among the species, and a negative association between OHCs and DNA DSB was obtained.

To my knowledge this is the first study assessing DNA DSB in relation to OHCs at an early developmental stage in wildlife, and the first study using egg material in the DNA DSB agarose gel electrophoresis analysis. From the modification of the method we realized how difficult it was to predict the amount of DNA in each sample as it varied between species and by the stage of development. Therefore, a dilution series of the samples made it possible to obtain samples with different amounts of DNA, and was successfully incorporated into the protocol. Furthermore, we realized that the optimal developmental stage for the method was a short window between developmental day two and four when the blastodisc was expanded, and blood vessels were visible. Less developed eggs will not yield the required amount of DNA for the analysis, and more developed eggs will have more developed tissue and organs that complicates the method. It was not possible to separate the developmental stages from each other with an egg flotation test. Eggs candling was not tested in the present study, however both flotation test and egg candling have shown to overestimate the developmental day early in incubation (Reiter and Andersen, 2008). Thus, to ensure an optimal developmental stage of the eggs, monitoring the nests during egg laying is recommended.

4.1 Levels and patterns of contaminants

The contaminant levels were expected to differ between the three species due to the assumption of elevated levels in species feeding higher up in the food web due to biomagnification. Overall, eggs from glaucous gull had the highest OHC levels followed

by kittiwake and common eiders. This was in accordance with the trophic positions of the species (Hop et al., 2002, Wold et al., 2011). Surprisingly, kittiwake eggs had similar levels to glaucous gull eggs of PCBs and PBDEs and higher levels of PCB metabolites and PFASs, although not significant. The legacy contaminants PCBs and OHCs were the main contributors to the total contaminant burden for all three species. This is consistent with previous studies from Svalbard (Borgå et al., 2001) and Bjørnøya (Verreault et al., 2005b).

The levels of OCPs in the present study were highest in glaucous gull eggs. The OCP levels in glaucous gull eggs were lower than previously reported levels in glaucous gull eggs from Bjørnøya (Verreault et al., 2005b) and higher compared to levels reported from the Canadian Arctic (Braune et al., 2018). Levels of OCPs in kittiwake eggs were somewhat lower compared to reported levels in kittiwake eggs from Kongsfjorden (Miljeteig and Gabrielsen, 2009). However, the levels were somewhat higher compared to reported levels in kittiwake eggs from the Canadian Arctic (Braune, 2007, Braune et al., 2018). OCP levels in common eider eggs were between previously reported levels for common eider eggs in Norway (Herzke et al., 2009, Huber et al., 2015), and lower compared to common eider eggs in the Canadian Arctic (Peck et al., 2016). The DDT metabolite p,p'-DDE has been reported as the most prominent OCP detected in eggs from several arctic seabirds, including glaucous gull, kittiwake and common eider (Braune, 2007, Miljeteig and Gabrielsen, 2009, Huber et al., 2014, Braune et al., 2018). This finding is in line with the species in the present study where p,p'-DDE was the major OCP contributing with 49-75 % of ΣOPCs. Following p,p'-DDE, the most prominent OCPs were oxy-chlordane and HCB which are consistent with these contaminants being the major contaminants in seabird eggs (Miljeteig and Gabrielsen, 2009, Huber et al., 2014).

Similar PCB levels were measured in glaucous gull and kittiwake eggs in the present study. This result was unexpected as PCBs have shown to have a high biomagnification factor and glaucous gull is considered to be at a higher trophic level than kittiwake (Borgå et al., 2001). PCB levels in glaucous gull eggs were lower compared to levels reported in glaucous gull eggs from Bjørnøya (Verreault et al., 2005b) and the Canadian Arctic (Braune et al., 2018). Levels in kittiwake eggs were within the range of previously reported

levels in kittiwake eggs from Kongsfjorden (Miljeteig and Gabrielsen, 2009), and higher compared to the Canadian Arctic (Braune, 2007, Braune et al., 2018). Levels in common eider eggs were lower compared to glaucous gull and kittiwake eggs in the present study. Similar levels have been reported in common eider eggs from Sklinna, Norway, and higher levels were reported from Røst, Ekne and Munkholmen, Norway (Herzke et al., 2009, Huber et al., 2015). Furthermore, the levels in common eider eggs were lower compared to a study from the Canadian Arctic (Peck et al., 2016). PCB 153 was the major congener in all three species in the present study. The congener PCB 153 is poorly metabolized in animals and considered as one of the most persistent PCB congeners (Wolkers et al., 2004). This is in accordance with PCB 153 reported as the major PCB congener found in several seabird eggs (Braune, 2007, Herzke et al., 2009, Miljeteig and Gabrielsen, 2009, Huber et al., 2015). PCB 153 was followed by PCB 138 and PCB 180 for glaucous gull and kittiwake eggs, which is consistent with the pattern seen in glaucous gull plasma from Bjørnøya (Verreault et al., 2004b) and kittiwake eggs from Kongsfjorden (Miljeteig and Gabrielsen, 2009) and the Canadian Arctic (Braune, 2007). Common eider eggs showed a different pattern with PCB 118 showing higher levels than PCB 180. This result is consistent with a previous study on common eider eggs from Norway (Huber et al., 2014)

The levels of $MeSO_2$-PCBs/DDE were higher compared to HPCs in glaucous gull and kittiwake and is consisted with other studies (Verreault et al., 2005b, Huber et al., 2015). On the other hand, common eider had higher levels of HPC than $MeSO_2$-PCBs/DDE. $MeSO_2$-PCBs/DDE levels in glaucous gull eggs were lower compared to previously reported levels in glaucous gull eggs from Bjørnøya (Verreault et al., 2005b). Studies reporting PCB metabolites in kittiwake eggs are scarce. However, $MeSO_2$-PCBs/DDE have been measured in kittiwake chicks livers from Kongsfjorden (Helgason et al., 2010). The levels were lower than levels in the present study, although the different tissues make it difficult to compare. $MeSO_2$-PCBs/DDE levels in common eider eggs were lower than reported levels in common eider eggs from Norway (Huber et al., 2015). Previous studies have reported $MeSO_2$-DDE and 4-$MeSO_2$-PCB 101 as the most abundant $MeSO_2$-PCBs/DDE, followed by different patterns of 3-$MeSO_2$-PCB 101, 4-$MeSO_2$-PCB 110 and 4-$MeSO_2$-PCB 149 (Verreault et al., 2005b, Helgason et al., 2010, Huber et al., 2014)

which is in accordance with the present study. HCP levels in glaucous gull eggs were higher compared to a study from Bjørnøya (Verreault et al., 2005b). Furthermore, detected HPC levels in kittiwake eggs were lower than previously reported plasma levels in kittiwake chicks (Helgason et al., 2010). HPCs levels in common eider eggs were higher compared to levels detected in common eider eggs from Sklinna in Norway, and similar to common eider eggs from Røst in Norway (Huber et al., 2015). In the present study PCP was the most prominent HCP in all three species, followed by 4-OH-PCB 187. In the studies by Verreault et al. (2005b), Helgason et al. (2010) and Huber et al. (2015), 4-OH-PCB 187 was the HCP with highest levels. As these studies did not analyse for PCP they are in line with the present study.

The detected levels of PFASs in glaucous gull eggs were considerably lower compared to previous studies on glaucous gull eggs from Bjørnøya (Verreault et al., 2005a, MOSJ, 2018b) and the Canadian Arctic (Braune and Letcher, 2012). Furthermore, the levels of PFOS and PFOA in the present study were similar to the reported levels for glaucous gull eggs in Kongsfjorden 2013 (MOSJ, 2018b). PFASs levels have shown to be higher at Bjørnøya compared to Kongsfjorden, especially for PFOS which was almost 33 times higher, and the reason for this is currently unknown (MOSJ, 2018b). However, the major PFAS exposure and uptake route is through diet (Huber et al., 2015). Hence, differences in diet between the areas might explain the higher levels in eggs from Bjørnøya. Moreover, in the present study PFASs levels were higher in kittiwake eggs than glaucous gull eggs which may indicates that kittiwakes breeding in Kongsfjorden are exposed to different food sources with higher PFAS levels compared to glaucous gulls. Furthermore, PFAS levels in kittiwake eggs were somewhat higher compared to levels reported for kittiwake eggs in the Canadian Arctic (Braune and Letcher, 2012). However, the kittiwake levels were lower than previously reported PFAS levels in kittiwake eggs from Kongsfjorden (Miljeteig and Gabrielsen, 2009, Herzke et al., 2013). The levels in common eider eggs were somewhat lower than a previous study from the same area (Herzke et al., 2013), and lower compared to levels reported from Norway (Herzke et al., 2009, Herzke et al., 2013, Huber et al., 2015). PFOS has been reported as the major PFAS (Verreault et al., 2005a, Miljeteig and Gabrielsen, 2009, Herzke et al., 2013) which is in accordance with the PFAS pattern

observed in glaucous gull and common eider eggs in the present study. On the contrary, the pattern was different for kittiwake eggs, as PFTriA was the major PFAS followed by PFOS.

The levels of PBDEs in glaucous gull eggs in the present study were lower than reported in glaucous gull egg from Bjørnøya (Verreault et al., 2004a, Knudsen et al., 2005, Verreault et al., 2007) and the Canadian Arctic (Braune et al., 2015). Levels in kittiwake eggs were in the higher range of previous studies on kittiwake eggs in Kongsfjorden (Miljeteig and Gabrielsen, 2009) and the Canadian Arctic (Braune et al., 2015). PBDEs have previously been detected in common eiders eggs from Kongsfjorden (Herzke et al., 2013) and in Norway (Herzke et al., 2009, Herzke et al., 2013, Huber et al., 2015). However, all PBDE congeners were detected in less than 60 % of the common eider eggs in the present study. PBDE 47, PBDE 99 and PBDE 100 have shown to particularly biomagnify in species (de Wit, 2002), which is in accordance with the pattern in the present study. Moreover, PBDE 47 has shown to have a higher biomagnification factor compared to PBDE 99 and PBDE 100 (de Wit, 2002). The high biomagnification factor of PBDE 47 could explain the pattern observed in the present and previous studies where PBDE 47 was the major PBDE congener (Herzke et al., 2005, Knudsen et al., 2005, Verreault et al., 2007, Miljeteig and Gabrielsen, 2009).

It is important to take into account that comparisons of OHC levels between studies can be difficult due to analytical procedures, differences in number of compounds or congeners measured and differences in matrices. In addition, food sources and adaptions to the different areas and climate are not taken into consideration. Overall, levels in kittiwake eggs and common eider eggs were similar to previous studies from Norway and Kongsfjorden, although both lower and higher levels are reported. The levels in common eider eggs and glaucous gull eggs from the Canadian Arctic were higher compared to the present study. Whereas kittiwake eggs in the present study had somewhat higher levels compared to kittiwake eggs from the Canadian Arctic. These differences could be a result of spatial differences, diet and migrating area. The levels detected in glaucous gull eggs were generally lower compared to previously reported levels in glaucous gull eggs from

Bjørnøya (Verreault et al., 2004a, Knudsen et al., 2005, Verreault et al., 2005a, Verreault et al., 2005b, Verreault et al., 2007). These studies are somewhat old, but was used for comparison due to lack of studies in glaucous gull eggs from Kongsfjorden and more recent studies on contamination levels in glaucous gull eggs. Many of the contaminants measured in the present study have been banned and regulated, and newer studies show a declining trend of both legacy OHCs such as PCBs and DDTs and emerging contaminants such as PFASs and PBDEs (UNEP, 2017, Rigét et al., 2018). Hence, decreasing levels over the last decade could explain the lower levels detected in glaucous gull eggs in the present study. In addition, glaucous gulls at Bjørnøya generally contain higher levels of OHCs compared to Kongsfjorden glaucous gulls presumably because of differences in diet which also explains the variation in levels between the areas (Bustnes et al., 2000, MOSJ, 2018b).

4.2 Associations between contaminants and biological variables

Investigating potential correlations between lipid and OHCs were conducted to see if lipid normalization was necessary. There was no correlation between OHCs and lipid, except for PCBs in kittiwake. Lipid normalization was not conducted and lipid content in relation to OHCs will not be discussed further.

Several studies have investigated the effect of OHCs on egg size (egg volume) at Bjørnøya (Verboven et al., 2009) and Norway (Helberg et al., 2005, Bustnes et al., 2008) and documented a negative effect of OHCs on egg size. In contrast, there was no relationship between OHCs and egg volume in the present study, which is in accordance with one study from Bjørnøya (Bustnes et al., 2003). However, when comparing areas in Norway with different food availability, a positive effect of OHCs on egg size was seen in the area with good condition and a negative effect was seen in the areas with poor conditions (Bustnes et al., 2008). Moreover, a study from Kongsfjorden showed that common eiders in poor condition laid smaller eggs than common eiders in good condition (Hanssen et al., 2002). These studies suggest a complex interrelationship between egg size and OHCs with changing environmental conditions (Bustnes et al., 2008). Thus, it is

important to consider the environmental variations, such as food availability, and the status of the female when investigating the effect of OHCs on egg size.

There was no association with OHCs and developmental stage for kittiwake in the present study. However, common eider showed a significant positive association with developmental stage and OCPs and PCBs, indicating increasing contaminant levels in the egg as the blastodisc developed. The egg is a relative closed environment, thus experience minimal external exposure. Therefore, this explanation seems unlikely. To my knowledge, the association between developmental stage and OHC levels has not been studied in wildlife. It is hard to compare this result with injection studies as the chemicals will transfer from the injection site to other compartments. However, in a feeding trial study there was a slight, but not significant, increase of PCB 156 and PCB 189 with the development of the egg in the yolk-albumen compartment (Bargar et al., 2001). This result is in accordance with the result seen in common eider in the present study. Bargar et al. (2001) did not record the laying order of the eggs, but proposed that decreasing contaminant load with the sequence of egg laying could cause the observed result. Van den Steen et al. (2009) found that the OHC levels decreased significantly with laying order in eggs from blue tits (*Cyanistes caeruleus*). This is in contrast with (Verreault et al., 2006) who showed no effect of laying order on OHC levels in glaucous gull. Van den Steen et al. (2009) did not detect a clear pattern when reviewing studies on the effect of laying order on OHC levels, and proposed that species differences could be the cause (Van den Steen et al., 2009). To my knowledge, laying order effect on OHC levels in common eider has not been investigated. Hence the explanation for the observed increasing OHC levels with developmental stage remains unknown.

4.3 Levels of DNA DSB

The eggs from the three species differed in the contaminant levels, and it was therefore expected to see different levels of DNA DSB. However, no significant differences in DNA-FTM or MML were detected between the species. Haarr et al. (2017) studied DNA DSB in blood samples collected from the same colonies during the same season. Their results

showed no differences in DNA DSB between the species, which is in accordance with the results in the present study.

To our knowledge, this is the first time DNA-FTM and MML have been measured in wildlife during early avian development. However, DNA-FTM and MML have been measured in blood from incubating common eiders. The DNA-FTM levels in the present study were higher compared to levels seen in common eider blood early and late in the incubation period from Kongsfjorden and the Baltic Sea (Fenstad et al., 2014, Beckhmann, 2016, Fenstad et al., 2016a). However, levels in late incubation period for common eiders in Kongsfjorden in 2008 (Fenstad et al., 2014) showed similar levels as the present study. The MML values in the present study were somewhat lower compared to previously reported MML levels in common eider blood (Beckhmann, 2016). Overall, these findings indicate a higher level of DNA DSB in eggs compared to blood, although one should take in to considerations that comparing these values are difficult due to the levels being measured in different matrices, and possible variations in the protocol. Furthermore, there is a relative large variation in DNA-FTM and MML levels between the studies reporting levels in common eider blood. Fenstad et al. (2014) measured DNA-FTM in 2008 and 2009 and showed relative different levels between the years which could be caused by annual variations. Therefore, annual variation in DNA DSB should also be taken into considerations. Hence, comparing DNA-FTM and MML levels in eggs and blood from the same year would be recommended. In addition, more studies reporting the levels in eggs are necessary to support the findings in the present study and to reveal any annual variation.

4.4 Associations between DNA DSB and contaminants

The present study aimed to investigate potential associations between DNA DSB and OHCs levels in eggs from arctic seabirds. The results show that MML significantly increased with OCPs and PBDEs in kittiwake. This result is supported by the significant positive relationship between MML and PC1 where PBDEs and OCPs contributed most to PC1. Although none of the models for DNA-FTM in kittiwake or DNA-FTM or MML in common eider were significant, increasing OHCs levels still showed a negative trend on

DNA DSB. The negative, and lack of associations between DNA DSB and OHCs in the present study was unexpected, and is in contrast with the existing knowledge that contaminants cause ROS formation and induces DNA DSB. Fenstad et al. (2016a) explored the effect of OHCs on DNA DSB in common eiders from the Baltic sea and Kongsfjorden. DNA DSB showed a positive association with levels of PCBs and OCP in the Baltic Sea. However, no associations between DNA DSB and OHCs were seen in common eiders in Kongsfjorden. The OHC levels in Kongsfjorden are lower compared to the Baltic Sea (Fenstad et al., 2016b), and the levels might be too low to cause genotoxic effects in Svalbard eggs.

Increasing evidence shows that the actual contaminant levels in the blastodisc are lower than the whole egg contaminant levels which are reported in the present study. When reviewing *in ovo* injection studies of PCBs in different avian species, they all show that less than 10 % are taken up by the embryo midway during incubation (developmental day 13) (de Roode and van den Brink, 2002, Maervoet et al., 2005, Dean et al., 2018). Towards the end of incubation there was reported a large increase of contaminant absorption by the embryo (11-18 % at developmental day 18-19) (de Roode and van den Brink, 2002, Maervoet et al., 2005). Dean et al. (2018) was the only study measuring contaminant absorption at a similar incubation stage as the present study (developmental day five), and the result showed that the embryonic uptake of PCB 77 was only 1-2 % in five-days-old embryos. Overall, these results show that the embryo only absorb a small percentage of the total contaminant burden in the egg during early and midway stages of development. Injection studies might be different from wildlife maternal transfer in sense of mobility of the contaminants between compartments. Hence, one should be careful when assessing these studies. However, Dean et al. (2018) implied that injection studies will reflect maternal transfer because the injection is given prior to incubation which allows the chemicals to move to the different egg compartments. This is supported by the similarity between the injections studies discussed above and a feeding trial by Bargar et al. (2001). The absorbed PCB 189 levels in 19-day old embryos were 17-30 % which was 77 times higher compared to absorbed PCB 189 levels in the nine-day-old embryos (Bargar et al., 2001). As the levels of OHCs in the present study represent the whole egg

contaminant burden, the actual levels in the blastodisc remains unknown. Therefore, one should be cautious when trying to predict the potential genotoxic effect and associations between OHCs and DNA DSB based on whole egg concentrations. However, based on the studies discussed in this section one would assume that the OHC levels in the blastodisc, where DNA DSB were measured, are much lower than the measured whole egg OHC levels. Hence, the low OHC levels might not cause much genotoxicity on the DNA.

The organisms encompass natural defence systems against DNA damage, such as detoxification of genotoxicants, antioxidant defence, DNA repair and apoptosis (Figure 2, section 1.3) (Jenkins et al., 2010). With low contaminant levels, repair system and antioxidant defence system are able to handle and repair the DNA damage until the levels increase to a point where the systems will be saturated and unable to handle all the DNA damage (Jenkins et al., 2010). Kleczkowska and Althaus (1996), Wang et al. (2012) and Gilmore (2015) have shown that low levels of genotoxic chemicals reduce levels of DNA DSB, and have linked the effect to upregulated defence systems. In the study by Wang et al. (2012) low exposure of PBDE 47 to human cells showed contaminant induced ROS formation and decreasing levels of DNA DSB. Moreover, Kleczkowska and Althaus (1996) showed reduced DNA DSB with low levels of N-methyl-N′-nitro-N-nitrosoguanidine in human cells, and elevated levels of DNA DSB after higher exposure. Gilmore (2015) who investigated relationships between OHCs and genotoxicity in polar bears, found a negative relationship between PBDE 47 and OH-PCB and DNA DSB and proposed an upregulation of repair pathways as a possible explanation for the observed negative relationship. Moreover, TRXR, a gene related to oxidative stress, was positively correlated with OHCs (Gilmore, 2015). Thus, a reduction in DNA DSB in relation to chemicals, such as PBDEs, have been detected *in vitro and in vivo,* and is in accordance with the results in the present study. However, these studies did not measure repair and antioxidant endpoints.

The accumulation of ROS and DNA DSB with OHC exposure activates signalling cascades which induces apoptosis, cell death, DNA repair and antioxidant defences to cancel the negative effects from reactive species (Mrema et al., 2013). The antioxidant defence system act as a first line of defence and try to reduce the increased levels of ROS to protect the cells against oxidative stress and DNA DSB. Fenstad et al. (2016a) did not detect any differences in DNA DSB (measured as DNA-FTM) between common eiders in a high polluted area (Baltic Sea) and a less polluted area (Svalbard). However, the Baltic Sea eiders had much higher OHC levels and elevated antioxidant levels compared to Svalbard eiders. Although Fenstad et al. (2016a) did not show a significant relationship between antioxidant levels and DNA DSB, Fenstad et al. (2016c) did show a positive relationship between OHCs and total antioxidant capacity, indicating chemical-induced ROS formation and an upregulated antioxidant defence system.

The repair response system in avian species have shown to be efficient and embryonic cells have shown to tolerate and survive oxidative stress better than mammalian cells (Takata et al., 1998, Ogburn et al., 2001). Avian kidney epithelial cells experienced less DNA damage and better survival compared to mouse cells when exposed to ROS, indicating a better defence system in birds (Ogburn et al., 1998). Furthermore, avian primary embryotic fibroblast cells (from 7 -14 days old embryos) were more resistant to oxidative stress than similar mouse and human cells (Ogburn et al., 2001). Avian species uses more HR than NHEJ to repair DNA DSB in comparison to mammals (Takata et al., 1998, Lieber and Karanjawala, 2004). HR is more precise compared to NHEJ, which results in very efficient and precise repair of DNA DSB during genotoxic exposure in avian species and will therefore not cause as much errors and mutations (Takata et al., 1998). Using HR over NHEJ could explain why avian cells experience less DNA damage and survive better than mammalian cells when exposed to oxidative stress. Overall, it is reasonable to assume that genotoxic compounds, especially in low levels, may result in reduction in DNA DSB due to upregulation of repair systems, especially HR, and antioxidant defence. Thus, this could be an explanation for the observed negative relationship between DNA DSB and OHCs in the present study. However, the mechanism behind needs to be further explored.

ROS are produced during biotransformation of xenobiotics (Gregus, 2013), which raises the question on the biotransformation capacity in the avian embryo during early developmental stage. Avian embryos show a relative early biotransformation of chemicals around day five when the liver differentiates (Hamilton et al., 1983). Aryl hydrocarbon hydroxylase (AHHase), involved in xenobiotic biotransformation, was induced by polycyclic aromatic hydrocarbons (PAH) at embryonic day five. Prior to day five, no induction was observed, and levels in exposed embryos were similar to basal AHHase activity in control embryos and adults (Hamilton et al., 1983). According to the study by Hamilton et al. (1983) one can assume that there might be low, or no, biotransformation in the eggs used in the present study. Thus, biotransformation capacity during the embryonic stages is questionable. If biotransformation is low, there might also be low levels of ROS, which could result in low levels of DNA DSB. On the other hand, OHCs may also induce DNA DSB directly and thereby induce repair mechanism.

Pesticides (such as DDTs, HCH and HCB) also have the ability to induce apoptosis, possibly through the mechanism of decreasing antioxidant defences and production of ROS (Mrema et al., 2013). During apoptosis and programmed cell death the DNA will be fragmented and degraded (Muscarella et al., 1998). In addition to apoptosis being a stress-induced response to contaminant induced DNA damage, it is also a natural event during avian development (Muscarella et al., 1998). If there is relative high frequency of naturally occurring apoptosis and consequently DNA fragmentation during embryonic development, this may be a source to some of the observed DNA DSB levels in the present study and could possibly explain the similar levels of DNA DSB among the species. However, there is evidence that apoptosis is detected in relatively low frequency during early stages of development (Bloom et al., 1998). Studies also indicate that avian blastodermal cells are highly resistance and more protected to stress-induced cell death and apoptosis compared to mammalian species (Bloom et al., 1998, Muscarella et al., 1998). In addition, Collins et al. (2008) stated that the DNA fragments from apoptosis are very small and will diffuse away during electrophoresis. Still, some fragments at early stages of apoptosis are larger and could be revealed during electrophoresis (Collins et al., 2008). Hence, the effect of DNA fragments produced in the early stages of apoptosis

could, to some degree, affect the level of DNA DSB. Since several studies suggest that apoptosis is low during the early stage of avian development, and most of the fragments produced are suggested to be too small to be detected, one would not expect that the measured DNA DSB arises from apoptosis in the present study. However, one cannot rule out that both stress-induced and natural occurring apoptosis may account for some of the DNA DSB measured. Hence, apoptosis, and the produced DNA fragments should be further investigated as it might be a confounding factor.

To summarize, chemical exposure can induce several responses (figure 2) which will affect the level of DNA DSB. None of these endpoints (ROS formation, antioxidants, apoptosis and repair) were measured in the present study and warrant further investigation. Still, a negative relationship between DNA DSB and contaminants have previously been shown (Kleczkowska and Althaus, 1996, Wang et al., 2012, Gilmore, 2015), which strengthens the results in the present study. Moreover, studies show that the avian embryo, in the early developmental stages, is exposed to very little of the total contaminant load in the egg. This may indicate that there is little or no genotoxic stress on the DNA during this developmental period. Furthermore, the effect of OHCs on DNA might be different at lower levels of exposure because the repair system and antioxidant defence system might still be able to handle the exposure resulting in less DNA DSB. There is a lack of studies exploring these mechanisms during early avian development, and the association between OHCs and DNA DSB and the possible explanations in the present study can only be implied. Another aspect is that chemical compounds have different uptake rates due to their different properties. For example, methyl mercury (MeHg) has shown to diffuse very easily into the egg content (Heinz et al., 2006). There are also indications that metabolites are easier taken up by the embryo compared to their parent compounds. They can get excreted and may be reabsorbed after excretion, causing double exposure to the embryo (Bargar et al., 2001). Hence, some compounds may be detected in somewhat high levels in early developmental stages. In addition, multiple stressors such as climate change, overall fitness, nutrition status of the maternal bird may also cause additional stress and affect DNA DSB. In wildlife studies confounding factors are difficult to account for. Although a larger sample size could give more reliable results.

4.5 Future prospects and recommendations

Future studies should measure OHC levels in the blastodisc and embryo and not use whole egg concentrations. This could be difficult in the early developmental stages due to the small size of the blastodisc. It would also be advantageous to include other compounds such as mercury (Hg) and PAH and PBDE metabolites. Hg is especially interesting as it previously has been associated with DNA DSB in avian blood (Fenstad et al., 2016a) and studies indicate that MeHg are more easily taken up by the embryo in the early stages of development (Heinz et al., 2006). PAHs are interesting because they are known as strong genotoxicants and have been detected in common eiders breeding in Kongsfjorden (Beckhmann, 2016). Furthermore, PBDE showed a significant relationship with MML in the present study. PBDE metabolites are reported to be of greater toxic potential compared to their parent compounds (Hakk and Letcher, 2003) which make them interesting to investigate.

The investigations of genotoxic effect and responses to chemical stress in avian embryos are scarce. The negative association between DNA DSB and PBDEs and OCPs in kittiwake eggs in the present study was contradicting previously results from common eider blood in the same area and should be further investigated. The response may be related to defence mechanisms such as antioxidant defence, repair and stress-induced apoptosis. On the other hand, the observed levels of DNA DSB could also be a result of natural processes, such as apoptosis, occurring during this early stage of development. It would be of interest to further explore levels of chemical induced ROS, apoptosis, antioxidant capacity and repair and their relationship with genotoxic agents and DNA damage in avian blastodisc and embryos to understand the complex mechanisms. It would also be of interest to investigate the relationship between OHCs and DNA DSB in midway and late in the developmental period when the embryo is exposed to much higher levels of OHCs.

5. Conclusion

Levels of OHCs varied between the three species. As expected glaucous gull had the highest OHC levels followed by kittiwake and common eider. However, the levels in glaucous gull for each contaminant group were not significantly different from the levels in kittiwake. The pattern was similar among the species and consistent with previous studies. There was no observed effect of OHCs on egg volume. However, developmental stage seemed to account for some variation in the contaminant data, at least for common eider.

There was no significant difference in MML or DNA-FTM between the species. A negative relationship between PBDEs and OCPs and MML was observed for kittiwake. No significant relationship was seen for DNA-FTM in kittiwake or for MML nor DNA-FTM in common eider, although the same negative trend between OHCs and DNA DSB was observed. The processes and mechanisms behind this negative relationship are unknown, but possible explanations are suggested to be low levels in the blastodisc at early developmental stage, induced repair responses and antioxidant responses. Thus, further exploration of these factors is recommended.

In the present study no OHC induced adverse genotoxic effect in form of DNA DSB was seen in the blastodisc during early developmental stage. Thus, DNA damage do not explain the rather high mortality during early development. However, there could still be a potential for adverse genotoxic effect with higher levels of OHC and at later developmental stages which warrants further studies.

References

AMAP. 2004. AMAP Assessment 2002: Persistent organic pollutants in the Arctic. Oslo, Norway: Arctic Monitoring and Assessment Programme (AMAP).

AMAP. 2017. AMAP Assessment 2016: Chemicals of Emerging Arctic Concern. Oslo, Norway: Arctic Monitoring and Assessment Programme (AMAP).

ANKER-NILSSEN, T., BAKKEN, V., STRØM, H., GOLOVKIN, A. N., BIANKI, V. V. & TATARINKOVA, I. P. 2000. *The Status of Marine Birds Breeding in the Barents Sea Region*.

ANKER-NILSSEN, T., BARRETT, R., CHRISTENSEN-DALSGAARD, S., HANSSEN, S. A., REIERTSEN, T. K., BUSTNES, J. O., DESCAMPS, S., ERIKSTAD, K.-E., FOLLESTAD, A., LANGSET, M., LORENTSEN, S.-H., LORENTZEN, E., STRØM, H. & SYSTAD, G. H. 2018. Key-site monitoring in Norway 2017, including Svalbard and Jan Mayen. *SEAPOP Short Report*, 1-2018.

ARMITAGE, J. M., ARNOT, J. A., WANIA, F. & MACKAY, D. 2013. Development and evaluation of a mechanistic bioconcentration model for ionogenic organic chemicals in fish. *Environmental Toxicology and Chemistry,* 32**,** 115-128.

BARGAR, T., SCOTT, G. & COBB, G. 2001. Uptake and distribution of three PCB congeners and endosulfan by developing white leghorn chicken embryos (Gallus domesticus). *Archives of Environmental Contamination and Toxicology,* 41**,** 508-514.

BARRON, M., GALBRAITH, H. & BELTMAN, D. 1995. Comparative reproductive and developmental toxicology of PCBs in birds. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology,* 112**,** 1-14.

BECKHMANN, A. S. 2016. *Genotoxic Effects in relation to Polycyclic Aromatic Hydrocarbons in Blood from Female Common Eiders (Somateria mollissima) in Arctic Environments.* Master thesis, Norwegian University of Science and Technology.

BICKHAM, J. W., SANDHU, S., HEBERT, P. D., CHIKHI, L. & ATHWAL, R. 2000. Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. *Mutation Research/Reviews in Mutation Research,* 463**,** 33-51.

BINELLI, A., RIVA, C., COGNI, D. & PROVINI, A. 2008. Genotoxic effects of p, p′-DDT (1, 1, 1-trichloro-2, 2-bis-(chlorophenyl) ethane) and its metabolites in Zebra mussel (D. polymorpha) by SCGE assay and micronucleus test. *Environmental and Molecular Mutagenesis,* 49**,** 406-415.

BLÉVIN, P., ANGELIER, F., TARTU, S., RUAULT, S., BUSTAMANTE, P., HERZKE, D., MOE, B., BECH, C., GABRIELSEN, G. W., BUSTNES, J. O. & CHASTEL, O. 2016. Exposure to oxychlordane is associated with shorter telomeres in arctic breeding kittiwakes. *Science of the Total Environment,* 563**,** 125-130.

BLOOM, S. E., MUSCARELLA, D. E., LEE, M. Y. & RACHLINSKI, M. 1998. Cell death in the avian blastoderm: resistance to stress-induced apoptosis and expression of anti-apoptotic genes. *Cell Death and Differentiation,* 5**,** 529-538.

BORGÅ, K., GABRIELSEN, G. & SKAARE, J. 2001. Biomagnification of organochlorines along a Barents Sea food chain. *Environmental Pollution,* 113**,** 187-198.

BRAUNE, B. M. 2007. Temporal trends of organochlorines and mercury in seabird eggs from the Canadian Arctic, 1975–2003. *Environmental Pollution,* 148**,** 599-613.

BRAUNE, B. M., DONALDSON, G. M. & HOBSON, K. A. 2001. Contaminant residues in seabird eggs from the Canadian Arctic. Part I. Temporal trends 1975–1998. *Environmental Pollution,* 114**,** 39-54.

BRAUNE, B. M., GASTON, A. J. & MALLORY, M. L. 2018. Temporal trends of legacy organochlorines in eggs of Canadian Arctic seabirds monitored over four decades. *Science of the Total Environment,* 646**,** 551-563.

BRAUNE, B. M. & LETCHER, R. J. 2012. Perfluorinated sulfonate and carboxylate compounds in eggs of seabirds breeding in the Canadian Arctic: temporal trends (1975–2011) and interspecies comparison. *Environmental Science & Technology,* 47**,** 616-624.

BRAUNE, B. M., LETCHER, R. J., GASTON, A. J. & MALLORY, M. L. 2015. Trends of polybrominated diphenyl ethers and hexabromocyclododecane in eggs of Canadian Arctic seabirds reflect changing use patterns. *Environmental Research,* 142**,** 651-661.

BURNHAM, K. P. & ANDERSON, D. R. 2004. Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods & Research,* 33**,** 261-304.

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

BUSTNES, J. O., ERIKSTAD, K. E., BAKKEN, V., MEHLUM, F. & SKAARE, J. U. 2000. Feeding ecology and the concentration of organochlorines in glaucous gulls. *Ecotoxicology,* 9**,** 179-186.

BUSTNES, J. O., ERIKSTAD, K. E., SKAARE, J. U., BAKKEN, V. & MEHLUM, F. 2003. Ecological effects of organochlorine pollutants in the Arctic: A study of the glaucous gull. *Ecological Applications,* 13**,** 504-515.

BUSTNES, J. O., FAUCHALD, P., TVERAA, T., HELBERG, M. & SKAARE, J. 2008. The potential impact of environmental variation on the concentrations and ecological effects of pollutants in a marine avian top predator. *Environment International,* 34**,** 193-201.

BÅRDSEN, B. J., HANSSEN, S. A. & BUSTNES, J. O. 2018. Multiple stressors: modeling the effect of pollution, climate, and predation on viability of a sub-arctic marine bird. *Ecosphere,* 9**,** 1-22.

CARLSON, R. W. & DUBY, R. 1973. Embryotoxic effects of three PCB's in the chicken. *Bulletin of Environmental Contamination and Toxicology,* 9**,** 261-266.

CHRISTENSEN, V. L. 2001. Factors associated with early embryonic mortality. *World's Poultry Science Journal,* 57**,** 359-372.

COLLINS, A. R., OSCOZ, A. A., BRUNBORG, G., GAIVAO, I., GIOVANNELLI, L., KRUSZEWSKI, M., SMITH, C. C. & ŠTĚTINA, R. 2008. The comet assay: topical issues. *Mutagenesis,* 23**,** 143-151.

DAHL, T. M., FALK-PETERSEN, S., GABRIELSEN, G. W., SARGENT, J. R., HOP, H. & MILLAR, R. M. 2003. Lipids and stable isotopes in common eider, black-legged kittiwake and northern fulmar: a trophic study from an Arctic fjord. *Marine Ecology Progress Series,* 256**,** 257-269.

DE ROODE, D. F. & VAN DEN BRINK, N. W. 2002. Uptake of injected PCBs from the yolk by the developing chicken embryo. *Chemosphere,* 48**,** 195-199.

DE WIT, C. A. 2002. An overview of brominated flame retardants in the environment. *Chemosphere,* 46**,** 583-624.

DE WIT, C. A., HERZKE, D. & VORKAMP, K. 2010. Brominated flame retardants in the Arctic environment—trends and new candidates. *Science of the Total Environment,* 408**,** 2885-2918.

DEAN, K. M., BALTOS, L. D., MARCELL, A. M., BOHANNON, M. E., IWANIUK, A. N. & OTTINGER, M. A. 2018. Uptake of radiolabeled 3, 3′, 4, 4′-tetrachlorobiphenyl into Japanese quail egg compartments and embryo following air cell and albumen injection. *Environmental Toxicology and Chemistry,* 37**,** 126-135.

DEROSA, C., RICHTER, P., POHL, H. & JONES, D. E. 1998. Environmental exposures that affect the endocrine system: public health implications. *Journal of Toxicology and Environmental Health, Part B Critical Reviews,* 1**,** 3-26.

DROUILLARD, K. G. & NORSTROM, R. J. 2001. Quantifying maternal and dietary sources of 2,2 ',4,4 ',5,5 '-hexachlorobiphenyl deposited in eggs of the ring dove (Streptopelia risoria). *Environmental Toxicology and Chemistry,* 20**,** 561-567.

EATON, D. L. & GILBERT, S. G. 2013. Principles of Toxicology. *In:* KLAASSEN, C. D. (ed.) *Casarett and Doull's Toxicology: The Basic Science of Poisons.* New York (NY): McGraw-Hill Education, p. 13-48.

ENCYCLOPÆDIA BRITANNICA. 2008. *The structural components of an egg* [Online]. Encyclopædia Britannica. Available: https://www.britannica.com/science/eggshell-zoology/images-videos/media/180259/182 [Accessed 20.09 2018].

ERIKSTAD, K. E. 1990. Winter diets of four seabird species in the Barents Sea after a crash in the capelin stock. *Polar Biology,* 10**,** 619-627.

ERIKSTAD, K. E., MOUM, T., BUSTNES, J. O. & REIERTSEN, T. K. 2011. High levels of organochlorines may affect hatching sex ratio and hatchling body mass in arctic glaucous gulls. *Functional Ecology,* 25**,** 289-296.

FAUCHALD, P., ANKER-NILSSEN, T., BARRETT, R. T., BUSTNES, J. O., BÅRDSEN, B.-J., CHRISTENSEN-DALSGAARD, S., DESCAMPS, S., ENGEN, S., ERIKSTAD, K. E., HANSSEN, S. A., LORENTSEN, S.-H., MOE, B., REIERTSEN, T. K., STRØM, H. & SYSTAD, G. H. 2015. The status and trends of seabirds breeding in Norway and Svalbard. *NINA Report 1151.* Tromsø, Norway: Norwegian Institute for Nature Research.

FENSTAD, A. A., BUSTNES, J. O., BINGHAM, C. G., ÖST, M., JAATINEN, K., MOE, B., HANSSEN, S. A., MOODY, A. J., GABRIELSEN, K. M., HERZKE, D., LIERHAGEN, S., JENSSEN, B. M. & KRØKJE, Å. 2016a. DNA double-strand breaks in incubating female common eiders (Somateria mollissima): Comparison between a low and a high polluted area. *Environmental Research,* 151**,** 297-303.

FENSTAD, A. A., JENSSEN, B. M., GABRIELSEN, K. M., ÖST, M., JAATINEN, K., BUSTNES, J. O., HANSSEN, S. A., MOE, B., HERZKE, D. & KRØKJE, Å. 2016b. Persistent organic pollutant levels and the importance of source proximity in Baltic and Svalbard breeding common eiders. *Environmental Toxicology and Chemistry,* 35**,** 1526-1533.

FENSTAD, A. A., JENSSEN, B. M., MOE, B., HANSSEN, S. A., BINGHAM, C., HERZKE, D., BUSTNES, J. O. & KRØKJE, Å. 2014. DNA double-strand breaks in relation to persistent organic pollutants in a fasting seabird. *Ecotoxicology and Environmental Safety,* 106**,** 68-75.

FENSTAD, A. A., MOODY, A. J., ÖST, M., JAATINEN, K., BUSTNES, J. O., MOE, B., HANSSEN, S. A., GABRIELSEN, K. M., HERZKE, D., LIERHAGEN, S., JENSSEN, B. M. & KRØKJE, Å. 2016c. Antioxidant Responses in Relation to Persistent Organic Pollutants and Metals in a Low-and a High-Exposure Population of Seabirds. *Environmental Science & Technology,* 50**,** 4817-4825.

FURNESS, R. W. 1993. Birds as monitors of pollutants. *In:* FURNESS, R. W. & GREENWOOD, J. J. D. (eds.) *Birds as Monitors of Environmental Change.* London: Chapmann and Hall.

FURNESS, R. W. & CAMPHUYSEN, K. 1997. Seabirds as monitors of the marine environment. *ICES Journal of Marine Science,* 54**,** 726-737.

GABRIELSEN, G. W. 2007. Levels and effects of persistent organic pollutants in arctic animals. *In:* ØRBÆK, J. B., KALLENBORN, R., TOMBRE, I., HEGSETH, E. N., FALK-PETERSEN, S. & HOEL, A. H. (eds.) *Arctic Alpine Ecosystems and People in a Changing Environment.* Berlin, Germany: Springer.

GABRIELSEN, G. W. 2009. Seabirds in the Barents Sea. *In:* SAKSHAUG, E., JOHNSEN, G. M. & KOVACS, K. M. (eds.) *Ecosystem Barents Sea.* Trondheim: Tapir Academic Press, p. 415-452.

GABRIELSEN, G. W., MEHLUM, F., KARLSEN, H. E., ANDRESEN, Ø. & PARKER, H. 1991. Energy cost during incubation and thermoregulation in the female common eider Somateria mollissima. *Norsk Polarinstitutt Skrifter,* 195**,** 51-62.

GILMORE, E. H. 2015. *Do contaminants in polar bear (Ursus maritimus) modulate the expression of selected genes and cause DNA strand breaks?* Master thesis, University of Oslo.

GOUTTE, A., BARBRAUD, C., HERZKE, D., BUSTAMANTE, P., ANGELIER, F., TARTU, S., CLÉMENT-CHASTEL, C., MOE, B., BECH, C., GABRIELSEN, G. W., BUSTNES, J. O. & CHASTEL, O. 2015. Survival rate and breeding outputs in a high Arctic seabird exposed to legacy persistent organic pollutants and mercury. *Environmental Pollution,* 200**,** 1-9.

GREGUS, Z. 2013. Mechanisms of Toxicology. *In:* KLAASSEN, C. D. (ed.) *Casarett and Doull's Toxicology: The Basic Science of Poisons.* 8th ed. New York (NY): McGraw-Hill Education, p. 49-122.

HAARR, A., HYLLAND, K., ECKBO, N., GABRIELSEN, G. W., HERZKE, D., BUSTNES, J. O., BLEVIN, P., CHASTEL, O., MOE, B., HANSSEN, S. A., SAGERUP, K. & BORGÅ, K. 2017. DNA damage in arctic seabirds: baseline, sensitivity to a genotoxic stressor and association to organohalogen contaminants. *Environmental Toxicology and Chemistry,* 37**,** 1084-1091.

HAFTORN, S. 1971. *Norges Fugler,* Trondheim, Universitetsforlaget.

HAKK, H. & LETCHER, R. J. 2003. Metabolism in the toxicokinetics and fate of brominated flame retardants—a review. *Environment International,* 29**,** 801-828.

HAMILTON, J. W., DENISON, M. S. & BLOOM, S. E. 1983. Development of basal and induced aryl hydrocarbon (benzo [a] pyrene) hydroxylase activity in the chicken embryo in ovo. *Proceedings of the National Academy of Sciences,* 80**,** 3372-3376.

HANSSEN, L., DUDAREV, A. A., HUBER, S., ODLAND, J. Ø., NIEBOER, E. & SANDANGER, T. M. 2013. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. *Science of the Total Environment,* 447**,** 430-437.

HANSSEN, S. A., ENGEBRETSEN, H. & ERIKSTAD, K. E. 2002. Incubation start and egg size in relation to body reserves in the common eider. *Behavioral Ecology and Sociobiology,* 52**,** 282-288.

HANSSEN, S. A., HASSELQUIST, D., FOLSTAD, I. & ERIKSTAD, K. E. 2005. Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. *Proceedings of the Royal Society B: Biological Sciences,* 272**,** 1039-46.

HEINZ, G., HOFFMAN, D., KONDRAD, S. & ERWIN, C. 2006. Factors affecting the toxicity of methylmercury injected into eggs. *Archives of Environmental Contamination and Toxicology,* 50**,** 264-279.

HELBERG, M., BUSTNES, J. O., ERIKSTAD, K. E., KRISTIANSEN, K. O. & SKAARE, J. U. 2005. Relationships between reproductive performance and organochlorine contaminants in great black-backed gulls (Larus marinus). *Environmental Pollution,* 134**,** 475-483.

HELGASON, L. B., ARUKWE, A., GABRIELSEN, G. W., HARJU, M., HEGSETH, M. N., HEIMSTAD, E. S., JORGENSEN, E. H., MORTENSEN, A. S. & WOLKERS, J. 2010. Biotransformation of PCBs in Arctic seabirds: Characterization of phase I and II pathways at transcriptional, translational and activity levels. *Comparative Biochemistry and Physiology, Part C-Toxicology & Pharmacology,* 152**,** 34-41.

HELGASON, L. B., SAGERUP, K. & GABRIELSEN, G. W. 2011. Organohalogen Pollutants in Seabird Eggs from Northern Norway and Svalbard. *In:* LOGANATHAN, B. G. & LAM, P. K. S. (eds.) *Global Contamination Trends of Persistent Organic Chemicals.* Boca Raton, FL: CRC Press, p. 547-569.

HERZKE, D., BERGER, U., KALLENBORN, R., NYGÅRD, T. & VETTER, W. 2005. Brominated flame retardants and other organobromines in Norwegian predatory bird eggs. *Chemosphere,* 61**,** 441-449.

HERZKE, D., KAASA, H., GRAVEM, F., GREGERSEN, H., JENSEN, J. B., HORN, J., HARJU, M., BORGEN, A., ENGE, E. & WARNER, N. 2013. Perfluorinated alkylated substances, brominated flame retardants and chlorinated paraffins in the Norwegian Environment - Screening 2013. *M-40.* Oslo, Norway: Norwegian Environment Agency.

HERZKE, D., NYGÅRD, T., BERGER, U., HUBER, S. & RØV, N. 2009. Perfluorinated and other persistent halogenated organic compounds in European shag (Phalacrocorax aristotelis) and common eider (Somateria mollissima) from Norway: a suburban to remote pollutant gradient. *Science of the Total Environment,* 408**,** 340-348.

HOP, H., BORGÅ, K., GABRIELSEN, G. W., KLEIVANE, L. & SKAARE, J. U. 2002. Food web magnification of persistent organic pollutants in poikilotherms and homeotherms from the Barents Sea. *Environmental Science & Technology,* 36**,** 2589-2597.

HOYT, D. F. 1979. Practical methods of estimating volume and fresh weight of bird eggs. *The Auk,* 96**,** 73-77.

HUBER, S., NYGÅRD, T., WARNER, N. A., REMBERGER, M., HARJU, M., UGGERUD, H. T., KAJ, L., SCHLABACH, M. & HANSSEN, L. 2014. Kartlegging av miljøgifter i sjøfuglegg fra Sklinna og Røst. *Oppdragsrapport 8/2014.* Tromsø: Norwegian Institute for Air Research.

HUBER, S., WARNER, N. A., NYGÅRD, T., REMBERGER, M., HARJU, M., UGGERUD, H. T., KAJ, L. & HANSSEN, L. 2015. A broad cocktail of environmental pollutants found in eggs of three seabird species from remote colonies in Norway. *Environmental Toxicology and Chemistry,* 34**,** 1296-1308.

HUNG, H., KATSOYIANNIS, A. A., BRORSTRÖM-LUNDÉN, E., OLAFSDOTTIR, K., AAS, W., BREIVIK, K., BOHLIN-NIZZETTO, P., SIGURDSSON, A., HAKOLA, H., BOSSI, R., SKOV, H., SVERKO, E., BARRESI, E., FELLIN, P. & WILSON, S. 2016. Temporal trends of Persistent Organic Pollutants (POPs) in arctic air: 20 years of monitoring under the Arctic Monitoring and Assessment Programme (AMAP). *Environmental Pollution,* 217**,** 52-61.

JACKSON, S. P. 2002. Sensing and repairing DNA double-strand breaks. *Carcinogenesis,* 23**,** 687-696.

JACOBSON, M. D., WEIL, M. & RAFF, M. C. 1997. Programmed cell death in animal development. *Cell,* 88**,** 347-354.

JENKINS, G. J., ZAÏR, Z., JOHNSON, G. E. & DOAK, S. H. 2010. Genotoxic thresholds, DNA repair, and susceptibility in human populations. *Toxicology,* 278**,** 305-310.

JOLLIFFE, I. 2011. *Principal component analysis,* Berlin, Heidelberg, Springer, p. 1094-1096.

KELLY, B. C., IKONOMOU, M. G., BLAIR, J. D., SURRIDGE, B., HOOVER, D., GRACE, R. & GOBAS, F. A. 2009. Perfluoroalkyl contaminants in an Arctic marine food web: trophic magnification and wildlife exposure. *Environmental Science & Technology,* 43**,** 4037-4043.

KERNS, G. J. 2010. Introduction to probability and statistics using R. USA: Youngstown State University.

KILPI, M. & LINDSTRÖM, K. 1997. Habitat-specific clutch size and cost of incubation in common eiders, Somateria mollissima. *Oecologia,* 111**,** 297-301.

KIMBERLY, D. A. & SALICE, C. J. 2015. Evolutionary responses to climate change and contaminants: Evidence and experimental approaches. *Current Zoology,* 61**,** 690-701.

KISSA, E. 2001. *Fluorinated Surfactants and Repellents,* New York, Marcel Dekker

KLAUNIG, J. E. 2013. Chemical Carsinogenesis. *In:* KLAASSEN, C. D. (ed.) *Casarett and Doull's Toxicology: The Basic Science of Poisons.* 8th ed. New York (NY): McGraw-Hill Education, p. 393-443.

KLECZKOWSKA, H. E. & ALTHAUS, F. R. 1996. Response of human keratinocytes to extremely low concentrations of N-methyl-N′-nitro-N-nitrosoguanidine. *Mutation Research/Genetic Toxicology,* 367**,** 151-159.

KNUDSEN, L. B., GABRIELSEN, G. W., VERREAULT, J., BARRETT, R., SKAARE, J. U., POLDER, A. & LIE, E. 2005. Temporal trends of brominated flame retardants, cyclododeca-1, 5, 9-triene and mercury in eggs of four seabird species from Northern Norway and Svalbard. *SPFO-Report 942/2005.* Oslo, Norway: Norwegian Pollution Control Authority.

KRØKJE, Å., BINGHAM, C., TUVEN, R. H. & GABRIELSEN, G. W. 2006. Chromosome aberrations and DNA strand breaks in glaucous gull (Larus hyperboreus) chicks fed environmentally contaminated gull eggs. *Journal of Toxicology and Environmental Health, Part A: Current Issues,* 69**,** 159-174.

LETCHER, R. J., BUSTNES, J. O., DIETZ, R., JENSSEN, B. M., JØRGENSEN, E. H., SONNE, C., VERREAULT, J., VIJAYAN, M. M. & GABRIELSEN, G. W. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of the Total Environment,* 408**,** 2995-3043.

LETCHER, R. J., KLASSON-WEHLER, E. & BERGMAN, A. 2000. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. *In:* PAASIVIRTA, J. (ed.) *The Handbook of Environment Chemistry: New Types of Persistent Halogenated Compounds, 3.* Heidelberg: Springer, p. 315-360.

LIEBER, M. R. & KARANJAWALA, Z. E. 2004. Ageing, repetitive genomes and DNA damage. *Nature reviews Molecular cell biology,* 5**,** 69-75.

LYDERSEN, C. & SMITH, T. G. 1989. Avian predation on ringed seal Phoca hispida pups. *Polar Biology,* 9**,** 489-490.

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

MAERVOET, J., BECK, V., ROELENS, S. A., COVACI, A., VOORSPOELS, S., GEUNS, J. M., DARRAS, V. M. & SCHEPENS, P. 2005. Uptake and tissue-specific distribution of selected polychlorinated biphenyls in developing chicken embryos. *Environmental Toxicology and Chemistry,* 24**,** 597-602.

MEHLUM, F. 1991. Egg predation in a breeding colony of the common eider Somateria mollissima in Kongsfjorden, Svalbard. *Norsk Polarinstitutt Skrifter,* 195**,** 37-45.
MEHLUM, F. & GABRIELSEN, G. W. 1993. The diet of high-arctic seabirds in coastal and ice-covered, pelagic areas near the Svalbard archipelago. *Polar Research,* 12**,** 1-20.
MILJETEIG, C. & GABRIELSEN, G. W. 2009. Contaminants in black-legged kittiwake eggs from Kongsfjorden, Barentsburg and Pyramiden. *Brief Report Series NO.14.* Tromsø, Norway: Norwegian Polar Institute.
MOSJ. 2018a. *Common eider (Somateria mollissima borealis)* [Online]. Environmental monitoring of Svalbard and Jan Mayen (MOSJ). Available: http://www.mosj.no/en/fauna/marine/common-eider.html [Accessed 01.09 2018].
MOSJ. 2018b. *Pollutants in glaucous gulls (Larus hyperboreus)* [Online]. Environmental monitoring of Svalbard and Jan Mayen (MOSJ). Available: http://www.mosj.no/en/influence/pollution/pollutants-glaucous-gull.html [Accessed 09.08 2018].
MREMA, E. J., RUBINO, F. M., BRAMBILLA, G., MORETTO, A., TSATSAKIS, A. M. & COLOSIO, C. 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. *Toxicology,* 307**,** 74-88.
MURRAY, H. A. 1925. Physiological ontogeny: A. Chicken embryos. II. Catabolism. Chemical changes in fertile eggs during incubation. Selection of standard conditions. *The Journal of General Physiology,* 9**,** 1-37.
MUSCARELLA, D. E., RACHLINSKI, M. K. & BLOOM, S. E. 1998. Expression of cell death regulatory genes and limited apoptosis induction in avian blastodermal cells. *Molecular Reproduction and Development: Incorporating Gamete Research,* 51**,** 130-142.
O'BRIEN, R. M. 2007. A caution regarding rules of thumb for variance inflation factors. *Quality & Quantity,* 41**,** 673-690.
OGBURN, C. E., AUSTAD, S. N., HOLMES, D. J., KIKLEVICH, J. V., GOLLAHON, K., RABINOVITCH, P. S. & MARTIN, G. M. 1998. Cultured renal epithelial cells from birds and mice: enhanced resistance of avian cells to oxidative stress and DNA damage. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 53**,** 287-292.
OGBURN, C. E., CARLBERG, K., OTTINGER, M. A., HOLMES, D. J., MARTIN, G. M. & AUSTAD, S. N. 2001. Exceptional cellular resistance to oxidative damage in long-lived birds requires active gene expression. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 56**,** 468-474.
PECK, L. E., GILCHRIST, H. G., MALLORY, C. D., BRAUNE, B. M. & MALLORY, M. L. 2016. Persistent organic pollutant and mercury concentrations in eggs of ground-nesting marine birds in the Canadian high Arctic. *Science of the Total Environment,* 556**,** 80-88.
PERRY, M., GILBERT, A. & EVANS, A. 1978. The structure of the germinal disc region of the hen's ovarian follicle during the rapid growth phase. *Journal of Anatomy,* 127**,** 379.
PFEIFFER, P. 1998. The mutagenic potential of DNA double-strand break repair. *Toxicology Letters,* 96**,** 119-129.
PRESTON, J. R. & HOFFMANN, G. R. 2013. Gene Toxicology. *In:* KLAASSEN, C. D. (ed.) *Casarett and Doull's Toxicology: The Basic Science of Poisons.* 8th ed. New York (NY): McGraw-Hill Education, p. 445-480.
PRESTRUD, P. & MELUM, F. 1991. Population size and summer distribution of the Comnlon Eider Somateria mollissima in Svalbard. *Norsk Polarinstitutt Skrifter,* 195**,** 9-20.

REITER, M. E. & ANDERSEN, D. E. 2008. Comparison of the egg flotation and egg candling techniques for estimating incubation day of Canada Goose nests. *Journal of Field Ornithology,* 79**,** 429-437.

RIGÉT, F., BIGNERT, A., BRAUNE, B., DAM, M., DIETZ, R., EVANS, M., GREEN, N., GUNNLAUGSDÓTTIR, H., HOYDAL, K. S., KUCKLICK, J., LETCHER, R. J., MUIR, D., SCHUUR, S., SONNE, C., STERN, G., TOMT, G., VORKAMP, K. & WILSON, S. 2018. Temporal trends of persistent organic pollutants in Arctic marine and freshwater biota. *Science of The Total Environment,* 649**,** 99-110.

ROMANOFF, A. L. & ROMANOFF, A. J. 1967. *Biochemistry of the Avian Embryo; A Quantitative Analysis of Prenatal Development,* New York, Wiley Interscience

SAGERUP, K., LARSEN, H. J. R. S., SKAARE, J. U., JOHANSEN, G. M. & GABRIELSEN, G. W. 2009. The toxic effects of multiple persistent organic pollutant exposures on the post-hatch immunity maturation of glaucous gulls. *Journal of Toxicology and Environmental Health, Part A,* 72**,** 870-883.

SELLSTRÖM, U., KIERKEGAARD, A., DE WIT, C. & JANSSON, B. 1998. Polybrominated diphenyl ethers and hexabromocyclododecane in sediment and fish from a Swedish river. *Environmental Toxicology and Chemistry,* 17**,** 1065-1072.

SHAPOSHNIKOV, S. A., SALENKO, V. B., BRUNBORG, G., NYGREN, J. & COLLINS, A. R. 2008. Single-cell gel electrophoresis (the comet assay): Loops or fragments? *Electrophoresis,* 29**,** 3005-3012.

SRINIVASAN, A., LEHMLER, H.-J., ROBERTSON, L. W. & LUDEWIG, G. 2001. Production of DNA strand breaks in vitro and reactive oxygen species in vitro and in HL-60 cells by PCB metabolites. *Toxicological Sciences,* 60**,** 92-102.

STRØM, H. 2007. Distribution of seabirds on Bjørnøya. *In:* ANKER-NILSSEN, T., BARRETT, R. T., BUSTNES, J. O., ERIKSTAD, K. E., FAUCHALD, P., LORENTSEN, S.-H., STEEN, H., SYSTAD, G. H. & TVERAA, T. (eds.) *SEAPOP studies in the Lofoten and Barents Sea area in 2006. NINA Rapport 249: 63.* Trondheim: Norwegian Institute for Nature Research

SWENNEN, C., URSEM, J. C. H. & DUIVEN, P. 1993. Determinate laying and egg attendance in common eiders. *Ornis Scandinavica,* 24**,** 48-52.

TAKATA, M., SASAKI, M. S., SONODA, E., MORRISON, C., HASHIMOTO, M., UTSUMI, H., YAMAGUCHI-IWAI, Y., SHINOHARA, A. & TAKEDA, S. 1998. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *The EMBO Journal,* 17**,** 5497-5508.

TARTU, S., ANGELIER, F., HERZKE, D., MOE, B., BECH, C., GABRIELSEN, G. W., BUSTNES, J. O. & CHASTEL, O. 2014. The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. *Science of The Total Environment,* 476**,** 553-560.

THEODORAKIS, C. W., D'SURNEY, S. J. & SHUGART, L. R. 1994. Detection of genotoxic insult as DNA strand breaks in fish blood cells by agarose gel electrophoresis. *Environmental Toxicology and Chemistry,* 13**,** 1023-1031.

UNEP. 2008a. *The 12 initial POPs under the Stockholm Convention* [Online]. Available: http://chm.pops.int/TheConvention/ThePOPs/The12InitialPOPs/tabid/296/Default.aspx [Accessed 31.08].

UNEP. 2008b. *What are POPs?* [Online]. Available: http://chm.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx [Accessed 31.08 2018].

UNEP. 2017. The 16 New POPs -An introduction to the chemicals added to the Stockholm Convention as Persistent Organic Pollutants by the Conference of the Parties. Geneva: United Nations Environment Programme.

VAMVAKAS, S., VOCK, E. H. & LUTZ, W. K. 1997. On the role of DNA double-strand breaks in toxicity and carcinogenesis. *Critical Reviews in Toxicology,* 27**,** 155-174.

VAN DEN STEEN, E., JASPERS, V. L., COVACI, A., NEELS, H., EENS, M. & PINXTEN, R. 2009. Maternal transfer of organochlorines and brominated flame retardants in blue tits (Cyanistes caeruleus). *Environment International,* 35**,** 69-75.

VERBOVEN, N., VERREAULT, J., LETCHER, R. J., GABRIELSEN, G. W. & EVANS, N. P. 2009. Differential investment in eggs by Arctic-breeding glaucous gulls (Larus hyperboreus) exposed to persistent organic pollutants. *Auk,* 126**,** 123-133.

VERREAULT, J., GABRIELSEN, G. W. & BUSTNES, J. O. 2010. The Svalbard glaucous gull as bioindicator species in the European Arctic: Insight from 35 years of contaminants research. *Reviews of Environmental Contamination and Toxicology,* 205**,** 77-116.

VERREAULT, J., GABRIELSEN, G. W., LETCHER, R. J., MUIR, D. C. & SHAOGANG, C. 2004a. New and established organohalogen contaminants and their metabolites in plasma and eggs of glaucous gulls from Bear Island. *SPFO-Report 914/2004.* Oslo, Norway: Norwegian Pollution Control Authority.

VERREAULT, J., GEBBINK, W. A., GAUTHIER, L. T., GABRIELSEN, G. W. & LETCHER, R. J. 2007. Brominated flame retardants in glaucous gulls from the Norwegian Arctic: more than just an issue of polybrominated diphenyl ethers. *Environmental Science & Technology,* 41**,** 4925-4931.

VERREAULT, J., HOUDE, M., GABRIELSEN, G. W., BERGER, U., HAUKÅS, M., LETCHER, R. J. & MUIR, D. C. 2005a. Perfluorinated alkyl substances in plasma, liver, brain, and eggs of glaucous gulls (Larus hyperboreus) from the Norwegian Arctic. *Environmental Science & Technology,* 39**,** 7439-7445.

VERREAULT, J., LETCHER, R. J., MUIR, D. C. G., CHU, S. G., GEBBINK, W. A. & GABRIELSEN, G. W. 2005b. New organochlorine contaminants and metabolites in plasma and eggs of glaucous gulls (Larus hyperboreus) from the Norwegian Arctic. *Environmental Toxicology and Chemistry,* 24**,** 2486-2499.

VERREAULT, J., SKAARE, J. U., JENSSEN, B. M. & GABRIELSEN, G. W. 2004b. Effects of organochlorine contaminants on thyroid hormone levels in Arctic breeding glaucous gulls, Larus hyperboreus. *Environmental Health Perspectives,* 112**,** 532-537.

VERREAULT, J., VILLA, R. A., GABRIELSEN, G. W., SKAARE, J. U. & LETCHER, R. J. 2006. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. *Environmental Pollution,* 144**,** 1053-1060.

WALDECK, P., HANSSEN, S. A. & ANDERSSON, M. 2011. Indeterminate laying and flexible clutch size in a capital breeder, the common eider. *Oecologia,* 165**,** 707-712.

WALKER, C. H., SIBLY, R., HOPKIN, S. & PEAKALL, D. B. 2006. *Principles of Ecotoxicology,* Boca Raton (FL), CRC Press.

WANG, L., ZOU, W., ZHONG, Y., AN, J., ZHANG, X., WU, M. & YU, Z. 2012. The hormesis effect of BDE-47 in HepG2 cells and the potential molecular mechanism. *Toxicology Letters,* 209**,** 193-201.

WATSON, M. D., ROBERTSON, G. J. & COOKE, F. 1993. Egg-laying time and laying interval in the common eider. *Condor,* 95**,** 869-878.

WOLD, A., JAEGER, I., HOP, H., GABRIELSEN, G. W. & FALK-PETERSEN, S. 2011. Arctic seabird food chains explored by fatty acid composition and stable isotopes in Kongsfjorden, Svalbard. *Polar Biology,* 34**,** 1147-1155.

WOLKERS, H., VAN BAVEL, B., DEROCHER, A. E., WIIG, Ø., KOVACS, K. M., LYDERSEN, C. & LINDSTRÖM, G. 2004. Congener-specific accumulation and food chain transfer of polybrominated diphenyl ethers in two Arctic food chains. *Environmental Science & Technology,* 38**,** 1667-1674.

WURGLER, F. & KRAMERS, P. 1992. Environmental effects of genotoxins (eco-genotoxicology). *Mutagenesis,* 7**,** 321-327.

ZHENG, X. B., LUO, X. J., ZENG, Y. H., WU, J. P., CHEN, S. J. & MAI, B. X. 2014. Halogenated flame retardants during egg formation and chicken embryo development: Maternal transfer, possible biotransformation, and tissue distribution. *Environmental Toxicology and Chemistry,* 33**,** 1712-1719.

ØSTBY, L., GABRIELSEN, G. W. & KRØKJE, Å. 2005. Cytochrome P4501A induction and DNA adduct formation in glaucous gulls (Larus hyperboreus), fed with environmentally contaminated gull eggs. *Ecotoxicology and Environmental Safety,* 62**,** 363-375.

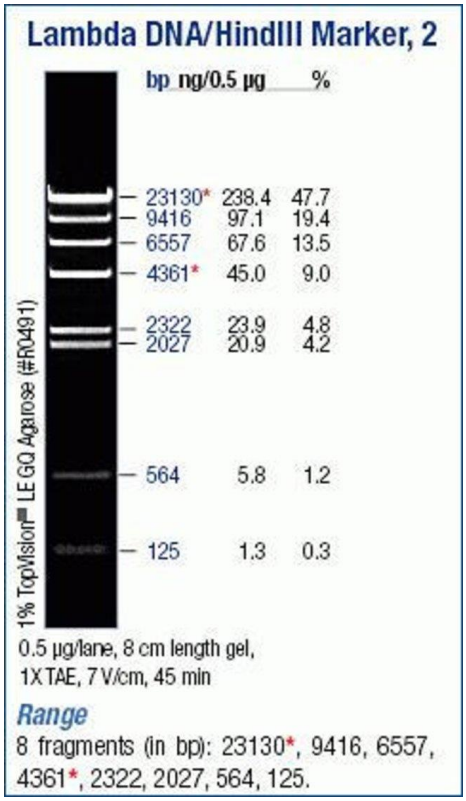
Appendices

Appendix A

A.1. Chemicals used in double strand break analysis

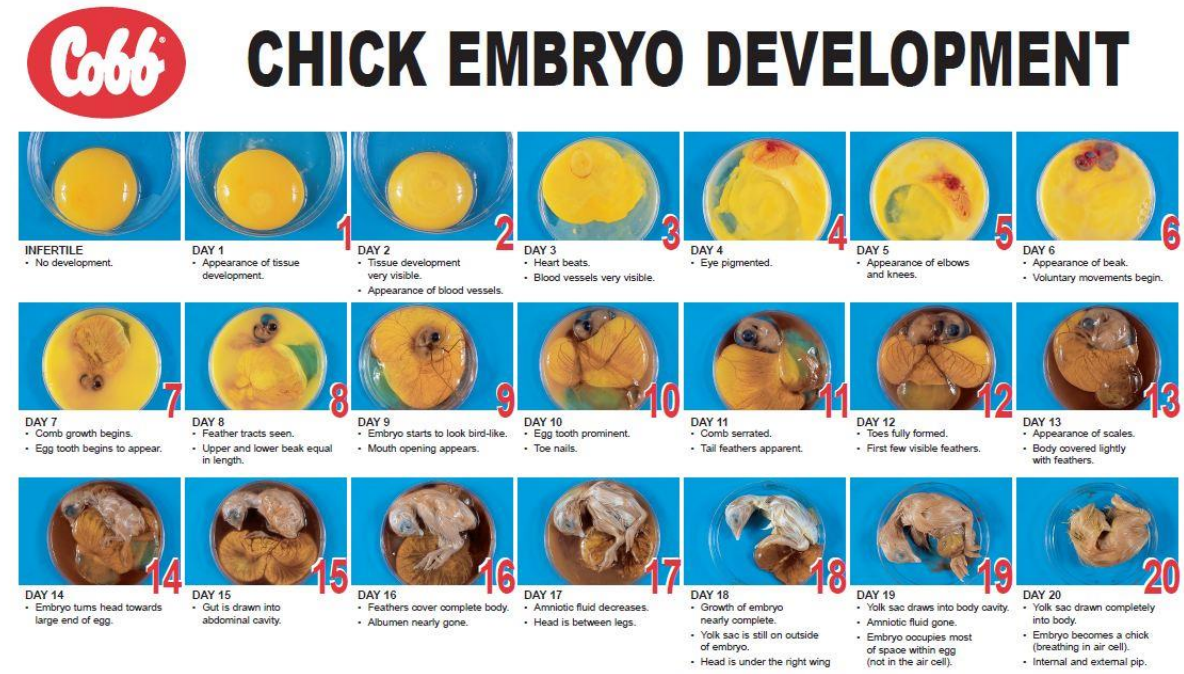
Chemical	Product number	Producer
Agarose	A9539	Sigma
Boric acid (H3BO3)	B7901	Sigma
Ethyl bromide	161-0433	Bio-Rad
Ethylenediaminetatraacetic acid (EDTA)	161-0729	Bio-Rad
HindIII-digested lambda DNA	#SM0101	Fermentas
Low melting point Agarose (LMPA)	162-0019	Bio-Rad
Lambda DNA	#SM0231	Fermentas
DNA Gel Loading Dye (6x)	#R0611	Fermentas
sodium chloride (NaCl)	S3014	Sigma
Proteinase K	P2308	Sigma
Sodium dodecyl sulphate (SDS)	161-0301	Bio-Rad
Trizma base	T6066	Sigma

A.2. The standard base pair sizes for the ladder: λ DNA: 483337, HindIII digested λ DNA; 23130, 9416, 6557, 4361, 2322, 2027, 564 and 125 bp.



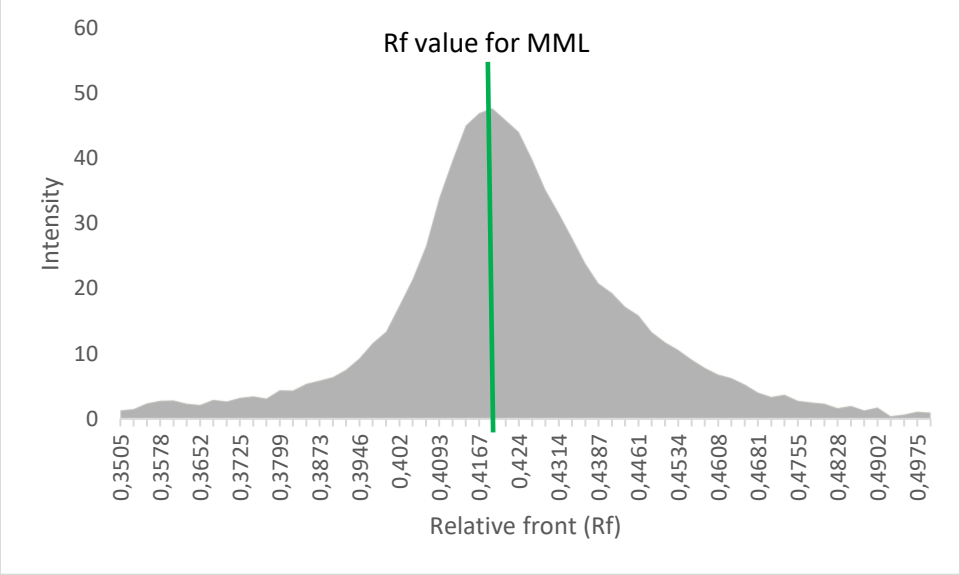
Appendix B

Scoring table for the developmental stage of the eggs

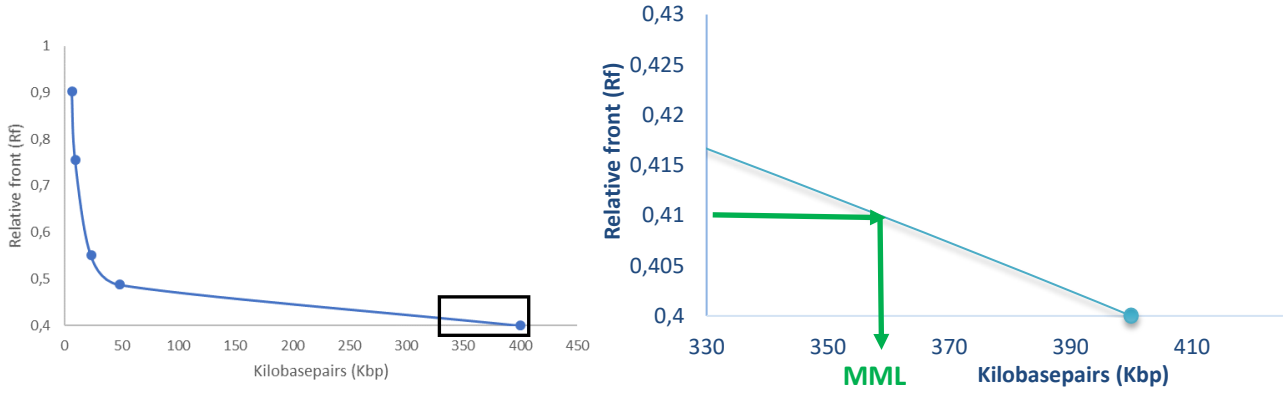


Appendix C

C.1. Rf-value of the MML acquired from the area corresponding to 50 % of the second peak of the intensity curve. Hence, the peak of the migrated DNA



C.2. The standard curve obtained from the Lambda DNA size markers and one extrapolated point at 400 Kbp. The MML value (kbp) was quantified from the standard curve and rf-vaue of the MML.



Appendix D

The chlorinated, brominated and fluorinated contaminants analysed in eggs from glaucous gull, kittiwake captured around Ny-Ålesund during the breeding season in June 2015.

Group	Acronym	Analyte/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	
DDTs	o,p'-DDT	o,p,'-Dichloro-α,α-diphenyl-β,β,β-trichloroethane
	p,p'-DDT	p,p,'-Dichloro-α,α-diphenyl-β,β,β-trichloroethane
	o,p'-DDD	o,p,'-Dichloro-diphenyl-dichloroethane
	p,p'-DDD	p,p,'-Dichloro-diphenyl-dichloroethane
	o,p'-DDE	o,p,'-Dichloro-diphenyl-dichloroethylene
	p,p'-DDE	p,p,'-Dichloro-diphenyl-dichloroethylene
PCBs	PCB 28	2,4,4'-Trichlorobiphenyl
	PCB 52	2,2',5,5'-Tetrachlorobiphenyl
	PCB 99	2,2',4,4',5-Pentachlorobiphenyl
	PCB 101	2,2',4,5,5'-Pentachlorobiphenyl
	PCB 105	2,3,3',4,4'-Pentachlorobiphenyl
	PCB 118	2,3',4,4',5-Pentachlorobiphenyl
	PCB 138	2,2',3,4,4',5'-Hexachlorobiphenyl
	PCB 153	2,2',4,4',5,5'-Hexachlorobiphenyl
	PCB 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl
	PCB 183	2,2',3,4,4',5',6-Heptachlorobiphenyl
	PCB 187	2,2',3,4',5,5',6'-Heptachlorobiphenyl
	PCB 194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
$MeSO_2$-PCBs/DDE	3-$MeSO_2$-DDE	3-Methylsulfonyl-2,2′-bis(4-chlorophenyl)-1,1′-dichloroethene
	3-$MeSO_2$-PCB49	3-Methylsufonyl-2,2′,4′,5-tetrachlorobiphenyl
	4-$MeSO_2$-PCB49	4-Methylsufonyl-2,2′,4′,5-tetrachlorobiphenyl
	3-$MeSO_2$-PCB52	3-Methylsufonyl-2,2′,5,5′-tetrachlorobiphenyl
	4-$MeSO_2$-PCB52	4-Methylsufonyl-2,2′,5,5′-tetrachlorobiphenyl
	3-$MeSO_2$-PCB91	3-Methylsufonyl-2,2′,3,4′,6-pentachlorobiphenyl
	4-$MeSO_2$-PCB91	4-Methylsufonyl-2,2′,3,4′,6-pentachlorobiphenyl
	3-$MeSO_2$-PCB101	3-Methylsufonyl-2,2′,4′,5,5′-pentachlorobiphenyl
	4-$MeSO_2$-PCB101	4-Methylsufonyl-2,2′,4′,5,5′-pentachlorobiphenyl
	3-$MeSO_2$-PCB87	3-Methylsufonyl-2,2′,3′,4′,5-pentachlorobiphenyl
	4-$MeSO_2$-PCB110	4-Methylsufonyl-2,2′,4′,5,5′-pentachlorobiphenyl

Group	Acronym	Analyte/structure
	3-MeSO$_2$-PCB110	3-Methylsufonyl-2,2′,4′,5,5′-pentachlorobiphenyl
	3-MeSO$_2$-PCB132	3-Methylsufonyl-2,2′,3′,4′,5,6-hexachlorobiphenyl
	4-MeSO$_2$-PCB132	4-Methylsufonyl-2,2′,3,3′,4′,6-hexachlorobiphenyl
	3-MeSO$_2$-PCB141	3-Methylsufonyl-2,5,2',3',4',5'-hexachlorobiphenyl
	4-MeSO$_2$-PCB141	4-Methylsufonyl-2,5,2',3',4',5'-hexachlorobiphenyl
	3-MeSO$_2$-PCB149	3-Methylsufonyl-2,2′,4′,5,5′,6-hexachlorobiphenyl
	4-MeSO$_2$-PCB149	4-Methylsufonyl-2,2′,3,4′,5′,6-hexachlorobiphenyl
	3-MeSO$_2$-PCB174	3-Methylsufonyl-2,2′,3′,4′,5,5′,6-heptachlorobiphenyl
	4-MeSO$_2$-PCB174	4-Methylsufonyl-2,2′,3,3′,4′,5′,6-heptachlorobiphenyl
HPCs	PCP	Pentachlorophenol
	4-OH-HpCS	4-hydroxyheptachlorostyrene
	4-OH-PCB 107	2,3,3’,4’,5-pentachloro-4-biphenylol
	3-OH-PCB 153	2,2’,4,4’,5,5’- hexachloro-3-biphenylol
	4-OH-PCB 146	2,2’,3,4’,5,5’- hexachloro-4-biphenylol
	3-OH-PCB138	2,2’,3’,4,4’,5- hexachloro-3-biphenylol
	4-OH-PCB130	2,2',3,3',4',5-hexachloro-4-biphenylol
	4-OH-PCB 163	2,3,3',4',5,6-hexachloro-4-biphenylol
	4-OH-PCB 187	2,2’,3,4’,5,5’,6-heptachloro-4-biphenylol
	4-OH-PCB172	2,2',3,3',4',5,5'-heptachloro-4-biphenylol
	4'-OH-PCB 193	2,3,3',4',5,5',6-heptachloro-4-biphenylol
PBDEs	PBDE28	2',4,4'-Tribromodiphenyl ether
	PBDE47	2,2’,4,4’-Tetrabromodiphenyl ether
	PBDE99	2,2’,4,4’,5'-Pentabromodiphenyl ether
	PBDE100	2,2′,4,4′,6’-Penta-bromodiphenyl ether
	PBDE153	2,2’,4,4’,5,5’-Hexabromobiphenyl ether
	PBDE154	2,2',4,4',5,6'-Hexabromodiphenyl ether
	PBDE183	2,2',3',4,4',5',6'-Heptabromodiphenyl ether
PFASs	PFOSA	Perfluorooctane sulphonamide
	PFBS	Perfluorobutane sulfonate
	PFHxS	Perfluorohexane sulfonate
	br-PFOS	Branched perfluorooctane sulfonate
	lin-PFOS	Linear perfluorooctane sulfonate
	PFDcS	Perfluorodecanoic sulfonate
	PFHxA	Perfluorohexanoic acid
	PFHpA	Perfluoroheptanoic acid
	PFOA	Perfluorooctanoic acid
	PFNA	Perfluorononanoic acid
	PFDcA	Perfluorodecanoic acid
	PFUnA	Perfluoroundecanoic acid
	PFDoA	Perfluorododecanoic acid
	PFTriA	Perfluorotridecanoic acid
	PFTeA	Perfluorotetradecanoic acid

Appendix E

Mean concentrations (ng/g ww) and standard error of compounds detected in 60 % or more of the samples. ND denotes not detected in 60 % or more of the samples.

Contaminant	GG (n=5)			KW (n=11)			CE (n=17)		
(ng/g ww)	mean		SE	mean		SE	mean		SE
HCB	9.65	±	0.39a	9.71	±	0.21a	5.00	±	0.37b
b-HCH	1.99	±	0.24a	2.81	±	0.24a	0.37	±	0.04b
oxy-chlordane	29.04	±	5.43	24.09	±	3.22	2.11	±	0.31
c-chlordane	0.13	±	0.05	0.21	±	0.03	0.06	±	0.01
t-Nonachlor	4.61	±	1.32	4.64	±	0.92	1.55	±	0.34
c-Nonachlor	4.76	±	0.94	2.88	±	0.42	0.62	±	0.12
Mirex	7.98	±	1.37	9.55	±	1.31	0.40	±	0.04
p,p'-DDD	0.69	±	0.07	0.51	±	0.08	0.30	±	0.03
p,p'-DDE	179.43	±	27.08	74.41	±	7.59	10.11	±	1.49
ΣOCPs	**238.27**	**±**	**36.03**	**128.82**	**±**	**10.83**	**20.54**	**±**	**2.61**
PCB 28	2.73	±	0.48	3.26	±	0.42	0.40	±	0.06
PCB 52	2.30	±	0.46	0.15	±	0.03	ND		
PCB 99	30.49	±	4.76	25.41	±	2.96	0.96	±	0.14
PCB 101	5.73	±	1.50	ND			ND		
PCB 105	12.13	±	1.79	8.21	±	0.90	0.57	±	0.06
PCB 118	41.91	±	6.74	34.40	±	3.19	1.91	±	0.22
PCB 138	101.39	±	15.67	116.03	±	11.92	3.64	±	0.48
PCB 153	166.43	±	27.87	157.49	±	15.80	6.27	±	0.74
PCB 180	66.71	±	13.54	61.05	±	6.88	1.12	±	0.19
PCB 183	11.46	±	2.04	11.74	±	1.28	0.37	±	0.07
PCB 187	19.90	±	3.18	23.44	±	2.05	1.09	±	0.16
PCB 194	6.41	±	1.51	5.29	±	0.64	ND		
ΣPCB	**467.60**	**±**	**78.79**	**446.47**	**±**	**44.83**	**16.35**	**±**	**2.02**
4-$MeSO_2$-PCB 91	ND			0.02	±	0.003	ND		
3-$MeSO_2$-PCB 101	0.21	±	0.04	0.24	±	0.09	0.02	±	0.003
4-$MeSO_2$-PCB 101	0.10	±	0.02	0.12	±	0.03	0.02	±	0.003
3-$MeSO_2$-DDE	0.07	±	0.01	0.90	±	0.05	ND		
3-$MeSO_2$-PCB 87	0.07	±	0.02	0.08	±	0.03	ND		
4-$MeSO_2$-PCB 110	0.05	±	0.01	0.07	±	0.01	0.02	±	0.002
3-$MeSO_2$-PCB 149	0.02	±	0.01	0.05	±	0.01	ND		
4-$MeSO_2$-PCB 149	0.07	±	0.01	0.10	±	0.01	ND		
4-$MeSO_2$-PCB 132	ND			0.05	±	0.004	ND		
3-$MeSO_2$-PCB 141	ND			0.01	±	0.004	ND		
4-$MeSO_2$-PCB 141	0.03	±	0.01	0.03	±	0.004	ND		
4-$MeSO_2$-PCB 174	ND			0.01	±	0.002	ND		
Σ$MeSO_2$-PCBs/DDE	**0.62**	**±**	**0.11**	**1.67**	**±**	**0.16**	**0.05**	**±**	**0.01**

Contaminant	GG (n=5)			KW (n=11)			CE (n=17)		
(ng/g ww)	**mean**		**SE**	**mean**		**SE**	**mean**		**SE**
PCP	0.18	±	0.07	0.31	±	0.15	0.17	±	0.01
3-OH-PCB 153	0.01	±	0.00	0.03	±	0.002	0.02	±	0.002
4-OH-PCB 187	0.04	±	0.01	0.22	±	0.10	0.03	±	0.004
4'-OH-PCB 193	0.005	±	0.002	ND			ND		
ΣHPC	**0.25**	**±**	**0.08**	**0.57**	**±**	**0.21**	**0.22**	**±**	**0.01**
PBDE 28	ND			0.27	±	0.04	ND		
PBDE 47	7.39	±	0.91	5.48	±	0.44	ND		
PBDE 99	0.46	±	0.02	1.03	±	0.11	ND		
PBDE 100	1.31	±	0.17	0.71	±	0.07	ND		
PBDE 153	0.66	±	0.08	0.77	±	0.08	ND		
PBDE 154	0.85	±	0.12	0.63	±	0.07	ND		
ΣPBDE	**10.67**	**±**	**1.26**	**8.89**	**±**	**0.77**	**ND**		
PFHxS	0.10	±	0.02	ND			0.55	±	0.17
br-PFoS	0.66	±	0.11	0.13	±	0.05	0.61	±	0.21
linPFOS	4.46	±	0.32	6.04	±	0.94	3.84	±	0.77
PFOA	0.51	±	0.06	0.06	±	0.03	0.29	±	0.06
PFNA	0.84	±	0.07	0.47	±	0.05	0.53	±	0.07
PFDcA	0.42	±	0.05	0.80	±	0.14	0.20	±	0.03
PFUnA	0.97	±	0.13	3.69	±	0.65	0.64	±	0.08
PFDoA	0.21	±	0.04	1.13	±	0.19	0.17	±	0.02
PFTriA	1.17	±	0.17	7.97	±	1.22	0.88	±	0.09
PFTeA	0.22	±	0.03	1.90	±	0.30	0.20	±	0.02
ΣPFAS	**9.57**	**±**	**0.72**	**22.19**	**±**	**3.29**	**7.91**	**±**	**1.18**
ΣOHC	**727,36**			**608,61**			**45,07**		

Appendix F

F.1. DNA-fraction of total DNA that migrated (DNA-FTM (%)) and Median molecular length (MML (Kbp)) in eggs from glaucous gull (GG), kittiwake (KW) and common eider (CE) collected at Svalbard 2015 presented as mean ± SE, median and range.

DNA-FTM (%)	**Mean ± SE**	**Median**	**Range**
GG (n=5)	54.72 ± 7.59	61.22	26.47 - 68.72
KW (n=11)	53.09 ± 4.50	49.16	30.86 - 74.49
CE (n=17)	56.57 ± 4.18	57.62	34.02 - 80.83
MML (Kbp)			
GG (n=5)	277.86 ± 25.40	287.4	187.61 - 343.74
KW (n=11)	288.95 ± 16.17	297.2	207.78 - 369.91
CE (n=17)	280.42 ± 16.95	280.5	183.18 - 398.10

F.2 Median concentrations (ng/g ww) and range of compounds detected in 60 % or more of the samples. ND denotes not detected in 60 % or more of the samples.

Contaminant	**GG (n=5)**		**KW (n=11)**		**CE (n=17)**	
(ng/g ww)	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
HCB	9.65	8.531-10.595	9.66	8.418-10.817	4.44	3.255-8.070
b-HCH	2.03	1.341-2.746	2.73	1.837-4.488	0.32	0.077-0.641
oxy-chlordane	30.40	13.993-43.588	21.22	12.671-47.666	1.55	0.844-4.488
c-chlordane	0.19	0.037-0.266	0.21	0.043-0.396	0.05	0.036-0.108
t-Nonachlor	3.40	1.797-9.476	3.82	0.367-9.405	0.99	0.544-6.028
c-Nonachlor	4.76	3.037-7.961	2.73	0.392-5.225	0.42	0.194-2.008
Mirex	8.49	4.279-11.211	7.96	5.903-20.394	2.01	0.229-0.902
p,p'-DDD	0.72	0.452-0.852	0.47	0.139-0.995	0.30	0.079-0.618
p,p'-DDE	183.07	95.482-237.99	75.49	41.438-131.04	7.17	4.287-24.623
ΣOCPs	**239.96**	**130.75-316.61**	**127.86**	**80.981-192.11**	**15.46**	**10.164-45.800**
PCB 28	2.23	1.842-4.262	2.91	2.098-6.766	0.29	0.174-0.933
PCB 52	1.89	1.173-3.811	0.12	0.036-0.420	ND	
PCB 99	32.98	16.023-40.515	23.07	15.139-44.348	0.70	0.342-2.206
PCB 101	6.14	1.363-8.969	ND		ND	
PCB 105	12.82	6.649-16.187	7.83	5.044-13.737	2.21	0.285-1.073
PCB 118	44.53	21.842-57.383	33.15	22.187-49.688	1.52	0.755-3.592
PCB 138	107.20	54.748-135.97	105.67	70.493-78.930	2.91	1.482-8.128
PCB 153	182.15	82.10-231.331	149.27	96.099-249.56	5.03	2.944-13.524
PCB 180	62.53	31.689-103.34	52.46	34.450-103.87	0.91	0.403-3.875
PCB 183	103.34	5.854-17.168	10.01	7.077-18.119	0.29	0.041-1.222
PCB 187	20.88	9.766-28.597	24.20	14.426-35.517	0.87	0.417-2.973
PCB 194	5.18	3.031-10.152	4.41	2.691-8.764	ND	
ΣPCB	**490.02**	**236.12-651.86**	**415.74**	**274.94-687.51**	**12.61**	**7.503-35.632**

Contaminant		GG (n=5)	KW (n=11)		CE (n=17)	
(ng/g ww)	Median	Range	Median	Range	Median	Range
4MeSO$_2$-PCB 91	ND		0.02	0.012-0.046	ND	
3MeSO$_2$-PCB 101	0.18	0.135-0.376	0.14	0.088-1.0005	0.01	0.001-0.044
4MeSO$_2$-PCB 101	0.07	0.066-0.189	0.12	0.067-0.348	0.02	0.008-0.030
3MeSO$_2$-DDE	0.06	0.049-0.107	0.85	0.681-1.169	ND	
3MeSO$_2$-PCB 87	0.06	0.005-0.120	0.05	0.002-0.047	ND	
4MeSO$_2$-PCB 110	0.05	0.029-0.076	0.07	0.057-0.100	0.01	0.002-0.035
3MeSO$_2$-PCB 149	0.02	0.003-0.038	0.05	0.021-0.076	ND	
4MeSO$_2$-PCB 149	0.05	0.044-0.105	0.10	0.068-0.157	ND	
4MeSO$_2$-PCB 132	ND		0.04	0.035-0.074	ND	
3MeSO$_2$-PCB 141	ND		0.01	0.002-0.047	ND	
4MeSO$_2$-PCB 141	0.04	0.002-0.049	0.03	0.0002-0.043	ND	
4MeSO$_2$-PCB 174	ND		0.01	0.005-0.027	ND	
ΣMeSO$_2$-PCBs/DDE	**0.49**	**0.433-1.031**	**1.601**	**1.127-2.932**	**0.04**	**0.018-0.109**
PCP	0.10	0.089-0.438	0.13	0.004-1.636	0.15	0.101-0.236
3-OH-PCB 153	0.02	0.003-0.025	0.03	0.023-0.043	0.02	0.001-0.040
4-OH-PCB 187	0.03	0.019-0.099	0.13	0.069-1.184	0.02	0.010-0.078
4'-OH-PCB 193	0.004	0.001-0.008	ND		ND	
ΣHPC	**0.16**	**0.127-0.544**	**0.29**	**0.164-2.063**	**0.21**	**0.168-0.297**
PBDE 28	ND		0.24	0.121-0.614	ND	
PBDE 47	7.77	4.437-9.385	5.11	3.809-8.030	ND	
PBDE 99	0.43	0.420-0.543	0.88	0.539-1.917	ND	
PBDE 100	1.39	0.741-1.732	0.69	0.463-1.153	ND	
PBDE 153	0.55	0.483-0.877	0.73	0.497-1.467	ND	
PBDE 154	1.006	0.454-1.075	0.58	0.388-1.187	ND	
ΣPBDE	**11.66**	**6.535-13.204**	**8.18**	**6.048-14.175**	**ND**	
PFHxS	0.09	0.054-0.175	ND		0.40	0.065-3.079
br-PFoS	0.61	0.453-1.099	0.05	0.005-0.452	0.43	0.002-3.498
linPFOS	4.59	3.244-5.176	5.20	3.120-13.088	3.22	1.214-14.265
PFOA	0.48	0.402-0.744	0.03	0.011-0.342	0.23	0.010-0.995
PFNA	0.83	0.627-1.021	0.39	0.257-0.773	0.41	0.164-1.125
PFDcA	0.39	0.298-0.574	0.68	0.171-1.896	0.15	0.062-0.473
PFUnA	0.81	0.695-1.365	3.10	0.469-8.275	0.59	0.264-1.474
PFDoA	0.21	0.087-0.314	1.07	0.086-2.438	0.17	0.021-0.362
PFTriA	1.20	0.749-1.709	7.08	0.723-14.542	0.78	0.425-1.534
PFTeA	0.22	0.137-0.326	1.66	0.186-3.540	0.17	0.082-0.361
ΣPFAS	**11.26**	**7.867-11.852**	**19.09**	**6.817-45.327**	**7.14**	**2.632-22.975**

Appendix G

Detection frequency (DF) and Limit of detection (LOD ng/g ww) for OHCs analysed in eggs from glaucous gull, kittiwake and common eider from Kongsfjorden, Svalbard in June 2015. Compounds denoted with * was detected in less than 60% of the samples for all three species.

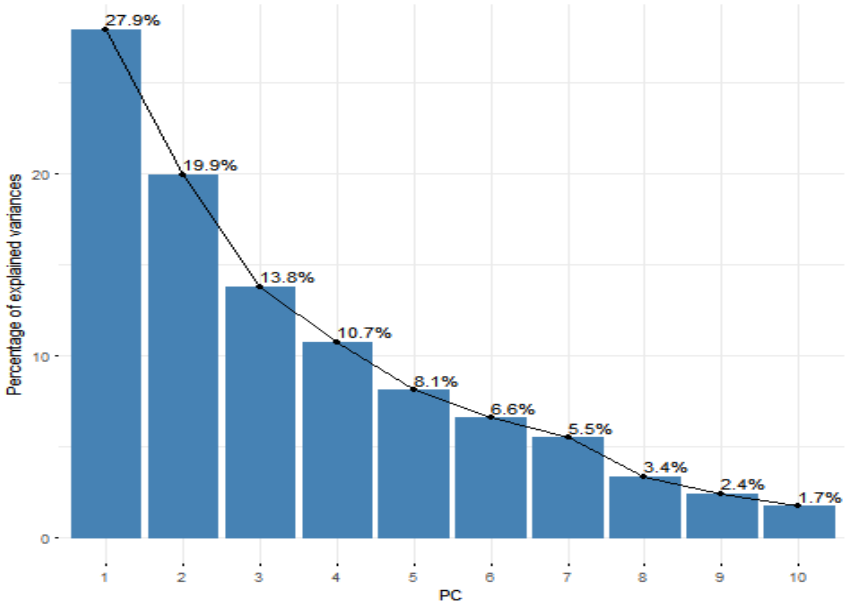
Compound	Glaucous G	Kittiwake	Common E.	Glaucous G.	Kittiwake	Common e.
	DF	DF	DF	LOD	LOD	LOD
HCB	1	1	1	0.037	0.037	0.037
α-HCH*	ND	ND	ND	0.155	0.160	0.107
β-HCH	1	1	0.8	0.215	0.176	0.201
γ-HCH *	ND	ND	ND	0.042	0.045	0.031
Heptachlor*	ND	ND	ND	0.080	0.076	0.027
oxy-chlordane	1	1	1	0.116	0.116	0.116
t-chlordane*	ND	ND	ND	0.014	0.014	0.007
c-chlordane	1	1	1	0.021	0.020	0.009
t-nonachlor	1	1	1	0.012	0.012	0.007
c-nonachlor	1	1	1	0.007	0.008	0.004
mirex	1	1	1	0.142	0.124	0.056
o,p'-DDT *	ND	ND	ND	0.643	0.477	0.407
p,p'-DDT *	0.4	0.09	0.29	0.665	0.495	0.421
o,p'-DDD*	ND	ND	ND	0.328	0.353	0.195
p,p'-DDD	1	0.82	0.82	0.345	0.298	0.205
o,p'-DDE*	ND	ND	ND	0.238	0.151	0.152
p,p'-DDE	1	1	1	0.322	0.205	0.206
PCB 28	1	1	1	0.113	0.064	0.054
PCB 52	1	0.91	0.24	0.079	0.064	0.062
PCB 99	1	1	1	0.195	0.164	0.132
PCB 101	1	0.27	ND	0.244	0.207	0.167
PCB 105	1	1	1	0.207	0.174	0.148
PCB 118	1	1	1	0.158	0.165	0.135
PCB 139	1	1	1	0.320	0.274	0.272
PCB 153	1	1	1	0.244	0.165	0.187
PCB 180	1	1	1	0.305	0.213	0.308
PCB 183	1	1	0.88	0.210	0.154	0.219
PCB 187	1	1	1	0.232	0.192	0.261
PCB 194	1	1	0.06	0.638	0.405	0.464
3MeSO2-PCB52*	ND	ND	ND	0.009	0.009	0.009
3MeSO2-PCB49 *	ND	0.30	ND	0.014	0.014	0.014
4MeSO2-PCB52 *	ND	ND	ND	0.006	0.006	0.006
4MeSO2-PCB49*	ND	0.20	ND	0.006	0.006	0.006
3MeSOPCB91*	ND	ND	ND	0.006	0.006	0.006
4-$MeSO_2$-PCB91	ND	1	ND	0.010	0.010	0.010

Compound	Glaucous G	Kittiwake	Common E.	Glaucous G.	Kittiwake	Common e.
	DF	DF	DF	LOD	LOD	LOD
3-MeSO2-PCB101	1	1	0.59	0.013	0.013	0.013
4-MeSO2-PCB101	1	1	1	0.006	0.006	0.006
3-MeSO$_2$-DDE	1	1	ND	0.022	0.022	0.022
3-MeSO$_2$-PCB87	0.80	0.90	ND	0.014	0.014	0.014
3MeSOPCB110*	ND	ND	ND	0.011	0.011	0.011
4MeSOPCB110	1	1	0.76	0.009	0.009	0.009
3-MeSO$_2$-PCB149	0.6	1	0.41	0.009	0.009	0.009
4-MeSO$_2$-PCB149	1	1	0.29	0.009	0.009	0.009
3MeSOPCB132*	ND	ND	ND	0.005	0.005	0.005
4MeSOPCB132	0.20	1	0.18	0.010	0.010	0.010
3MeSOPCB141	ND	0.6	ND	0.008	0.008	0.008
4-MeSO$_2$-PCB 141	0.8	0.9	0.06	0.008	0.008	0.008
3MeSOPCB174*	ND	ND	ND	0.005	0.005	0.005
4MeSOPCB174	ND	0.7	ND	0.006	0.006	0.006
PCP	1	0.73	1	0.088	0.088	0.088
4-OH-HpCS *	ND	0.09	ND	0.0008	0.0008	0.0008
4-OH-PCB 107*	ND	0.18	0.12	0.018	0.018	0.018
3-OH-PCB 153	0.6	1	0.94	0.039	0.039	0.039
4-OH-PCB 146*	ND	ND	ND	0.006	0.006	0.006
3-OH-PCB 138*	ND	0.27	ND	0.013	0.013	0.013
4-OH-PCB 130*	ND	ND	ND	0.014	0.014	0.014
4-OH-PCB 163*	0.40	ND	0.53	0.004	0.004	0.004
4-OH-PCB 187	1	1	1	0.003	0.003	0.003
4-OH-PCB 172*	ND	ND	ND	0.012	0.012	0.012
4'-OH-PCB 193	0.6	0.18	ND	0.002	0.002	0.002
PBDE 28	0.20	1	0.12	0.031	0.031	0.031
PBDE47	1	1	0.29	0.013	0.013	0.013
PBDE99	1	1	0.06	0.073	0.073	0.073
PBDE100	1	1	0.47	0.098	0.098	0.098
PBDE153	1	1	0.18	0.026	0.026	0.026
PBDE154	1	1	0.18	0.026	0.026	0.026
PBDE183*	ND	ND	ND	0.409	0.409	0.409
PFOSA *	ND	0.09	0.06	0.025	0.025	0.025
PFBS*	ND	ND	ND	0.025	0.025	0.025
PFHxS	1	0.18	1	0.025	0.025	0.025
br-PFOS	1	0.45	0.71	0.050	0.050	0.050
lin-PFOS	1	1	0.94	0.050	0.050	0.050
PFDcS *	ND	ND	ND	0.050	0.050	0.050
PFHxA	ND	ND	ND	0.030	0.030	0.030

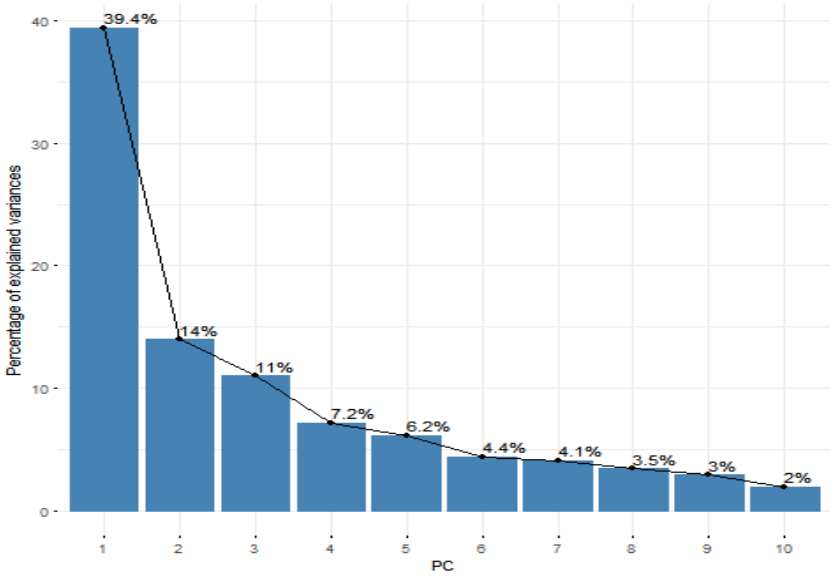
Compound	Glaucous G	Kittiwake	Common E.	Glaucous G.	Kittiwake	Common e.
	DF	DF	DF	LOD	LOD	LOD
PFHpA*	ND	ND	ND	0.030	0.030	0.030
PFOA	1	1	1	0.010	0.010	0.010
PFNA	1	1	1	0.100	0.100	0.100
PFDcA	1	1	1	0.050	0.050	0.050
PFUnA	1	1	1	0.050	0.050	0.050
PFDoA	1	1	1	0.050	0.050	0.050
PFTriA	1	1	1	0.050	0.050	0.050
PFTeA	1	1	1	0.070	0.070	0.070

Appendix H

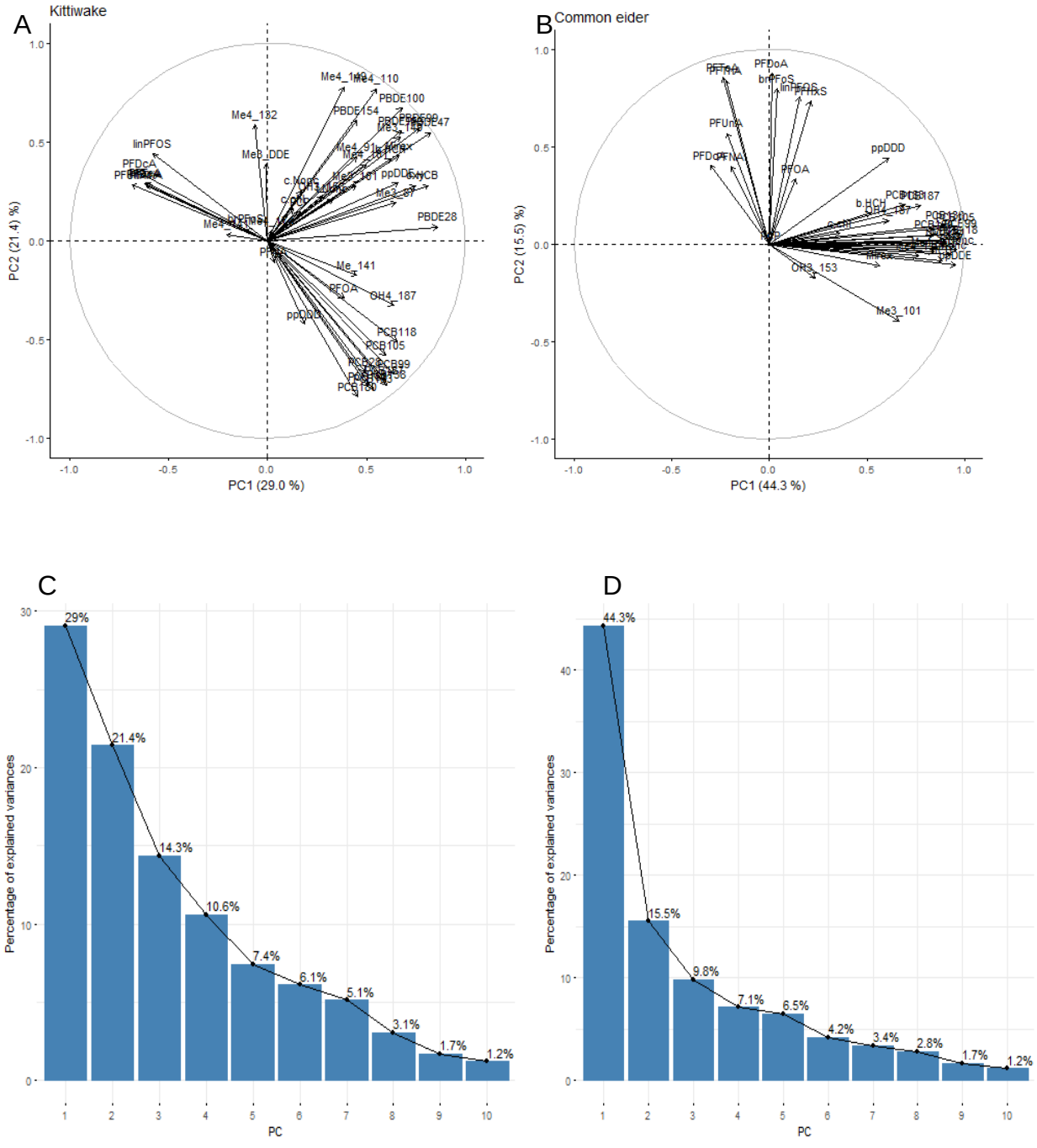
H.1. Contribution (%) of each PC to the total variance in the dataset for the PCA including all variables for kittiwake.



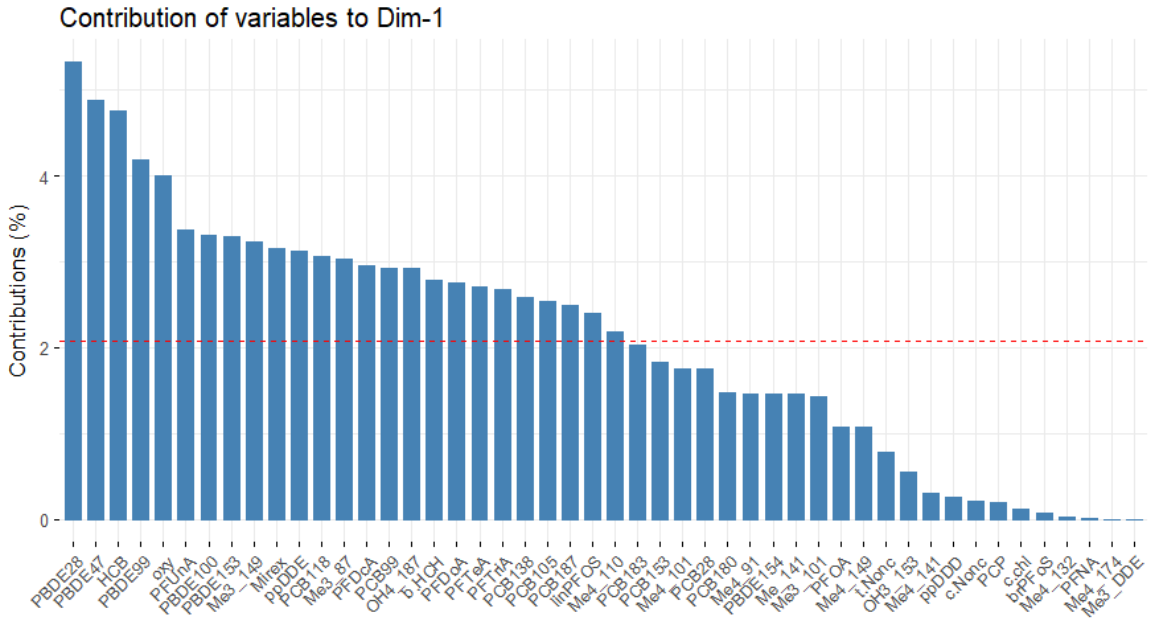
H.2. Contribution (%) of each PC to the total variance in the dataset for the PCA including all variables for common eider.



H.3. Loading plot for the PCA with only contaminants for a) kittiwake and b) common eider, and screeplot showing the contribution (%) of each PC to the total variance in the dataset for c) kittiwake and d) common eider.



H.4. The contaminants contribution to PC1 for Kittiwake with only contaminants in the PCA plot.



Appendix I

I.1. Variance inflation factor (VIF) for the predictor variables in multiple regression for kittiwake. All chemicals were ln transformed.

	OCP	PCB	MeSO2	HPC	PFAS	PBDE	Lipid	Age
Kittiwake	5.921	5.079	1.958	2.217	3.184	5.458	5.170	2.417
Common eider	30.003	8.349	10.812	1.413	1.273	-	1.275	2.269

I.2. Candidate models for multiple regression analysis. All chemicals were ln transformed

Model1 <- lm(MML/FTM ~ OCP)

Mode[2 <- lm(MML/FTM ~ PCB)

Model3 <- lm(MML/FTM ~ MeSO2-PCBs/DDE)

Model4 <- lm(MML/FTM ~ HPC)

Model5 <- lm(MML/FTM ~ PFAS)

Model6 <- lm(MML/FTM ~ Age)

Model7 <- lm(MML/FTM ~ Lipid)

Model8 <- lm(MML/FTM ~ OCP + Age)

Model9 <- lm(MML/FTM ~ PCB + Age)

Model10 <- lm(MML/FTM ~ MeSO2-PCBs/DDE + Age)

Model11 <- lm(MML/FTM ~ OH_PCB + Age)

Model12 <- lm(MML/FTM ~ PFAS + Age)

Model13 <- lm(MML/FTM ~ OCP + MeSO2-PCBs/DDE)

Model14 <- lm(MML/FTM ~ OCP + OH_PCB)

Model15 <- lm(MML/FTM ~ OCP+ PFAS)

Model16 <- lm(MML/FTM ~ OCP + PCB)

Model17 <- lm(MML/FTM ~ PCB+ MeSO2-PCBs/DDE)

Model18 <- lm(MML/FTM ~ PCB + HPC)

Model19 <- lm(MML/FTM ~ PCB + PFAS)

Model20 <- lm(MML/FTM ~ MeSO2-PCBs/DDE + HPC)

Model21 <- lm(MML/FTM ~ MeSO2-PCBs/DDE + PFAS)

Model22 <- lm(MML/FTM ~ HPC + PFAS)

Model23 <- lm(MML/FTM ~ OCP + Lipid)

Model24 <- lm(MML/FTM ~ PCB + Lipid)

Model25 <- lm(MML/FTM ~ MeSO2-PCBs/DDE + Lipid)

Model26 <- lm(MML/FTM ~ OH_PCB + Lipid)

Model27 <- lm(MML/FTM ~ PFAS + Lipid)

Model28 <- lm(MML/FTM ~ Age + Lipid)

Model29 <- lm(MML/FTM ~ OCP + PCB+ MeSO2-PCBs/DDE + HPC + PFAS + PBDE + Lipid + Age)

Model30 <- lm(MML/FTM ~ OCP + PCB + MeSO2-PCBs/DDE + HPC + PFAS + PBDE)

Candidate models with PBDE, only for kittiwake:

Model31 <- lm(MML/FTM ~ PBDE)

Model32 <- lm(MML/FTM ~ PBDE + Age)

Model33 <- lm(MML/FTM ~ OCP+ PBDE)

Model34 <- lm(MML/FTM ~ PCB + PBDE)

Model35 <- lm(MML/FTM ~ MeSO2-PCBs/DDE + PBDE)

Model36 <- lm(MML/FTM ~ HPC + PBDE)

Model37 <- lm(MML/FTM ~ PFAS + PBDE)

Model38 <- lm(MML/FTM ~ PBDE + Lipid)

Appendix J

J.1. Linear regression for kittiwake with DNA-FTM as response variable. Linear regression parameter estimates and model statistics for the top-ranked models based on the second-order Akaike`s Information Criterion, explaining variation in FTM (Kbp) in kittiwake (n=11) are presented. All contaminants were transformed by natural logarithm (ln ng/g ww) to meet the assumption for the model. Significant models and parameters are denoted with *.

Model 1	**FTM ~ $MeSO_2$-PCBs/DDE**		**Adj R2 = 0.03**	***p* = 0.314**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	62.205	9.643	6.45	0.000*
MeSO2-PCB	-19.371	18.146	-1.07	0.314
Model 2	**FTM ~ PBDE**		**Adj R2 = -0.01**	***p* = 0.379**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	87.58	37.56	2.33	0.044*
PBDE	-16.04	17.34	-0.93	0.379
Model 3	**FTM~ OCP**		**Adj R2 = -0.04**	***p* = 0.449**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	116.07	79.73	1.46	0.179
OCP	-13.06	16.51	-0.79	0.449
Model 4	**FTM~ PFAS**		**Adj R2 = -0.05**	***p* = 0.519**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	33.981	28.811	1.18	0.268
PFAS	6.333	9.426	0.67	0.519
Model 5	**FTM ~ HPC**		**Adj R2 = -0.07**	***p* = 0.572**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	49.784	7.310	6.81	0.000
HPC	-3.320	5.662	-0.59	0.572
Model 6	**FTM~ PCB**		**Adj R2 = -0.07**	***p* = 0.607**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	6.027	88.335	0.067	0.947
PCB	7.778	14.580	0.53	0.607
Model 7	**FTM~ Age**		**Adj R2 = -0.10**	***p* = 0.790**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	57.179	15.685	3.65	0.005*
Age	-1.501	5.483	-0.27	0.790
Model 8	**FTM~ Lipid**		**Adj R2 = -0.11**	***p* = 0.843**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	50.2662	14.651	3.43	0.008
Lipid	0.3813	1.875	0.20	0.843

J.2. Linear regression for common eider with MML as response variable. Linear regression parameter estimates and model statistics for the top-ranked models based on the second-order Akaike`s Information Criterion, explaining variation in MML (Kbp) in common eider (n=17) are presented. All contaminants were transformed by natural logarithm (ln ng/g ww) to meet the assumption for the model. Significant models and parameters are denoted with *.

Model 1	**MML ~ HPC**		**Adj R2 = 0.09**	***p* = 0.131**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	508.89	143.98	3.53	0.003*
HPC	147.47	92.35	1.59	0.131
Model 2	**MML ~ Age**		**Adj R2 = 0.09**	***p* = 0.133**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	229.84	35.68	6.44	0.000*
Age	23.88	15.02	1.59	0.133
Model 3	**MML~ HPC + Age**		**Adj R2 = 0.18**	***p* = 0.092**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	486.49	136.70	3.56	0.003*
HPC	151.51	87.29	1.74	0.105
Age	47.10	28.13	1.67	0.116
Model 4	**MML~ OCP**		**Adj R2 = 0.06**	***p* = 0.175**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	129.38	107.31	1.21	0.247
OCP	51.87	36.42	1.42	0.175
Model 5	**MML ~ PCB**		**Adj R2 = 0.007**	***p* = 0.309**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	168.07	107.99	1.56	0.140
PCB	41.70	39.59	1.05	0.309
Model 6	**MML~ MeSO$_2$-PCBs/DDE**		**Adj R2 = -0.004**	***p* = 0.348**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	337.46	101.62	3.74	0.002*
Meso2	30.91	31.92	0.97	0.348
Model 7	**MML~ Lipid**		**Adj R2 = -0.01**	***p* = 0.409**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	212.956	81.262	2.62	0.019*
Lipid	3.241	3.816	0.85	0.409
Model 8	**MML~ PFAS**		**Adj R2 = -0.02**	***p* = 0.451**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	328.14	63.97	5.13	0.000*
PFAS	-24.84	32.08	-0.77	0.451

J.3. Linear regression for common eider with DNA-FTM as response variable. Linear regression parameter estimates and model statistics for the top-ranked models based on the second-order Akaike`s Information Criterion, explaining variation in MML (Kbp) in common eider (n=17) are presented. All contaminants were transformed by natural logarithm (ln ng/g ww) to meet the assumption for the model. Significant models and parameters are denoted with *.

Model 1	**FTM ~ PCB**		**Adj R2 = 0.04**	***p* = 0.206**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	90.60	26.10	3.47	0.003
PCB	-12.63	9.56	-1.32	0.206
Model 2	**FTM ~ Lipid**		**Adj R2 = 0.02**	***p* = 0.250**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	79.48	19.58	4.06	0.001*
Lipid	-1.10	0.92	-1.20	0.250
Model 3	**FTM ~ PFAS**		**Adj R2 = -0.02**	***p* = 0.463**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	45.124	15.775	2.86	0.012*
PFAS	5.959	7.912	0.73	0.463
Model 4	**FTM ~ OCP**		**Adj R2 = -0.03**	***p* = 0.499**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	75.575	27.724	2.73	0.016*
OCP	-6.527	9.408	-0.69	0.499
Model 5	**FTM ~ Age**		**Adj R2 = -0.04**	***p* = 0.539**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	61.824	9.380	6.59	0.000*
Age	-2.481	3.947	-0.63	0.539
Model 6	**FTM ~ HPC**		**Adj R2 = -0.06**	***p* = 0.724**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	42.92	38.20	1.12	0.279
HPC	-8.81	24.50	-0.36	0.724
Model 7	**FTM ~ $MeSO_2$-PCBs/DDE**		**Adj R2 = -0.07**	***p* = 0.885**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	52.830	25.786	2.05	0,058
$MeSO_2$-PCBs/DDE	-1.191	8.098	-0.15	0.885
Model 8	**FTM ~ PCB + $MeSO_2$-PCBs/DDE**		**Adj R2 = 0.07**	***p* = 0.244**
Parameter:	Estimate:	SE:	t value	*p:*
Intercept	160.86	65.95	2.44	0.029
PCB	-23.98	13.62	-1.76	0.100
$MeSO_2$-PCBs/DDE	12.64	10.92	1.59	0.266

Appendix K

K.1. Individual biological measurements, location, MML, DNA-FTM levels and Coefficient of variation (CV, %)

Species	Id	Open (day)	Age (day)	MML (Kbp)	CV MML	FTM (%)	CV FTM	Volume (Cm3)	Lipid (%)
GG	GG02	2	4	343.74	4.26	26.47	9.17	114.29	9.96
GG	GG05	2	1	295.61	14.11	61.22	8.18	110.11	9.86
GG	GG10	3	3	287.39	13.54	52.11	4.96	131.86	11.70
GG	GG11	3	3	274.97	19.35	65.08	5.68	123.37	11.79
GG	GG13	1	2	187.61	23.16	68.73	7.88	105.49	8.97
KW	KW01	2	3	339.03	10.34	44.78	11.76	48.08	4.86
KW	KW02	2	4	306.13	26.11	41.60	17.40	47.90	6.88
KW	KW03	2	4	306.35	11.24	51.27	19.93	53.11	2.42
KW	KW04	2	3	259.64	2.17	74.48	6.87	47.92	7.49
KW	KW05	2	3	270.84	3.11	40.13	11.11	42.92	7.79
KW	KW07	2	2	226.99	42.32	66.99	12.97	47.68	8.95
KW	KW09	2	3	297.25	3.22	72.66	3.09	47.59	8.30
KW	KW10	2	2	236.85	8.53	68.24	8.68	50.19	9.59
KW	KW16	2	1	207.78	19.5	49.16	50.48	46.45	4.08
KW	KW17	2	2	369.92	6.11	30.86	5.34	39.99	10.05
KW	KW20	2	3	357.62	19.38	43.75	0.89	47.64	10.92
CE	CE01	1	3	316.03	13.94	34.35	10.91	99.59	33.87
CE	CE02	1	1	265.36	10.36	34.97	5.52	110.34	21.89
CE	CE05	5	3	211.81	11.89	80.33	0.07	99.43	16.76
CE	CE06	5	1	280.54	2.57	34.11	1.74	104.27	15.31
CE	CE07	5	4	329.18	14.46	41.48	11.88	91.86	21.82
CE	CE14	5	3	183.18	4.04	80.76	0.48	102.51	22.35
CE	CE16	2	1	323.80	2.52	69.31	5.05	100.93	23.61
CE	CE20	4	3	239.38	5.01	67.86	13.27	93.56	21.55
CE	CE73	1	1	302.79	5.82	46.84	1.06	102.12	13.64
CE	CE74	1	1	188.73	20.23	65.46	16.20	95.02	23.62
CE	CE78	2	2	265.56	6.48	57.62	3.45	82.04	19.20
CE	CE79	2	2	300.99	0.12	68.51	1.46	98.36	17.94
CE	CE81	1	3	398.10	20.98	47.23	2.07	94.69	20.47
CE	CE82	1	1	192.29	6.96	65.91	10.62	95.31	20.02
CE	CE90	1	1	209.19	41.69	80.83	0.35	97.28	16.09
CE	CE91	1	4	394.38	1.43	34.02	3.69	98.85	25.06
CE	CE92	1	2	365.86	29.11	52.08	9.75	93.85	20.71

K.2. Individual contaminant concentrations for OCPs (ng/g ww)

Id	HCB	b-HCH	oxy	c-chl	t-Nonc	c-Nonc	Mirex	ppDDD	ppDDE
GG02	9.60	2.03	30.40	0.05	1.80	3.67	8.49	0.85	183.07
GG05	10.43	2.74	37.27	0.27	9.48	7.96	11.21	0.81	234.67
GG10	8.53	1.34	13.99	0.04	3.33	3.04	4.28	0.72	95.48
GG11	10.60	2.24	43.59	0.21	5.03	5.83	10.52	0.61	237.99
GG13	9.08	1.61	19.94	0.08	3.40	3.33	5.38	0.45	145.95
KW01	10.82	4.49	40.12	0.23	5.13	3.62	20.39	0.14	75.49
KW02	9.62	2.38	24.88	0.04	0.37	0.39	7.96	0.14	82.07
KW03	9.66	2.99	22.36	0.15	2.64	2.73	6.39	0.31	69.94
KW04	9.87	3.20	17.11	0.10	1.45	1.68	6.17	0.36	42.76
KW05	9.04	2.05	15.92	0.40	9.40	4.66	8.91	1.00	73.96
KW07	10.11	3.39	20.45	0.25	6.64	5.23	9.30	0.80	84.68
KW09	10.30	3.34	25.87	0.30	6.73	2.15	11.80	0.57	131.04
KW10	8.42	1.84	16.69	0.21	2.75	2.41	6.74	0.47	41.45
KW16	9.03	1.91	12.67	0.26	3.82	3.09	5.90	0.47	48.75
KW17	9.52	2.65	21.22	0.18	9.28	3.68	7.74	0.68	84.68
KW20	10.40	2.73	47.67	0.17	2.85	2.05	13.79	0.69	83.68
CE01	4.43	0.40	1.15	0.11	1.16	0.46	0.25	0.36	6.34
CE02	3.95	0.47	1.15	0.04	0.54	0.25	0.34	0.30	4.31
CE05	4.75	0.32	1.55	0.04	0.99	0.45	0.23	0.31	6.05
CE06	3.28	0.14	0.84	0.06	0.58	0.24	0.34	0.25	5.20
CE07	5.53	0.32	3.57	0.10	2.32	1.11	0.53	0.62	19.65
CE14	3.64	0.30	1.18	0.08	0.65	0.28	0.39	0.35	7.06
CE16	3.26	0.23	0.96	0.04	0.68	0.19	0.27	0.25	4.29
CE20	8.07	0.64	3.86	0.05	2.35	1.12	0.26	0.42	14.28
CE73	4.44	0.30	1.44	0.04	0.69	0.29	0.48	0.25	8.76
CE74	7.65	0.48	3.87	0.08	2.41	1.12	0.38	0.40	15.91
CE78	4.45	0.26	1.57	0.05	0.89	0.34	0.38	0.08	8.29
CE79	4.88	0.39	1.68	0.06	0.99	0.51	0.32	0.23	6.40
CE81	6.59	0.56	4.49	0.11	6.03	2.01	0.90	0.50	24.62
CE82	3.87	0.58	1.43	0.08	0.89	0.37	0.48	0.26	6.36
CE90	4.41	0.29	1.43	0.04	0.88	0.30	0.41	0.10	7.17
CE91	7.55	0.58	4.01	0.04	3.17	1.04	0.63	0.38	18.93
CE92	4.29	0.08	1.67	0.05	1.17	0.42	0.30	0.11	8.23

K.3. Individual contaminant concentrations for PCB congeners (ng/g ww)

Id	CB28	CB52	CB99	CB101	CB105	CB118	CB138	CB153	CB180	CB183	CB187	CB194
GG02	2.23	1.85	32.98	6.14	12.82	44.53	107.20	182.15	62.53	11.53	20.88	5.18
GG05	3.42	3.81	39.72	8.97	15.41	54.27	135.97	231.33	103.34	17.17	28.60	9.85
GG10	1.89	1.17	16.02	1.36	6.65	21.84	54.75	82.10	31.69	5.85	9.77	3.03
GG11	4.26	2.77	40.52	8.84	16.19	57.38	131.80	212.75	91.22	14.46	23.51	10.15
GG13	1.84	1.89	23.23	3.36	9.59	31.52	77.22	123.84	44.79	8.31	16.75	3.84
KW01	2.49	0.07	16.14	NA	5.46	23.25	70.49	96.10	36.42	7.08	14.43	3.01
KW02	2.99	0.04	20.39	NA	8.14	33.15	96.59	133.96	48.79	9.48	17.87	4.41
KW03	2.39	0.07	18.68	NA	6.14	25.44	76.42	100.95	34.45	7.13	16.63	2.69
KW04	6.77	0.05	44.35	NA	13.74	46.88	165.96	249.56	103.87	18.12	29.46	8.76
KW05	2.55	0.42	23.07	NA	6.43	29.00	129.77	171.75	77.55	15.20	28.05	8.10
KW07	2.90	0.20	23.42	NA	7.83	35.75	105.67	149.27	52.46	10.01	24.20	4.04
KW09	2.94	0.27	32.25	NA	12.42	49.69	158.49	212.15	80.15	15.53	28.61	6.57
KW10	2.34	0.12	16.30	NA	5.21	23.37	81.56	118.12	43.14	7.85	16.19	3.67
KW16	2.10	0.14	15.14	NA	5.04	22.19	77.33	102.15	42.04	7.74	20.17	3.75
KW17	3.38	0.15	31.30	NA	9.24	45.50	135.09	191.59	71.43	14.41	26.73	6.82
KW20	5.02	0.11	38.49	NA	10.69	44.21	178.93	206.83	81.19	16.60	35.52	6.38
CE01	0.25	NA	0.72	NA	0.47	1.52	3.08	4.75	0.71	0.24	0.70	NA
CE02	0.22	NA	0.51	NA	0.40	1.34	2.84	5.67	0.91	0.24	0.94	NA
CE05	0.41	NA	0.93	NA	0.73	2.06	3.77	4.68	0.92	0.35	1.09	NA
CE06	0.20	NA	0.50	NA	0.40	1.32	2.91	4.59	0.77	0.29	0.87	NA
CE07	0.49	NA	1.81	NA	0.97	3.59	8.13	12.57	3.88	1.22	2.97	NA
CE14	0.20	NA	0.62	NA	0.34	1.39	2.82	5.03	0.68	0.32	0.92	NA
CE16	0.17	NA	0.34	NA	0.29	0.76	1.76	2.94	0.40	0.21	0.64	NA
CE20	0.93	NA	1.43	NA	0.81	2.66	4.17	5.60	0.98	0.35	0.81	NA
CE73	0.23	NA	0.62	NA	0.39	1.52	3.38	6.68	0.82	0.35	1.13	NA
CE74	0.87	NA	1.70	NA	0.91	3.47	6.52	10.02	1.34	0.43	1.52	NA
CE78	0.29	NA	0.62	NA	0.39	1.56	2.67	5.05	0.94	0.22	0.86	NA
CE79	0.31	NA	0.70	NA	0.47	1.10	1.48	3.68	1.05	0.04	0.42	NA
CE81	0.74	NA	2.21	NA	1.07	3.50	7.49	13.52	1.91	0.86	2.42	NA
CE82	0.30	NA	0.67	NA	0.41	1.19	2.12	4.04	0.74	0.17	0.85	NA
CE90	0.19	NA	0.50	NA	0.36	1.28	2.28	4.67	0.79	0.25	0.67	NA
CE91	0.79	NA	1.72	NA	0.87	2.83	4.31	8.30	1.50	0.46	1.13	NA
CE92	0.24	NA	0.77	NA	0.45	1.40	2.23	4.80	0.70	0.24	0.67	NA

K.4. Individual contaminant concentrations for $MeSO_2$-PCBs/DDE and HCPs (ng/g ww)

Id	4M 91	3M 101	4M 101	3Me DDE	3Me 87	4M1 10	3Me 149	4M1 49	4Me 132	3M1 41	4Me 141	4Me 174	PCP	3OH 153	4OH 187	4OH 193
GG:																
02	NA	0.16	0.07	0.05	0.06	0.05	0.02	0.05	NA	NA	0.00	NA	0.09	0.03	0.03	0.01
05	NA	0.20	0.11	0.05	0.08	0.08	0.04	0.11	NA	NA	0.04	NA	0.09	0.01	0.02	0.00
10	NA	0.14	0.07	0.06	0.06	0.04	0.01	0.04	NA	NA	0.02	NA	0.21	0.02	0.03	0.01
11	NA	0.38	0.19	0.09	0.12	0.07	0.03	0.10	NA	NA	0.05	NA	0.44	0.00	0.10	0.00
13	NA	0.18	0.07	0.11	0.00	0.03	0.00	0.05	NA	NA	0.04	NA	0.10	0.00	0.03	0.00
KW:																
01	0.02	0.16	0.12	0.85	0.06	0.10	0.06	0.13	0.06	0.01	0.04	0.01	0.19	0.03	0.16	NA
02	0.02	0.13	0.08	1.03	0.04	0.07	0.04	0.10	0.05	0.00	0.03	0.01	1.64	0.03	0.16	NA
03	0.03	0.32	0.14	0.84	0.10	0.07	0.06	0.09	0.04	0.02	0.03	0.01	0.07	0.03	0.08	NA
04	0.01	0.10	0.07	0.68	0.03	0.06	0.02	0.07	0.03	0.01	0.03	0.01	0.10	0.03	0.12	NA
05	0.02	0.09	0.08	0.74	0.05	0.06	0.04	0.07	0.04	0.01	0.02	0.01	0.00	0.03	0.13	NA
07	0.02	0.15	0.10	1.00	0.05	0.09	0.05	0.16	0.06	0.00	0.03	0.03	0.22	0.04	0.07	NA
09	0.03	0.21	0.13	1.17	0.08	0.08	0.05	0.10	0.04	0.02	0.04	0.01	0.10	0.02	0.22	NA
10	0.02	0.09	0.07	1.00	0.00	0.06	0.02	0.08	0.04	0.00	0.02	0.01	0.13	0.02	0.10	NA
16	0.01	0.09	0.07	0.83	0.03	0.06	0.02	0.10	0.07	0.02	0.03	0.02	0.13	0.04	0.13	NA
17	0.05	1.00	0.35	0.84	0.33	0.09	0.08	0.11	0.04	0.05	0.00	0.01	0.05	0.03	0.11	NA
20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.84	0.04	1.18	NA
CE:																
01	NA	0.01	0.01	NA	NA	0.01	NA	NA	NA	NA	NA	NA	0.15	0.02	0.04	NA
02	NA	0.01	0.02	NA	NA	0.00	NA	NA	NA	NA	NA	NA	0.13	0.02	0.02	NA
05	NA	0.01	0.02	NA	NA	0.02	NA	NA	NA	NA	NA	NA	0.15	0.02	0.02	NA
06	NA	0.00	0.01	NA	NA	0.00	NA	NA	NA	NA	NA	NA	0.19	0.02	0.01	NA
07	NA	0.02	0.02	NA	NA	0.02	NA	NA	NA	NA	NA	NA	0.16	0.02	0.06	NA
14	NA	0.00	0.01	NA	NA	0.01	NA	NA	NA	NA	NA	NA	0.15	0.02	0.01	NA
16	NA	0.01	0.01	NA	NA	0.00	NA	NA	NA	NA	NA	NA	0.22	0.02	0.02	NA
20	NA	0.04	0.03	NA	NA	0.03	NA	NA	NA	NA	NA	NA	0.18	0.02	0.02	NA
73	NA	0.02	0.02	NA	NA	0.01	NA	NA	NA	NA	NA	NA	0.18	0.02	0.03	NA
74	NA	0.04	0.03	NA	NA	0.03	NA	NA	NA	NA	NA	NA	0.22	0.03	0.02	NA
78	NA	0.02	0.01	NA	NA	0.01	NA	NA	NA	NA	NA	NA	0.13	0.02	0.02	NA
79	NA	0.02	0.02	NA	NA	0.02	NA	NA	NA	NA	NA	NA	0.15	0.04	0.02	NA
81	NA	0.03	0.03	NA	NA	0.03	NA	NA	NA	NA	NA	NA	0.19	0.03	0.08	NA
82	NA	0.00	0.02	NA	NA	0.01	NA	NA	NA	NA	NA	NA	0.15	0.01	0.02	NA
90	NA	0.01	0.01	NA	NA	0.01	NA	NA	NA	NA	NA	NA	0.13	0.02	0.03	NA
91	NA	0.04	0.03	NA	NA	0.03	NA	NA	NA	NA	NA	NA	0.10	0.02	0.04	NA
92	NA	0.01	0.02	NA	NA	0.01	NA	NA	NA	NA	NA	NA	0.24	0.00	0.02	NA

K.5. Individual contaminant concentrations for PBDE congeners (ng/g ww)

Id	PBDE28	PBDE47	PBDE99	PBDE100	PBDE153	PBDE154
GG02	NA	9.04	0.50	1.73	0.55	1.01
GG05	NA	9.39	0.42	1.55	0.84	1.01
GG10	NA	4.44	0.42	0.74	0.48	0.45
GG11	NA	7.77	0.54	1.39	0.88	1.07
GG13	NA	6.31	0.43	1.13	0.55	0.72
KW01	0.42	8.03	1.92	1.15	1.47	1.19
KW02	0.13	4.63	0.87	0.53	0.57	0.52
KW03	0.16	5.10	0.88	0.69	0.74	0.59
KW04	0.24	3.83	0.73	0.52	0.54	0.45
KW05	0.21	4.32	0.85	0.46	0.73	0.47
KW07	0.32	6.25	1.20	0.96	0.72	0.89
KW09	0.27	6.08	1.11	0.76	0.90	0.67
KW10	0.17	3.81	0.54	0.51	0.57	0.45
KW16	0.12	4.35	0.85	0.55	0.50	0.58
KW17	0.29	6.71	1.13	0.84	0.86	0.39
KW20	0.61	7.18	1.26	0.79	0.88	0.79
CE01	NA	NA	NA	NA	NA	NA
CE02	NA	NA	NA	NA	NA	NA
CE05	NA	NA	NA	NA	NA	NA
CE06	NA	NA	NA	NA	NA	NA
CE07	NA	NA	NA	NA	NA	NA
CE14	NA	NA	NA	NA	NA	NA
CE16	NA	NA	NA	NA	NA	NA
CE20	NA	NA	NA	NA	NA	NA
CE73	NA	NA	NA	NA	NA	NA
CE74	NA	NA	NA	NA	NA	NA
CE78	NA	NA	NA	NA	NA	NA
CE79	NA	NA	NA	NA	NA	NA
CE81	NA	NA	NA	NA	NA	NA
CE82	NA	NA	NA	NA	NA	NA
CE90	NA	NA	NA	NA	NA	NA
CE91	NA	NA	NA	NA	NA	NA
CE92	NA	NA	NA	NA	NA	NA

K.6. Individual contaminant concentrations for PFASs congeners (ng/g ww)

Id	PFHxS	brPFOS	linPFOS	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFTriA	PFTeA
GG02	0.13	0.50	5.18	0.40	0.94	0.57	1.37	0.31	1.27	0.17
GG05	0.07	0.45	3.24	0.50	0.63	0.30	0.69	0.09	0.75	0.14
GG10	0.09	0.61	4.57	0.48	0.80	0.38	0.81	0.21	1.20	0.22
GG11	0.05	1.10	4.69	0.74	1.02	0.44	0.80	0.18	0.91	0.24
GG13	0.18	0.66	4.60	0.41	0.83	0.39	1.20	0.27	1.71	0.33
KW01	NA	0.05	5.66	0.02	0.34	0.68	2.73	0.84	6.05	1.48
KW02	NA	0.45	9.81	0.03	0.53	1.24	6.09	1.48	9.96	2.26
KW03	NA	0.01	6.38	0.03	0.26	0.63	3.10	0.92	5.77	1.40
KW04	NA	0.02	3.14	0.02	0.30	0.48	2.83	0.86	7.08	1.65
KW05	NA	0.01	5.19	0.03	0.39	0.83	3.71	1.25	8.36	2.03
KW07	NA	0.02	7.65	0.03	0.55	1.10	5.31	1.69	13.94	3.45
KW09	NA	0.01	4.63	0.07	0.64	0.68	2.76	1.07	6.71	1.44
KW10	NA	0.27	13.09	0.05	0.77	1.90	8.27	2.44	14.54	3.54
KW16	NA	0.16	3.28	0.02	0.33	0.36	1.75	0.48	4.44	1.06
KW17	NA	0.19	4.56	0.01	0.36	0.67	3.60	1.26	10.08	2.42
KW20	NA	0.29	3.12	0.34	0.64	0.17	0.47	0.09	0.72	0.19
CE01	0.55	0.41	3.22	0.34	0.74	0.12	0.44	0.18	0.82	0.17
CE02	0.46	0.45	4.49	0.08	0.34	0.27	1.20	0.35	1.53	0.36
CE05	3.08	3.50	14.26	0.07	0.24	0.09	0.40	0.20	0.90	0.24
CE06	0.09	0.02	1.66	0.01	0.16	0.14	0.58	0.05	0.49	0.16
CE07	1.11	1.59	7.94	0.13	0.29	0.11	0.64	0.28	1.44	0.34
CE14	0.54	0.43	2.28	0.17	0.41	0.06	0.27	0.20	1.32	0.29
CE16	0.40	1.01	4.39	0.64	1.12	0.47	0.89	0.13	0.85	0.21
CE20	0.56	0.44	5.40	0.23	0.26	0.09	0.34	0.09	0.57	0.15
CE73	0.22	0.29	1.85	0.41	0.55	0.20	0.57	0.13	0.58	0.09
CE74	0.63	0.58	3.58	0.33	0.53	0.17	0.59	0.16	0.76	0.16
CE78	0.15	0.01	1.21	0.11	0.28	0.08	0.26	0.02	0.42	0.08
CE79	0.07	0.01	1.65	0.13	0.33	0.13	0.62	0.17	0.75	0.15
CE81	0.19	0.01	1.33	0.33	0.38	0.11	0.42	0.08	0.46	0.10
CE82	0.54	0.58	3.11	1.00	0.99	0.45	1.47	0.36	1.49	0.35
CE90	0.26	0.00	2.05	0.23	0.52	0.22	0.64	0.17	1.05	0.17
CE91	0.21	0.24	3.30	0.33	0.75	0.37	0.90	0.23	0.68	0.13
CE92	0.30	0.84	3.63	0.42	1.06	0.30	0.71	0.15	0.78	0.20