Levels and effects of environmental contaminants in herring gull (*Larus argentatus*) from an urban and a rural colony in Norway

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Master thesis in toxicology

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A flock of gulls is called a squabble

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Abstract

Persistent and lipophilic contaminants present in the oceans will accumulate in organisms through transfer in foodwebs. Many seabirds occupy high trophic positions in marine foodwebs, and may be used as bioindicators for marine ocean health. There is limited knowledge on how and whether environmental pollutants affect DNA integrity in seabirds, including herring gulls. By analysing blood samples from female herring gulls from an urban (Oslofjord) and a rural (Hornøya, Northern Norway) location, with different presumed diets and exposure to contaminants the presence and levels of contaminants in the blood, biomarkers for DNA damage and recovery, as well as biomarkers for general health was quantified.

The objectives of this thesis were (1) to compare levels of contaminants in the two herring gull colonies, (2) to clarify whether exposure to contaminants affects the integrity of DNA in white blood cells (WBCs) and (3) to quantify the sensitivity of herring gull WBC DNA to oxidative stress. In addition, the diet of the herring gulls was evaluated by use of stable isotope ratios, to control for dietary influences in the urbanised colony.

Fifteen herring gulls were sampled at each location during the breeding season and whole blood was used for chemical analyses of persistent organic pollutants (POPs), perfluorinated alkylsubstances (PFASs) and cyclic volatile methylsiloxanes (cVMSs), while relative trophic position and primary carbon source was determined by use of nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and sulfur ($\delta^{32}S$) stable isotopes (SI). White blood cells were isolated from whole blood, and the DNA damage assessed by use of the comet assay (% DNA fragmentation). Sensitivity to oxidative stress was evaluated through *ex vivo* exposure to hydrogen peroxide and subsequent comet analysis.

The overall trend for contaminant concentrations showed significantly higher levels in the Hornøya colony and Oslofjord herring gulls with enriched SI ratios, for ΣPCB, ploybrominateddiphenyl ethers (PBDEs) and perfluorooctane sulfonate (PFOS), compared to the Oslofjord herring gulls with depleted SI ratios. This indicates that the exposure to the POPs is different depending on the diet, and is virtually independent of the habitat.

The baseline DNA damage was significantly higher in the Oslofjord population compared to the Hornøya population, meaning the DNA damage was caused by other stressors or contaminants not quantified in this thesis. A similar difference was observed for sensitivity to oxidative stress, indicating a stressed herring gull might respond negatively to additional stressors.

Abbreviations

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
Aov	Analysis of variance
BCI	Body condition index
BFR	Brominated flame retardant
cVMS	Cyclic volatile methylsiloxanes
DCM	Dichloromethane
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
dH_2O	distilled water
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FPG	Formamidopyrimidine DNA glycosylase
GC	Gas chromatography
H_2O_2	Hydrogen peroxide
HBB	Hexabromobiphenyl
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
IFE	Institute for Energy Technology
ISTD	Internal standard
KS-test	Kolmogorov-Smirnov test
LC	Liquid chromatography
Lm	Linear model

LOD	Limit of detection
Log	Logarithm
LOQ	Limit of quantification
MS	Mass spectrometry
N_2	Nitrogen gas
NA	Not analysed
n.d	Not detected
n.i	Not included
NILU	Norwegian Institute for Air Research
NINA	Norwegian Institute for Nature Research
NIVA	Norwegian Institute for Water Research
NPI	Norwegian Polar Institute
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybrominatediphenyl ether
PBS	Phosfatebuffered saline
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCN	Polychlorinated naphtalenes
PCP	Personal care products
PeCB	Pentachlorobenzene
PFAS	Perfluorinated alkylsubstances
PFOS	Perfluorooctane sulfonate
PFOSA	Perfluorooctanesulfonamide
PFOSF	Perfluoro-octanesulfonyl fluoride
N-EtPFOSA	N-ethyl perfluorooctanesulfonamide
POPs	Persistent organic pollutants

RBC	Red blood cell
RDA	Redundancy analysis
Rpm	Rotation per minute
RSTD	Recovery standard
SD	Standard deviation
SRM	Standard reference material
TL	Trophic level
TMU	Tromsø Museum
UHPLC	Ultra-high performance liquid chromatography
UiO	University of Oslo
WBC	White blood cell
W.w.	Wet weight

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1 Introduction

1.1 Contaminants

In 1962, the book *Silent Spring* by Rachel Carson alerted the public to the toxic side effects of insecticides, like DDT (dichlorodiphenyltrichloroethane), and the rapid decline in various organisms linked to the uninhibited application of pesticides in agriculture. Woodwell (1967) also warned against certain compounds' toxicity and persistence, predicting they might concentrate up the food chain and be distributed unexpectedly in the environment. These are characteristics of persistent organic pollutants (POPs): industrial compounds, by-product of industrial processes, pesticides, consumer products and waste products. POPs are bioaccumulative, reaching higher concentrations in organisms than in the ambient environment; they are toxic, either directly or through formation of metabolites; and they are persistent, degrading slowly and thus remaining in the environment for a long time (Lunde & Gether, 1976). In addition, POPs may be subject to long range transportation, by air, i.e. through the atmosphere, by ocean currents and rivers, through biota, and by anthropogenic transportation (Wania, 2003; Butt et al., 2010; de Wit et al., 2010).

A major proportion of POPs eventually end up in the oceans, brought there by river or surface run-off, atmospheric deposition, effluents, accidental and intentional spills or legal and illegal dumping. Many of these compounds are lipophilic and hydrophobic, thus, a contaminant in the ocean will shy away from water and quickly adsorb to organic matter and, following uptake in an organism, to a large extent associate with adipose tissues (Jones & de Voogt, 1999). Due to bioaccumulation into species at low trophic levels of marine ecosystems, and subsequent transfer through the food chain, this leads to trophic magnification (Borgå et al., 2004). Trophic magnification puts apex predators, including many seabirds, at risk for high exposure to these contaminants (Fisk et al., 1998; Hobson et al., 2002; Jepson & Law, 2016).

The Stockholm Convention (2001) has defined a list of legacy POPs that were banned or severely restricted. The "dirty dozen" includes pesticides: DDTs, aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, and toxaphene; and industrial chemicals: polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (dioxins) and dibenzofurans (furans). Recent addition to this list, the "new" POPs, are other halogenated compounds, such as the polybrominated diphenyl ethers (PBDEs); tetra-, penta-, hexa- and hepta-bromodiphenyl ether, α-, β-, and ɣ-hexachlorocyclohexane (HCH), chlordecone,

hexabromobiphenyl (HBB), pentachlorobenzene (PeCB), perfluorooactanesulfonic acid (PFOS), perfluoro-octanesulfonyl fluoride (PFOSF), endosulfan, hexabromocyclododecane (HBCD), hexachlorobutadiene, polychlorinated naphtalenes (PCN) and pentachlorophenol (Stockholm Convention, 2009). Restrictions and bans put in place over the past 20-30 years has led to a change in the global levels of many legacy contaminants. DDTs, PCBs, HCHs and chlordanes have exhibited a downward trend in biota and air. The more recent additions to the Stockholm Convention, the PBDEs and PFOS show a more mixed pattern, but the concentrations of both groups of contaminants have generally been decreasing in biota after the beginning of the 2000s. Some compounds show different trends due to continuing long distance transport and re-emission, caused by snow melt, ocean warming and loss of ice sheets. Among these are some PCBs, HCB, ∑DDT and PFASs, showing weak increasing trends in biota (AMAP, 2016).

Recently Howard and Muir (2010) identified cyclic volatile methylsiloxanes (cVMS) as products that may potentially be an environmental concern due to predicted persistence and bioaccumulation. The cVMSs are used in industry, commercially in car wax and polishes, and in personal care products (PCP)(Wang et al., 2013). Although not having POP classification, octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), cause the largest concerns for carcinogenic effects and effects on fertility (Lassen et al., 2005; Brooke et al., 2009a, 2009c). Borgå et al. (2012) showed biomagnification of D5 in an aquatic ecosystem. Dodecamethylcyclohexasiloxane (D6) have been shown to be very persistent, but does not meet toxic and bioaccumulative criteria (Brooke et al., 2009b).

The contaminants in focus in thesis are HCB, PCBs, PBDEs, PFOS and cVMS.

1.2 Effects

The toxicity of POPs is measured by different endpoints: some affect reproductive success in organisms, causing e.g. eggshell thinning (Lundholm, 1997) or endocrine disruption (Letcher et al., 2010), while others affect immune systems or behaviour, particularly in apex predator-species (Jones & de Voogt, 1999). The observed damage can rarely be linked to the exposure to a single contaminant, as natural populations of animals will experience a concoction of different compounds. An important endpoint is genotoxicity. By altering DNA structure and integrity, genotoxic compounds may cause serious damage and consequences could be cell

death, mutations or carcinogenesis. Damage to the DNA have predominantly been identified through quantification of DNA adducts (Ericson & Larsson, 2000), i.e. binding of reactive metabolites to the DNA backbone; micronucleus, which is fragmentation of chromosomes during cell division, leading to small secondary structures of chromatin in the cytoplasm (Baršienė et al., 2006); and DNA strand breaks (Collins, 2015). DNA strand breaks occur naturally during repair processes, and by stressors acting directly on the DNA backbone (Azqueta et al., 2009). One of these stressors is oxidative damage. The DNA is constantly exposed to oxidative damage, either endogenously, as the by-product of natural processes in the cells including oxygen (Azqueta et al., 2009), or through exogenous sources, for instance by genotoxic contaminants (Mitchelmore & Chipman, 1998). Oxidative stress arises due to imbalance between reactive oxygen species causing oxidation and anti-oxidant functions and repair in the cells, where the former is prevalent and cause damage (El-Bibany et al., 2014).

Reduced food availability and quality, stress in relation to reproduction and "natural" starvation following migration to the breeding colony will exert extra pressure on seabird's internal repair and maintenance systems (Alonso-Alvarez et al., 2004). With the additional stress of environmental contaminant exposure through food or through mobilisation of lipid storages the animal's anti-oxidant capacity may be strongly reduced. Causing organisms become vulnerable to the deleterious effects of genotoxicity and worst-case scenario, reproductive failure or death may follow (Fenstad et al., 2014).

Quantifying DNA strand breaks as an indication of DNA damage caused by genotoxic contaminants is considered a sensitive and intuitively relevant biomarker. Single cell gel electrophoresis, also called the comet assay, is a simple and efficient method of detecting DNA strand breaks in cells, including animal tissues, nucleated blood cells, cell cultures and to some extent plant tissues (Collins, 2014). The principles of the comet assay were first described by Östling and Johanson (1984). The original method requires a small number of cells and identifies double strand breaks in DNA. The method was modified by Singh et al. (1988) enabling the detection of single strand breaks. Maness and Emslie (2001) were the first to use of the comet assay for birds, in red blood cells from royal terns (*Sterna maxima*) linking increased amount of DNA damage to the exposure to previously undetected contaminants. Pastor et al. (2001) applied the comet assay to peripheral blood lymphocytes in white storks (*Ciconia ciconia*), observing higher levels of DNA damage due to a one-time exposure after an accidental spill of acid waste rich in heavy metals. More recently Haarr (2016) optimised the comet assay for use on avian white blood cells, measuring the DNA damage and contaminant

levels in Arctic seabirds, black-legged kittiwakes (*Rissa tridactyla*), common eiders (*Somateria mollissima*), Arctic skua (*Stercorarius parasiticus*) and glaucous gulls (*Larus hyperboreus*). No connection between DNA strand breaks and contaminant concentrations was established.

1.3 Monitoring

The sources of POPs to the environment are somewhat diffuse, several point sources remain, contaminants trapped in soils and sediments slowly leach into the ambient air and waters (Berge et al., 2013). These may be remnants of old industry, or the general run-off from large, densely populated areas and agriculture (Tieyu et al., 2005). An unknown amount is also currently circulating the globe in the atmosphere, eventually being deposited by precipitation at the poles, locked in the tissues of biota, or in ocean currents.

International agreements to reduce discharges of pollution and improve the health of marine organisms calls for monitoring. Several different marine species have historically been used to monitor ocean health by observing the effects of multiple stressors, both natural and anthropogenic (Tabor & Aguirre, 2004). For POPs, the contaminant concentrations in lower trophic levels are, by means of bioaccumulation and biomagnification, more clearly reflected in apex predators, like marine mammals and seabirds. Seabirds can be used as monitoring species as they accumulate the contaminants up the food web and may reach hazardous levels (Furness & Camphuysen, 1997). Borgå et al. (2001) showed that pollutant levels in the Arctic environment are well reflected in an apex predator, the glaucous gull. The Great Lakes monitoring of herring gull (*Larus argentatus*) in Canada found contaminant levels correlated with dietary uptake, and use the herring gull as an indicator of temporal and spatial contaminant levels (Ewins et al., 1994; Hebert et al., 2000). Most contaminant studies on herring gull have been conducted on eggs e.g. Bjerk and Holt (1971); Helgason et al. (2009); (Huber et al., 2015), giving the integrated contaminant load of the females before the breeding season. As mentioned above, time series on e.g. DDTs and PCBs illustrate the steady decrease observed for the unwanted POPs, both in areas with massive urban influence (Hebert et al., 2000) and the supposedly pristine Arctic (Rigét et al., 2010).

1.4 Study species

Based on earlier studies, and on Borgå et al. (2001), the European herring gull (*Larus argentatus argentatus*) was selected as a study species for this thesis, comparing levels and effects of POPs and cVMSs in a rural and an urban seabird colony.

Figure 1. Herring gull (*Larus argentatus*) from egg and newly hatched chick (left), to juvenile (1 year, middle), and mature adult (right). Photos: E. K. Keilen.

The herring gull is a large species of seagull distributed throughout north-temperate latitudes, it is found along the entire coast of Norway (Barth, 1975). They are surface feeders and rely on open bodies of water, preferably sea water, but are also found near lakes inland. The diet of this seagull includes fish, crustaceans, carcasses, insects and eggs, and also plants. The herring gull is a highly opportunistic feeder, and with proximity to human settlements, it will scavenge food from human waste dumps (Götmark, 1984; Coulson, 2015). Increases in herring gull populations has been observed since the 1900 (Kadlec & Drury, 1968; Harris, 1970), and is contributed to bans on hunting and egg collection, but more importantly, the food resources made available by increased human activities, left-overs and garbage (Hunt, 1972; Burger et al., 1980).

Trophic position and food source

Trophic position and food source have been linked to the contaminant pattern and total load in gull species (Borgå et al., 2001; Ruus et al., 2002; Haukås et al., 2007; Sørmo et al., 2011). To give an indication of the relative trophic position of an organism, the ratio of ^{15}N to ^{14}N ($\delta^{15}N$) is used. This signature is mainly caused by the excretion of the lighter ^{14}N and the consequent retention of the heavier ^{15}N isotope (Peterson & Fry, 1987). The $\delta^{15}N$ (‰) is shown to increase with higher trophic level (Minagawa & Wada, 1984). Dietary carbon source of an organism may be determined by estimating the ratio of the two stable isotopes of carbon, ^{13}C and ^{12}C.

This ratio, $\delta^{13}C$ (‰), varies between primary producers due to different photosynthetic pathways in plants i.e. C_3 and C_4 carbon fixation. C_3 plant will have a more depleted $\delta^{13}C$ than C_4 plants, while planktonic photosynthesis results in a carbon fractionation giving more enriched $\delta^{13}C$ (Peterson & Fry, 1987). The different isotopic characteristics are retained in consumers and predators (Vogel & van der Merwe, 1977), and a high $\delta^{13}C$ indicates a marine-based, pelagic diet, while an animal with a low $\delta^{13}C$ has more terrestrial influences in the diet, meaning they feed to a larger extent on either terrestrial or fresh water organisms (Elliott et al., 2014). Similarly, the signature ratio of sulfur isotopes, $\delta^{34}S$, is a good indication of food sources as it too varies between primary produces (Peterson & Fry, 1987): marine organisms have a higher $\delta^{34}S$ (‰) signature than terrestrial organisms (Lott et al., 2003) with no change with trophic level. $\delta^{34}S$ has also been shown to decline with increasing urbanization (Morrissey et al., 2013; Eulaers et al., 2014).

The adaptive nature of herring gulls has shown that they opportunistically will feed on human waste when it is easily accessible (Hunt, 1972; Chudzik et al., 1994). As exposure to, and possible biomagnification of many contaminants are linked to dietary input (Hebert et al., 2009), knowing the feeding preferences of the herring gulls is essential to understand patterns and presence of contaminants. An urban influenced colony may incorporate more food items of anthropogenic origins (Burger et al., 1980), drastically changing the expected exposure to e.g. PCB153, which is found in higher concentrations in lipid-rich marine organisms, than leftover dinner from a family in Oslo. By measuring stable isotopes, indication of feeding preferences is visualised through the C and S ratios, giving more or less marine signatures, and the $\delta^{15}N$, giving an estimation of trophic level. Pierotti and Annett (1991) noted herring gulls may specialise in e.g. intertidal species, scavenging human waste, or predation on other seabirds. Thus, the foraging preferences of, for instance, the urbanised herring gulls may result in very different isotopic signatures within the colony. Even marine-based foraging in the inner Oslofjord might affect stable isotope ratios, compared to marine-based foraging on the open ocean. In bald eagles (*Haliaeetus leucocephalus*), $\delta^{15}N$ and $\delta^{13}C$ have been shown to be higher in off-shore marine areas, followed by inshore, and lower in estuarine and freshwater sites (Elliott et al., 2009).

1.5 Aims

The main aim of this thesis was to clarify whether urbanisation causes increased contaminant concentrations in herring gull and whether such exposure leads to increased levels of DNA strand breaks. In addition, the diet of the herring gulls was evaluated by use of stable isotope ratios, to control for dietary influences in the urbanised colony. A second aim was to identify non-contaminant factors that may affect DNA strand breaks as well as the resilience of the target cells, white blood cells, to oxidative stress.

The objectives were:

I) To compare levels of contaminants in two different herring gull populations, one with urban influence and one from a rural area.
Expectation: the population with an urban influence may rely on unnatural food items in their diet, resulting in a higher or lower contaminant exposure than a natural population. Trophic level might also be affected by the diet.

II) To analyse the relationship between contaminant load and DNA damage.
Expectation: many contaminants are known to be genotoxic and will exert stress on the herring gull, especially during the already stressful breeding season. A higher contaminant load is predicted to result in higher DNA damage.

III) To identify exogenous or endogenous factors that affect levels of baseline DNA damage, resilience to oxidative stress, and/or the ability of white blood cells to recover following oxidative stress.
Expectation: herring gulls exposed to more oxidative stress through contaminant exposure and/or physiological processes may also be more efficient at repair and maintain a higher resilience to any added stressors.

2 Materials and Methods

2.1 Study areas

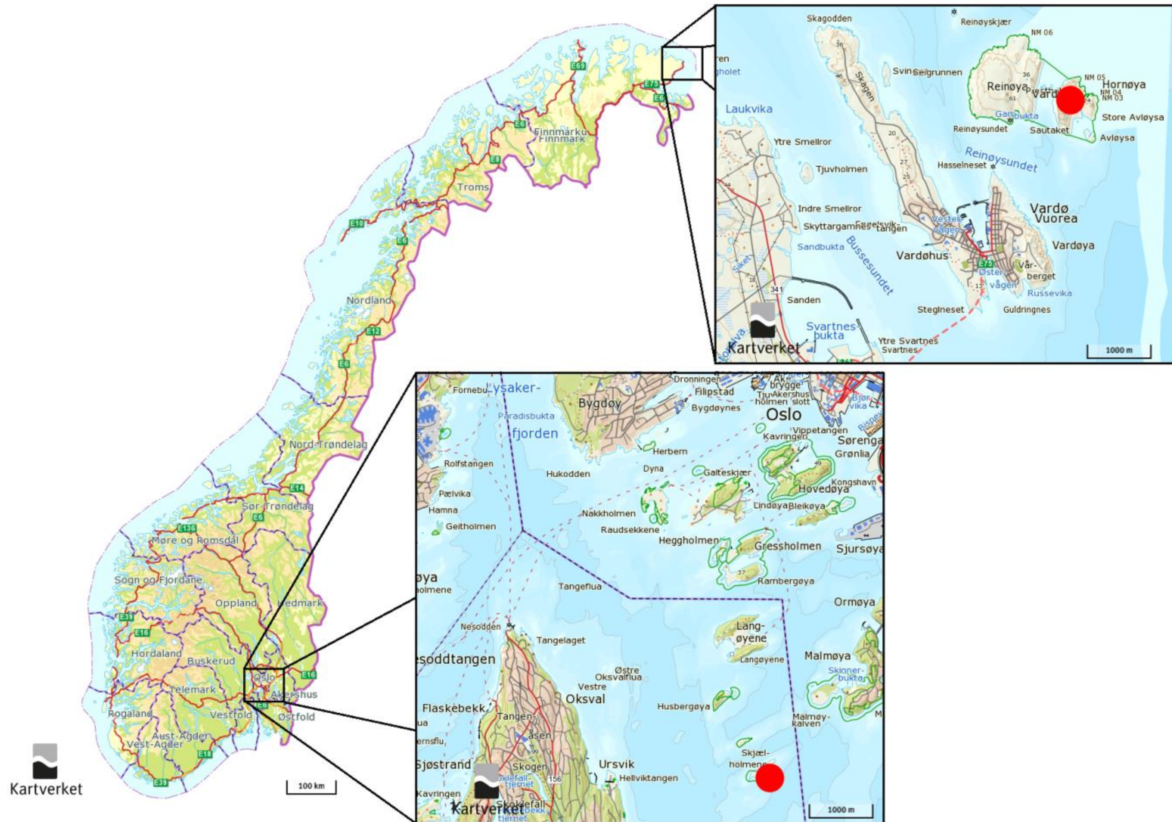

Figure 2. Map of Norway with sample areas highlighted: the rural location, Hornøya (north) and the urban location Søndre Skjælholmen (south). Map from Kartverket.no

2.1.1 Søndre Skjælholmen

Søndre Skjælholmen (Nesodden municipality, Akershus county; 59°85’ N, 10°73’ E, Figure 2, hereafter referred to as Oslofjord) is a locally listed islet about 6 km off the Oslo city centre, and 2 km from Nesodden in the Oslofjord. Part of the islet is a nature reserve, and during the spring and early summer several different seabirds nest on the islet; the barnacle goose (*Branta leucopsis*), common eiders (*Somateria mollissima*), Eurasian oystercatchers (*Haematopus ostralegus*), lesser black-backed gulls (*Larus fuscus*), herring gulls, common gulls (*Larus canus*) and a few pairs of great black-backed gulls (*Larus marinus*). The public are free to stay on and use the islet, even during nesting season, but there is a traffic restriction of motoring boats around the south-western part of the reserve (Climate and Environmental Agency, 2017).

Sampling

Blood of herring gull was sampled 2-3 weeks into egg incubation to prevent abandonment of the nests. Nests with chicks or hatching chicks were avoided. Herring gulls in the Oslofjord were sampled for the Climate and Environmental Agency monitoring programme Urban fjord on the 18th of May and the 25th of May 2016.

The handling and sampling of the birds was done by Morten Helberg, University of Oslo (UiO). Sterile syringes (5-mL, BD Plastipak™) were used in conjunction with orange cannulas (0.5x25mm BD Microlance™ 3) flushed with heparin (Heparin sodium salt from porcine intestinal mucosa, H3393, SIGMA-ALDRICH). Fifteen female herring gulls were sampled, the sex of the bird was determined by measuring the head length. Adult, breeding herring gulls with head length <120 mm are females, while a head length of >125 mm most likely male (Helberg 2016, pers.com.). Herring gulls with head length in-between these measurements were not sampled as they could be either large females, or small males.

Whole blood was transferred from syringe to 15-mL tubes (sterile centrifuge tubes, VWR), and between 200 and 300 µL was aliquoted from each tube into separate 1.5 mL Eppendorf tubes. To avoid clotting of the blood in these tubes, a few drops of heparin was added and the tubes were tilted to mix well. The samples were kept in a lidded Styrofoam box filled with ice for up to 8 hours prior to further processing.

2.1.2 Hornøya

Hornøya (Vardø municipality, Finnmark county; 70°23' N, 31°10' E, Figure 2) is a nature reserve (since 28.01.83) and popular tourist attraction with a large colony of seabirds nesting in bird cliffs and elsewhere on the island. In total around 40 000 pairs of black-legged kittiwakes (*Rissa tridactyla*), razorbills (*Alca torda*), Atlantic puffins (*Fratercula arctica*), black guillemots (*Cepphus grylle*), common guillemots (*Uria aalge*), Brünnich's guillemots (*Uria lomvia*), European shags (*Phalacrocorax aristotelis*), greater black-backed gulls, herring gulls and even a few pairs of common raven (*Corvus corax*) nest on the island 1 km from the town of Vardø. People are allowed on the island, but restricted to marked paths as to not disturb the nesting birds too much (Reiertsen, 2015).

The island has since 1980 been a reference area for monitoring of seabirds, and since 2005 a key location for SEAPOP (Seabird Populations) a monitoring programme by the Norwegian

Institute for Nature Research (NINA), Norwegian Polar Institute (NPI), Tromsø Museum (TMU). The island has one of the longest running time series of data on seabird- population, number and breeding biology (Reiertsen, 2015).

Sampling

The sampling on Hornøya was done simultaneously as NINA were tagging herring gulls, from 28th of May to 1st of June. There were few breeding herring gulls, and as the incubation period was coming to an end, capture was attempted for every nest with brooding parents.

Capture, tagging and handling of the birds was done by Tanguy Deville on behalf of NINA, while blood sampling was done by me. The blood samples were kept in the syringes and stored in a styrofoam box on ice for 2 to 6 hours prior to further processing.

2.2 Field procedures

All birds captured were tagged with two different kinds of identification rings, measured and sampled. Herring gulls were mostly caught on the nest with a walk-in trap; the traps were left on the nests for a maximum of 10 minutes at a time before each was checked. Trapped gulls were removed from the traps and put in cotton bags to limit their movement to prevent injuries and reduce stress. On Hornøya, the walk-in traps were largely unsuccessful, and most captures were therefore made with an automatic triggered snare trap placed around the nest. When the snares successfully captured a bird, the bird was removed immediately, and taken to a sheltered location for tagging and measurements.

When weighing the gulls, they were kept in a cotton bag and suspended on a spring scale (g). Wing and head length (mm) were measured using a ruler and sliding callipers respectively. Maximum handling time for each individual was 15-20 min.

Approximately 5 mL of blood was sampled from the basilic vein, also called the wing vein, located under the wing. If not sufficient blood was acquired at the first attempt the other wing was sampled as well. Sterile syringes (5 or 10 mL) were used with either orange cannulas (0.5x25mm) or blue cannulas (0.6x30mm BD Microlance™ 3). Prior to the sampling the syringe and cannula were flushed with heparin. Almost all heparin was then extruded leaving only a small amount in the syringe to avoid immediate blood clotting. The veins were exposed

either through plucking off some of the down and applying water or saliva to get a better visualization, or using a hand sanitizer in gel form (Antibac Pharma 85% ethanol).

Ethical considerations

Permission to handle and sample the birds at both locations was granted by the Norwegian Food Safety Authority and followed the guidelines of the Norwegian Animal Welfare Act. In the Oslofjord 15 female herring gull were sampled, and on Hornøya 17 herring gulls were sampled, the individuals were a mix of males and females, and only 15 are included in this thesis.

Special care was taken to prevent stressing the trapped herring gulls before tagging. If a bird was evaluated as being too hot or stressed, it was released without any further measurements or tagging. The traps were never left for too long at a time without being checked, and if the parents did not return to warm and protect the eggs/chicks after a maximum of 30 min the traps were moved to a different nest. This was to avoid nest abandonment. On the small islet of Søndre Skjælholmen in the Oslofjord, every location was close to nesting birds, so our camp was hidden in bushes and relocated after about 5 hours to minimize disturbance. The camp on Hornøya was on the path where tourists were also present during the day, and nests were monitored with binoculars.

2.3 Analysis of stable isotopes

For both sample batches (Oslofjord and Hornøya) stable isotopes of nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) were analysed by Institute for Energy Technology (IFE), Kjeller, Norway. Analysis of nitrogen and carbon isotopes was done by combustion in an element analyser, reduction of NO_x in Cuoven, separation of N_2 and CO_2 on a GC-column and determination of $\delta^{13}C$ and $\delta^{15}N$ at IRMS (Isotope Ratio Mass Spectrometer). Analysis of sulfur isotopes was done by combustion in an element analyser with V_2O_5 to increase the amount of available oxygen reduction of SO_x to SO_2, separation of SO_2 from other products of combustion on a GC-column, and determination of $\delta^{34}S$ at IRMS.

2.4 Analysis of POPs

2.4.1 Hornøya samples: procedure for extraction and clean-up of POPs in whole blood

The samples from Hornøya were prepared by me at NILU (Fram Centre, Tromsø), and instrument analyses and quantification were done by staff at NILU.

The analytes in this procedure include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane and metabolites (DDTs), non-DDT pesticides (e.g. chlordanes and metabolites) and brominated flame retardants (BFRs). Internal (ISTD) and recovery standard (RSTD) used in this procedure is included in Appendix D.

Extraction and lipid determination

This procedure targets lipid-soluble contaminants and uses a non-polar solvent, n-hexane, to extract them. The blood samples were thawed on the bench at room temperature. Two grams of blood was added to a 15-mL glass tube, each sample was weighed. Exactly 100 µL of the internal standard for POPs was added and thoroughly mixed with vortex machine. Two mL deionised water saturated with ammonium sulphate and 2 mL of ethanol was added to precipitate non-polar compounds and denaturize proteins. Six mL of n-hexane was added to the mix and the tube was vortexed for 45 seconds, and then left in a fume hood for 15 min to allow for phase separation. The supernatant was extracted, using a glass pipette, and added to a new pre-weighed 15 mL glass tube. Another 6 mL of n-hexane was added to the first glass tube to further extract the lipid-soluble components, vortexed for 45 sec and left for 15 min. The second supernatant was added to the first supernatant in the second glass tube. The combined extracts were further concentrated to a volume of about 200 µL using the RapidVap (LabConco RapidVap, Model 790001, Kansas City, MO, US). Each sample was carefully monitored during this process to avoid drying out causing loss of analyte.

For lipid determination, an N_2-evaporation unit was used to gently evaporate samples to dryness (N_2 purity 99.995%, quality 5.0, Yara Praxair AS, Porsgrunn, Norway). The samples were then weighed and recorded, and immediately re-dissolved in 500 µL of n-hexane. Lipid weight was the difference between the glass tube with the dried extract and the empty pre-weighed glass tube. Lipid percentages were calculated using Equation 1.

$$\text{lipid \%} = \frac{\text{lipid weight}}{\text{wet weight}} \times 100 \qquad \text{Equation 1}$$

Clean-up

The clean-up step was to remove lipids and other compounds and contaminants that are not the analytes. Florisil (magnesium silicate, particle size 150-250 µm) was burnt at 450°C for 8 hours to activate it and remove any moisture from the adsorbant. Glass columns were packed with one cotton frit in the tip cleaned with dichloromethane (DCM), 1 g Florisil, and a second cotton frit at the top. These columns were then run in the Rapidtrace (robot) following a program called FLOKORT.SPE. This program used DCM with 20% methanol, hexane with 10% DCM and hexane with 10% acetone.

After the clean-up, isooctane was added as a keeper to the extracts, and samples were concentrated to 200 µL using the RapidVap. The concentrated extracts were added to GC vials with insert, the test tubes were rinsed with n-hexane and the rinsing solvents were added to the vials as well. Using the N_2-evaporation unit the extracts were further reduced to about 30 µL. 10 µL of recovery standard was added to each sample and the vials were capped, labelled and stored at 4°C until analysis.

Instrument analysis

PCBs, PBDEs, DDTs and BFRs were analysed using an Agilent 7890 gas chromatograph equipped with a triple quadrupole mass spectrometer, Quattro Micro GC (Waters Corporation, Manchester, UK). Analysis of non-DDT pesticides was conducted using an Agilent 7890A gas chromatograph equipped with a 5975C mass spectrometer (Agilent Technology, Boblingen, Germany). A DB-5MS column (length 30m, 0.25 µm film thickness, 0.25 mm inner diameter) with pre-column (0.53 mm inner diameter deactivated) and restriction capillary column (0.18 mm inner diameter) was used. Carrier gas was helium (6.0 quality; Yara Praxair AS, Porsgrunn, Norway) with a flow rate of 1 mL/min. Temperature program was 70°C for 2 min, then temperature was increased by 15°C per min until 180°C, and after this temperature was increased by 5°C per min to 280°C were temperature was kept constant for 10 min.

2.4.2 Oslofjord samples: analysis of PCBs, DDT, HCH and PBDE

The samples from the Oslofjord were analysed by staff at the Norwegian Institute for Air Research (NILU) at Kjeller, Oslo. The analysis included PCBs, DDT, HCH and PBDE-compounds, and was principally the same as the procedure described above

Prior to extraction, the samples were added a mixture of isotope labelled PCBs, and DDT standards, for quantification purposes. The samples were extracted with organic solvents and concentrated under nitrogen flow, followed by a clean-up procedure using concentrated sulphuric acid and a silica column to remove lipids and other interferences prior to analysis. The compounds were quantified on GC-HRMS (Waters Autospec).

2.5 Analysis of PFASs

2.5.1 Hornøya samples: analytical method for PFAS in seabird whole blood

The samples from Hornøya were prepared by me at NILU (Fram Centre, Tromsø), and instrument analysis Instrument analyses and quantification were done by staff at NILU. Internal and recovery standard used in this procedure is included in Appendix D.

Extraction

This procedure targets the amphipathic PFAS, that have both water and grease repelling characteristics, and methanol, a polar solvent, was used for extraction. The samples were thawed at room temperature, and 200 µL blood was transferred to Eppendorf-centrifuge tubes. Twenty µL of the internal standard was added to each sample. One mL methanol was added to each tube and immediately mixed using vortex, the addition of methanol induces clumping of blood and it is important that the solvent has contact with as much surface area as possible. The samples were placed in an ultrasonic bath for 10 min three times with vortex in-between, this was to further ensure that the methanol could extract as much of the analytes from the blood cells as possible. After the ultrasonic baths the samples were centrifuged at 10 000 rpm for 5 min for sedimentation.

Clean-up

The PFAS extracts were cleaned using active carbon (Envi-Carb) as an adsorbent (Powley et al., 2005). About 750 µL of supernatant (methanol) was transferred to a 1.7-mL Eppendorf tube containing 25 mg Superclean Envi-Carb (120/400) and 50 µL acetic acid. The Eppendorf tubes were mixed by vortexing and centrifuged for 10 min at 10 000 rpm for sedimentation. Exactly 500 µL of the supernatant was transferred to vial, and 20 µL of recovery standard added. Vials were capped and vortexed and stored at 4°C until analysis.

Instrument analysis

Fifty µl of supernatant was transferred to an autosampler with insert, and 50 µL 2 mM NH_4OAc in water was added to the same autosampler. The autosampler was capped and vortexed, ready for analysis.

The instrumental analysis of PFASs was conducted as described by (Hanssen et al., 2013a) using ultrahigh pressure liquid chromatography triple-quadrupole mass spectrometry (UHPLC-MS/MS). Analysis was performed on a Thermo Scientific quaternary Accela 1250 pump (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a PAL Sample Manager (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a Thermo Scientific Vantage MS/MS (Vantage TSQ) (Thermo Fisher Scientific Inc., Waltham, MA, USA). 10 µL sample was injected to a Waters Acquity UPLC HSS 3 T column (2.1×100 mm, 1.8 µm) (Waters Corporation, Milford, MA, USA) equipped with a Waters Van guard HSS T3 guard column (2.1×5 mm, 1.8 µm) (Waters Corporation, Milford, MA, USA). To separate the different compounds, 2 mM NH4OAc in 90:10 methanol/water and 2 mM NH4OAc in methanol were used as the mobile phases. To avoid PFAS contamination from the pump and degasser, a Waters XBridge C18 column (2.1×50 mm, 5 µm) (Waters Corporation, Milford, MA, USA) was installed.

2.5.2 Oslofjord samples: analysis of PFAS

PFAS were analysed by NIVA (CIENS/Oslo Innovation Centre), following a procedure that is principally the same as described above. Extraction medium and analysis method differed.

Prior to extraction, the samples were added a mixture of isotope labelled PFAS, for quantification purposes. The samples were then extracted twice with acetonitrile and the extracts were cleaned using active coal if needed. PFAS compounds were analysed using LC/QToF (ESI negative mode).

2.6 Analysis of cVMS

The cVMS; D_4, D_5 and D_6, were analysed by the staff at NILU (Fram Centre, Tromsø) for both the Oslofjord and Hornøya samples. The sample preparation for the Hornøya samples was done

by me, but the procedure was identical for both sample batches. Internal and recovery standard used in this procedure is included in Appendix D.

Extraction

Samples were thawed at room temperature. 1 mL of blood was transferred to a pre-weighed 12 mL centrifuge glass tube, and weight was recorded. Samples were spiked with 20 µL internal standard, and tube was sealed with aluminium foil and capped securely, before vortexed briefly. 2 mL acetonitrile and 2 mL hexane was added to the centrifuge tube and re-capped tightly with aluminium foil. The tubes were placed in an ultrasonic bath for 15 min. After the ultrasonication the samples are placed on rotation (or rolling) mixer to extract for a minimum time of 1 hour – or overnight. After mixing, the samples were centrifuged at 3000 rpm for 10 min. 200 µL supernatant was transferred to a GC vial with insert. 20 µL recovery standard was added and vials were capped. Any remaining supernatant was transferred to a clean 4 mL glass vial, capped, labelled and stored at -20°C as back-up.

Analysis

Collected extracts were analysed using Concurrent solvent recondensation large volume injection gas chromatography mass spectrometry (CSR-VIGCMS) using a modified method recently published by Companioni-Damas et al. (2012). Instrument specifications and details are described by Krogseth et al. (2016)

2.7 Quantification and quality control

Quantification

Quantification of all compounds was done by use of an internal (ISTD) and a recovery standard (RSTD). A known amount of ISTD is added to the samples before any clean-up or extraction. When the final extract is ready the remaining ISTD is quantified. Due to the differenced in the extract volumes, a RSTD is added to correct for any changes in response that could be due to difference in volume. After this response correction, the correct mass of the ISTD at the end of the analysis can be calculated as well as an extraction recovery. This ensures that the analyte concentrations are correct.

Quality assurance

The laboratories at NIVA and NILU is accredited by Norwegian Accreditation for ISO/IEC 17025. The laboratories have general quality procedures in place to minimize any form of contamination from across samples or from personnel. For all analyses methods, field and lab blanks are run with the actual samples to register any noise from the handling or the instrument during the analysis. A reference material sample was run and analysed with the samples as additional quality control. The reference material contains a known amount of analytes and the levels of the test samples may be corrected according to the reference.

Analysis of cVMSs is the most delicate method as there are multiple sources of background contamination of these compounds present in the ambient environment. cVMSs are used extensively in personal care products (PCPs), and to avoid contaminating the samples during the sample preparation, the use of these products was abstained. Procedural blanks were run before and after the samples to ensure the background variation did not contribute to the detection levels of the compounds.

Field blanks

MilliQ water from the lab at UiO was kept in 50 mL falcon tubes and used as a field blank for both Oslofjord and Hornøya samples. The herring gull sampling procedure was mimicked on the water with use of heparin, cannulas and syringes and transferred to the same tubes the blood samples were kept in. Field blanks for both Hornøya and Oslofjord were analysed alongside the Hornøya samples.

Hornøya lab blanks

In the POPs procedure for every 10th sample, one blank (dH_2O) and one SRM (standard reference material 1958 human serum from NIST) was extracted alongside samples.

In the PFAS procedure, a blank (dH_2O) and a reference material sample were run with each sample batch, up to 20 samples.

In the cVMS procedure, 3 lab blanks (dH_2O) were run per 10 samples.

Limits of Detection

The limit of detection (LOD) is usually calculated after instrumental analysis using the average lab blank response or instrumental noise value plus 3 times standard deviation of blank or instrumental noise value.

A more conservative limit of quantification (LOQ) is reported for the cVMSs, and is calculated using the average of the blanks plus 10 times the standard deviation for blanks.

2.8 Method preparation

A pilot study of the comet assay initiated in April 2016, with blood from chicken (*Gallus gallus domesticus*) from the animal facility at the Institute of Biosciences, UiO. The sampling procedure of the chicken was principally the same as it would be for the herring gulls; blood was taken from the basilic vein using 5-mL syringes with orange cannula. The syringe and cannula was flushed with heparin before sampling.

Percoll (™GE Healthcare) was used as separation medium to extract white blood cells (WBCs) from the blood samples. Different densities of the Percoll solution would allow the different components of whole blood to penetrate the medium differently. Centrifugation at 2000 rpm for 15 min at 4°C would leave the red blood cells at the bottom, the plasma at the top and the WBCs right underneath the plasma layer (Figure 3a). The WBCs are then easily extracted using a plastic transfer pipette (3.5 mL, SARSTEDT).

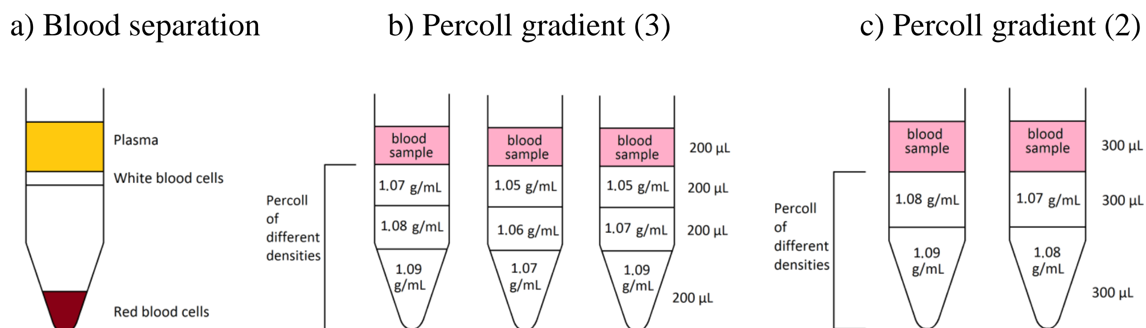

Figure 3. a) Eppendorf tube with separated blood after centrifugation. b) Test runs of three different Percoll densities and whole blood on top. c) Test runs of two different Percoll densities with whole blood on top.

Five different densities of Percoll were tested, 1.05 g/mL, 1.06 g/mL, 1.07 g/mL, 1.08 g/mL, and 1.09 g/mL. The heaviest density was placed at the bottom of a 1.5 mL Eppendorf tube, and the other consecutively lighter densities were layered carefully on top of each other. All layers

were 200 µL and the blood sample was gently placed on top of the gradient (Figure 3b), and the tube was centrifuged according to protocol.

Separation worked for all different test runs, but the initial three layers seemed superfluous, and were reduced to two layers of 300 µL each. In earlier studies 1.09 g/mL and 1.08 g/mL were utilised (Haarr, 2016), so these concentrations were tested along with 1.08 g/mL and 1.07 g/mL (Figure 3c). The former was deemed adequate as it allowed for a clean separation with a clear layer of WBCs. These initial tests were first performed on chicken blood, and when herring gull blood was obtained the 1.08 g/mL - 1.09 g/mL and 1.07 g/mL - 1.08 g/mL densities were tested yielding similar results. For the fieldwork, the densities chosen were 1.09 g/mL and 1.08 g/mL, 300 µL of each.

Test-sampling on herring gull took place simultaneously as routinely tagging of birds at Tøyen, Oslo. The birds were handled by Morten Helberg (UiO) and blood samples were taken by me. The herring gulls and one lesser black-backed gull were caught by hand or by manual snare after being lured close with pieces of bread. 5 mL syringes were used, with either orange cannula or blue cannula. The blue is larger, at 0.6 mm diameter, and prevents the blood clogging the needle, while the orange is smaller, at 0.5 mm diameter, and gentler on smaller individuals. The blood samples were taken from the basilic vein, transferred to heparinized tubes (Greiner bio-one, VACUETTE®), and stored in a styrofoam box on ice.

The equipment available on Hornøya was known to be limited, so the centrifugation step of the blood separation could not be performed at 4°C. Thus, blood separation was tested at room temperature with a pre-frozen rotor. The blood samples were kept in heparinised tubes on ice to minimize any DNA degradation and maintain constant temperature. The separation of both chicken and herring gull blood was run at 2000 rpm for 15 min, with the pre-frozen rotor. Some of the samples separated acceptably, but others were unsuccessful. When measuring the temperature of the rotor after centrifugation it was found to have reached room temperature by the end of the 15-min spin. Testing of separation with a room temperature rotor was also conducted, with fresh chicken blood, about 5 minutes old, and 2-hr old herring gull blood. The separation was equally poor for both species. The handling of the blood samples and the sampling itself had no clear differences between samples, so there was no clear indication as to why the separation was either partly successful or a failure. Varying degrees of coagulation could have been the reason to the poor separation. The transfer of the blood from syringe, to tube and further to Eppendorf tube was also considered to be a significant factor.

The separation worked flawlessly when in field on Hornøya. The blood was kept in the syringes until processing proving to be adequate to get excellent separation.

To use the herring gull WBCs in the comet assay, several dilutions were tested. Phosphate-buffered saline with ethylenediamine-tetraacetic acid (PBS-EDTA) was used as dilution medium. 10, 20, 40, and 50 times dilutions of herring gull blood were visually inspected using the comet assay. At least 50 scoreable cells are required for the comet assay (Collins, 2004), and the cells and tails should not be overlapping. The results were varying with no indication as to which dilution resulted in the better distribution of cells in the gels.

The dilution step was relatively easy and quick to perform and did not require addition of ore films, so several dilutions were decided upon; 10, 20 and 40. With the 40 times dilution being the least important and eligible to being dropped if shortage of time, chemicals, or space on the films. WBCs have a tendency to aggregate, and therefore, when extracted, one cannot be sure how many or few cells will be obtained.

Herring gull whole blood was included in the assay as a positive control, and diluted 5000-, and 10 000-fold. Most cells could not be scored, and only the 5000-fold dilution was included for the field work.

2.9 Comet assay

This procedure is based on Gutzkow et al. (2013) as modified by Haarr (2016).

Blood preparation

From the initial 5 mL whole blood sample, 300 µL was centrifuged in a Percoll gradient (™GE Healthcare) at 2000 rpm for 15 min to obtain white blood cells used for the comet assay, according to preliminary testing. The remaining whole blood was transferred to a 15-mL tube (sterile centrifuge tubes, VWR) and kept at -20°C for contaminant analysis. At the lab in UiO this was conducted on a centrifuge pre-cooled to 4°C. This centrifuge was not available at Hornøya, where the centrifugation step was successfully executed at the local room temperature, which was between 10-15°C. The layer of WBCs was extracted with a plastic pipette and diluted 10, 20 and 40 times in PBS-EDTA. Whole blood was diluted 5000 times also in PBS-EDTA. All pipette tips were cut to minimize mechanical damage to the blood cells.

Due to logistic difficulties with the transportation of the reagents to the location on Hornøya, the PBS, used as a dilution medium and in the agarose mix, did not contain EDTA as it should have according to protocol. For the first 13 individuals, all dilution steps were done with PBS without EDTA, only the last two samples were diluted in PBS-EDTA. This is addressed further in sections 3 and 4.

Induction of oxidative stress using hydrogen peroxide (H_2O_2) is commonly applied to the cells in the comet assay to act as a positive control. For this procedure, it was also applied to assess the sensitivity of DNA to oxidative stress. A H_2O_2 concentration of 5 µM was tested on herring gull blood and the preliminary results of the concentration proved adequate, with the resulting damage being measurable and not exceeding 80%.

An incubation step in PBS after the H_2O_2-treatment was included to test the cells' capability to recover after the induced oxidative stress. The incubation was between 4 and 8 hours.

Human blood (own) was used as a reference. The blood was obtained using a finger prick device (VITREX STERILANCE Lite II), and run alongside all samples, receiving all treatments (Sareisian, 2014; Gilmore, 2015).

Gels and film treatment

75 mg of low melting point (LMP) agarose (SIGMA-ALDRICH) was mixed with 10 mL PBS-EDTA in a 15-mL tube, this mix was then heated until it was clear without crystals.

Exactly 90 µL of agarose mix for each blood sample was added to Eppendorf tubes (1.5 mL) prepared on a heating block at 37°C. Ten µL WBCs from each sample was added to the agarose in the Eppendorf tubes. The agarose and WBCs were mixed by pipetting up and down, and swirling pipette tip gently around in the Eppendorf tube. From this WBC-agarose mix, the samples were applied as 20-µL gels on Gelbond® films. The films were placed on pre-refrigerated aluminium plates at 4°C to allow the gels to set. Each individual herring gull had WBC 10-, 20-, and 40 dilutions, and whole blood 5000-fold diluted. After application, the gels were left for a little while to set completely.

Identical gels were moulded on three films for each sample, the first was for baseline DNA damage, the second was for the H_2O_2 exposure, and the third was for the recovery treatment, following H_2O_2 exposure. Films receiving no treatment were placed directly into lysis buffer after the gels had set. The lysis buffer was prepared fresh from stock solution for each use, and refrigerated to be used at 4°C. The films with H_2O_2 treatment and recovery treatment were

placed in boxes containing 5 µM H_2O_2 at 4°C for 15 min in the dark. The H_2O_2 treatment-films were then placed in lysis buffer. The recovery treatment was rinsed in PBS and placed in a box containing new PBS for 4-8 hours. After this incubation step, the recovery-films were placed in lysis buffer. The films were incubated in the lysis buffer overnight in the dark at 4°C.

Electrophoresis and fixation

After the lysis, all films were rinsed for 5 minutes in freshly made, 4°C electrophoresis buffer prepared from stock solution. Then films were mounted on special racks and placed into the electrophoresis chamber, parallel to the chamber platform and all cut corners to the lower right. A spirit level was used to ensure the positioning of the films was level. The electrophoresis chamber contained about 1.4 L of electrophoresis buffer at 4°C, at Hornøya it was placed in a large styrofoam box containing water and ice to keep the buffer cool. In the lab at UiO, the electrophoresis chamber was placed into a refrigerator. The films were left for 15 min in the chamber with a pump circulating the buffer to keep constant temperature. After 20 min total in electrophoresis buffer, the electrophoresis was run for 25 min at 24 V. DNA is negatively charged and will be pulled from the cell nucleus and drawn towards the positively charged anode. The fragments of the DNA will travel differently through the porous agarose gel depending on their size, more fragmented DNA will give the cells the characteristic "comet" appearance when stained and observed under fluorescence microscope.

After electrophoresis, all films were rinsed for 5 minutes at room temp in neutralizing buffer, and then placed in fresh neutralizing buffer for 10 min, leaving the films in the buffer for a total of 15 min. The films were fixated by first rinsing them briefly in dH_2O, and then in ethanol for 5 min, before being left in fresh ethanol for 3-24 hours. After the fixation, the films were left to air dry overnight, and were stored, separated by sheets of paper, in a box and kept away from direct sun light.

Stain and score

Staining and scoring of the films were performed at the Department of Biosciences (UiO). Films were incubated in 50-mL of a room-tempered staining solution containing TE-buffer (Tris-EDTA) mixed with 50 µL SYBR® Gold, on a rocking table for 20 min. The SYBR® Gold binds to double and single stranded DNA and will emit a fluorescent light making it possible to measure using a fluorescence microscope. The SYBR® Gold is light sensitive and will thus degrade if exposed to light, the staining and scoring was therefore performed in the dark, limiting any exposure to direct light.

For scoring the cells (nuclei) and determining the DNA damage the films were placed on a plexi glass plate covered with dH_2O. After removing the excess water the capillary forces will ensure the film do not move during scoring. A cover slide was placed on top of the film in a similar manner. Any excess water was removed with tissue paper touching the edges of the plexi glass. To visualize the DNA a fluorescence microscope (Zeiss Axio Scope A1, ex/em 520/610) with a 20x objective coupled to a camera (Allied Vision Technologies) was used. The scoring of the cells was conducted using the Comet Assay IV software (Perspective Instruments, version 4.2). This software that determines DNA fragmentation (%). Fifty cells per sample was scored, and overlapping or irregular cells were avoided, as well as cells near the edges of the gels or near any air bubbles or gel irregularities.

2.10 Molecular determination of sex

Hornøya herring gulls were generally larger than the Oslofjord individuals. Barth (1968) showed an increase in herring gull body size from south to north in Norway, indicating the females might be generally larger in the northern location. Using previous sex determination by measuring the total length of head and bill was considered unsatisfactory for these larger gulls, and molecular determination of sex was performed.

2.10.1 DNA clean-up

The DNA clean-up followed the steps of the DNeasy kit from QIAGEN. Th blood samples for sex determination were mixed with ethanol (70%) and stored at 4°C. Prior to the procedure the ethanol was carefully pipetted off, leaving the whole blood samples.

Twenty µL proteinase K (DNeasy kit) was pipetted into a 1.5 mL Eppendorf tube. Between 5-10 µL anticoagulated blood was added to the proteinase K, the mix was pipetted up and down to ensure no blood was left in the pipette tip. The procedure suggests using 10 µL of anticoagulated blood for bird samples, but for some of the samples there was not enough material. Any interfering and contaminating proteins in the blood is lysed by the proteinase K, the nucleases that would degrade the DNA is also deactivated by this enzyme. The next step was addition of PBS to a total volume of 220 µL.

Exactly 200 µL buffer AL from the kit was added to the mixture and the tube was capped and thoroughly mixed by vortex. The buffer AL is a lysis buffer that will remove the cell membranes in the samples, leaving DNA free to bind to the DNeasy Mini spin column used in the next step. Incubation at 56°C for 10 min allowed for optimal DNA-binding conditions. Next, 200 µL of ethanol (96%) was added to the sample and the mix was thoroughly vortexed.

The mixture was pipetted into a DNeasy Mini spin column placed in a 2-mL collection tube. The tubes were centrifuged at 8000 rpm for 1 min, and the flow-through and collection tube was discarded. During centrifugation, the DNA in the mixture will selectively bind to the membrane in the column while other contaminators pass through. For the first washing step the DNeasy Mini spin column was placed in a new 2-mL collection tube and 500 µL of the buffer AW1 (DNeasy kit) was added. The tubes were centrifuged for 1 min at 8000 rpm, and flow-through and collection tube was discarded. For the second washing step the spin column was placed in a new 2-mL collection tube and 500 µL Buffer AW2 (DNeasy kit) was added. The tubes were then centrifuged for 3 min at 14 000 rpm, after this step the DNeasy membrane should also be dry. The flow-through and collection tube was discarded. The two washing buffers were added to remove any remains of the proteinase K and ethanol. Residual ethanol may interfere with subsequent reaction steps with the DNA, so any membranes that were not dry were centrifuged one more time at 14 000 rpm for 1 min.

The DNeasy Mini spin column was placed in a clean, pre-marked 1.5 ml Eppendorf tube, and 200 µL buffer AE (DNeasy kit) was pipetted directly onto the DNeasy membrane. The membrane was incubated at room temperature for 1 min and then centrifuged at 8000 rpm for 1 min to elute DNA into the Eppendorf tube. The DNA concentrate was stored at -20°C.

Quantification of DNA

To measure DNA concentration of the DNA extract Qubit™ 2.0 and 3.0 Fluorometers (ThermoFisher, SCIENTIFIC) were used.

In fume hood, 198 µL buffer (Qubit dsDNA HS) was mixed with 1 µL fluorochrome (Qubit dsDNA HS reagent 200x concentration in DMSO). One µL sample was added to 199 µL mix and vortexed briefly. To calibrate the Qubit, a buffer-fluorochrome mix with two different standards (STD 1 and STD 2) had to be measured before the samples. The Qubit uses a fluorescent dye that will only emit a signal when bound to the target, in this case DNA. Readings are accurate even at low concentrations, but in order for the following polymerase chain reaction (PCR) to work adequately the desired concentrations should not be lower than 20 ng/mL.

2.10.2 PCR and visualization

The PCR mix contained 12.5 µL GreenMix (GoTaq®), H_2O (adjustable), DNA concentrate between 1-10 µL and 1.5 µL of each primer for determination of sex; 2550F and 2718R (Fridolfsson & Ellegren, 1999). The PCR-program was set to run 94°C for 3 minutes, then 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 2 minutes, these three steps were repeated for 35 cycles. This allows for denaturation of the double stranded DNA, the annealing of polymerases and the extension of new DNA. The following step is 10 minutes of 72°C before a storing stage at 8°C, the samples may be left overnight at this storing stage.

To run electrophoresis on the PCR products a 2% agarose gel was made with 2 g agarose (SeaKem®LE) mixed with 100 mL TAE-buffer (Tris-acetate-EDTA). Five µL GelGreen was added in fume hood, and mixed gently. The gel was left to set for 60 min. Ten µL of each sample were loaded in separate wells, a quick load 100 bp DNA ladder was put in the first well for reference. Electrophoresis was run for 60 min. The sex of the herring gulls was determined by the number of bands on the gel. The heterogametic female (ZW) had two bands, while the homogametic male (ZZ) had only one band (Fridolfsson & Ellegren, 1999).

2.11 Data treatment and statistical analyses

All statistics were done using *R* (R Core Team, 2017), with significance level set to 0.05. Figures were created using both *R* and Microsoft Excel (2016).

Normal distribution of data was assessed using the Shapiro-Wilk test. Homogeneity of variance was assessed using the Fligner-Killeen test, this test was used for both normal distribution and non-normal distributed data as it is robust against departures from normality. When the normality assumption was met, both two sample t-test (t-test) and Welch two sample t-test (Welch test) was used to compare two groups. The Welch test makes no assumption of the variance of the data. In a few instances a paired two sample t-test was used. When the data did not fit the normality assumption, the Wilcoxon rank sum test (Wilcoxon test) was used, comparing the sample means of two independent groups. The Wilcoxon test was used in conjunction with Kolmogorov-Smirnov test (KS-test), which tests if the two groups have the same continuous distribution. The KS-test is good for small datasets, and was included to further assure the strength of the non-parametric tests, which are less robust than the parametric tests.

2.11.1 Data below the limit of detection and limit of quantification

Contaminants with >20% values below the limit of detection were excluded from the data treatment in this thesis to avoid the creation of false patterns, false correlations and false significant differences that often follows fabrication of large amount of data (Helsel, 2006). Thus, any contaminant included in the statistical analyses were above LOD for 80% or more of the samples. When as much as 80% of the data is above LOD, the remaining non-detects are assumed to be rather close to LOD. A random value between 0.5×LOD and LOD was generated using the RAND-function in Excel. A total of 14 (of 781 data points) substitutions were made, representing 1.8% of the dataset used in the statistical treatment. For the cVMS results, the limit of quantification (LOQ) was reported, and these values are used, but results must be treated with caution, this is addressed in section 4.2.5.

2.11.2 Contaminants

According to Henriksen et al. (1998) blood concentrations are comparable to tissues, and blood sampling is a non-destructive method allowing for repeated measurements and integration in long term studies. Contaminant levels were measured and used in ng/g wet weight (w.w.). The herring gull blood contains very low amount of lipids and small volumes were used, thus, the gravimetric determination of lipid content in blood becomes unprecise, and might be influenced by e.g. particles from the clean-up processes.

The PCBs were grouped according to the number of chlorine substitutions. The PBC homologs present in the samples of this thesis are tetraCB (4), pentaCB (5), hexaCB (6) and heptaCB (7), the lower chlorinated congeners (tetraCB) have been shown to constitute a larger proportion of the PCBs in water, while the higher chlorinated (penta-, hexa- and hepta-) biphenyls are the most bioaccumulative congeners (McFarland & Clarke, 1989).

Multivariate analyses were conducted using the *vegan* package in *R* (Oksanen et al., 2017), and the contaminant concentrations were log10 transformed when used in this analysis to ensure normality.

Principal component analysis

Principal component analysis (PCA) is a multivariate method that works well with large datasets with many different variables, as is often seen in ecotoxicological studies. The set of samples, having possibly correlated variables, are converted into a set of orthogonal variables,

called principle components (PC). The first PC accounts for as much variation in the data as possible. The following PCs account for progressively as much variation as possible, while still upholding the orthogonal constraint (Ruus et al., 2002). The PCA in this thesis was used to analyse the pattern and variation of contaminant concentrations in the individual herring gulls, and is represented by triplots. PC1 is presented as the x-axis and PC2 as the y-axis. Sites (in this case individual birds) are represented as points, response variables (contaminants) represented by vectors, and explanatory variables (location, TL, sex, baseline tail intensity, lipid content) are represented as passive vectors. The variables (contaminants) loads the PC1 and PC2 in the plot, and the length of the vectors indicate how much each variable contributes to the variation in the data. The cosine of the angle between vectors corresponds to the correlation between the vectors, meaning for instance vectors perpendicular to each other are uncorrelated, as the cosine of 90° equals 0. Vectors pointing same or opposite directions have a high positive or negative correlation, respectively (Sparks et al., 1999).

Redundancy analysis

Redundancy analysis (RDA) is a direct gradient analysis technique, summarizing linear relationships between response variables that are explained by, or redundant with, explanatory variables. RDA is a multivariate analogue of multiple linear regression, extending it by performing a regression of multiple response variables on multiple explanatory variables (Ramette, 2007). The RDA was conducted with location, TL, sex, baseline tail intensity and lipid content as explanatory variables, against contaminant concentrations as response variables. Through the RDA, the significant explanatory variables are determined, and used for further statistical analyses.

Post hoc analysis

Analysis of covariance (ANCOVA) was used to quantify relationships between response and explanatory variables. Any significance indicated by the RDA of the PCA, was tested using analysis of variance (aov and ANOVA), to determine significance of the explanatory variables that appeared to have an effect in the response variables.

Intercorrelation of DNA and contaminants

To assess association between the baseline DNA damage in herring gulls and contaminant levels, PC1, PC2 and PC3 site scores (individual herring gulls) from the PCA were extracted and tested with baseline DNA damage using linear regression analyses. Linear models (lm) were used to test if the baseline DNA damage could be explained by any of the PCs.

2.11.3 Body condition index

Body condition index (BCI) was calculated for each herring gull to control for any individual differences body mass, size, sex and location. The BCI was calculated using a multiple linear regression model. The body mass was the response variable, and head length + wing length were predictor variables, sex and location were included as significant factors (Fox et al., 2007). The residuals of the regression were defined as body condition index (Jakob et al., 1996). A negative BCI indicate that the gull has below average body condition than expected for that size and sex, while a positive BCI indicates above average body condition as expected from the regression. The BCI is per definition unit less and was included in the statistical analyses.

2.11.4 Trophic level

The $\delta^{15}N$ signature was used to calculate the trophic level (TL) of the herring gulls, which was included in the statistical analyses. Developed by Hobson and Welch (1992), Equation 2 is used to determine the trophic level of a primary consumer. $TL_{consumer}$ is determined by use of the consumer's $\delta^{15}N$; the number 3.8, which is the isotopic enrichment factor between trophic levels; and the number 1, which is added to indicate the movement from TL 1 (primary producer) to TL 2 (consumer). The number 3.8 has been shown to be the permille (‰) increase of the $\delta^{15}N$ signature from one trophic level to the next in the marine food webs (Hobson & Welch, 1992).

$$TL_{consumer} = 1 + (\delta^{15}N_{consumer} - \delta^{15}N_{primary\ producer})/3.8 \qquad \text{Equation 2}$$

To calculate the TL of the herring gull in this thesis, Equation 2 was modified, with values suggested by Mizutani et al. (1991), according to Fisk et al. (2001) and Ruus et al. (2002) to Equation 3. The number 2.4 the isotopic enrichment factor between the diet and the seabird, as Mizutani et al. (1991) showed an increase in $\delta^{15}N$ from piscivorous birds' diet to their tissue of 2.4‰. A possible explanation for this lower fractionation is the produce of uric acid as waste rather than urea (Mizutani et al., 1991). The $TL_{herring\ gull}$ is defined as $TL_{consumer}$ + 1, and $\delta^{15}N$ signature for herring gull is $\delta^{15}N_{consumer}$ + 2.4. $TL_{consumer}$ is in this case $TL_{blue\ mussel}$, and $\delta^{15}N_{consumer}$ is $\delta^{15}N_{blue\ mussel}$. The blue mussel (*Mytilus edulis*) was chosen as the representative baseline of the primary consumer with TL defined as 2.

$$TL_{herring\ gull} = 3 + (\delta^{15}N_{herring\ gull} - (\delta^{15}N_{blue\ mussel} + 2.4))/3.8 \qquad \text{Equation 3}$$

The $\delta^{15}N$ signature in blue mussel have been shown to differ along the Norwegian coast according to a report by Green et al. (2016), signatures from a northern location and an urban location were obtained from this report. The northern location, Brashavn ($\delta^{15}N$ = 6.46), is relatively close to Hornøya, and the urban location, Gressholmen ($\delta^{15}N$ = 7.3), is close to Søndre Skjælholmen (Figure 4).

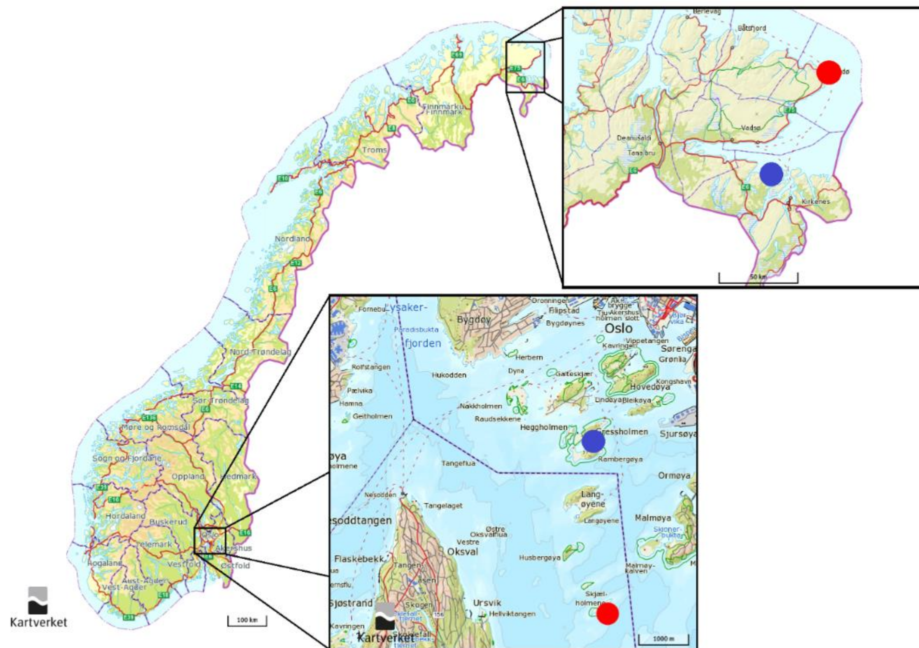

Figure 4. Map of Norway, the red dots indicate herring gull sampling locations for this thesis, Hornøya in the north, and Oslofjord (Søndre Skjælholmen) in the south. The blue dots indicate the location of the blue mussels sampled for the report by Green et al. (2016), Brashavn in the north, and Gressholmen in the south. Map from Kartverket.no.

2.11.5 DNA damage

For each individual gel 50 cells are scored. The Comet Assay IV computer software uses calculates the tail intensity (%), and this is used as measure for DNA damage. The median of these 50 cells is calculated and used for representation of the DNA damage in each individual gull. The tail intensity was log10 transformed to fit a normal distribution and stabilize variance as suggested by Mitchell and Brice (1986). If any difference was detected between males and female in the Hornøya samples, the location comparison was conducted using only the female herring gulls. Whenever there was no difference between genders, the males and females were pooled for further comparison.

3 Results

3.1 Biometric data

Biometric measurements of herring gulls are shown in Table 1, with mean ± standard deviation (SD), range (Min – Max) is also shown. For individual measurement see Appendix B.

Table 1. Mean ± standard deviation (SD) and range (Min – Max) measures of biometric variables in the herring gull of Oslofjord (n=15) and Hornøya (n=15), the latter split into females (n=7) and males (n=8).

	Oslofjord	Hornøya	
Mean ± SD Min – Max	Females n=15	Females n=7	Males n=8
Body mass (g)	927.9 ± 50.07 810.0 – 1010	1010 ± 34.64 960.0 – 1070	1277 ± 41.12 1230 – 1340
Wing (mm)	427 ± 11.5 411 – 448	426 ± 7.56 415 – 436	454 ± 12.8 437 – 474
Head (mm)	117 ± 2.77 112 – 121	121 ± 4.00 116 – 128	134 ± 3.93 131 – 142
Lipid (%)	1.3 ± 1.1 0.40 – 4.2	0.79 ± 0.22 0.46 – 1.1	0.64 ± 0.14 0.47 – 0.87
Body Condition Index (BCI)	<0.0010 ± 37 -74 – 59	0.014 ± 33 -45 – 54	<0.0010 ± 41 -44 – 63
Trophic level (TL)	2.9 ± 0.40 2.4 – 3.7	4.5 ± 0.10 4.3 – 4.7	4.7 ± 0.12 4.6 – 4.9

Hornøya females and males did not differ in body condition index (BCI) (t-test, $p>0.05$). Similarly, there was no difference between Hornøya and Oslofjord in BCI (t-test, $p>0.05$).

The males of this species are larger than the females, thus, only the females of both locations were compared by size.

Female herring gulls from Hornøya had a higher body mass, and larger head-beak size than the females from the Oslofjord (t-tests, $p<0.05$). The wing length of the females showed no difference between the colonies (t-test, $p>0.05$).

One herring gull individual from Oslofjord had an estimated lipid content of 14.8%, about 13 SDs from the mean, 1.3% (excluding the outlier). There was not enough material to run a new analysis and recalculate the estimate, but it is assumed to be an analytical error. In the following results, this data point has been removed, but the other variables measured for the individual are included as this assumed error was not reflected in the contaminant levels in this individual.

There was no difference in lipid % between males and females from Hornøya (t-test, $p>0.05$). Thus, the males and females from Hornøya were pooled, and had lower lipid content in their blood compared to the Oslofjord gulls (t-test, $p<0.05$).

3.2 Stable isotopes

Stable isotope ratios of C, N and S was analysed in the blood samples. The Oslofjord gulls appear to be separated into two groups by the $\delta^{13}C$ (t-test, $p<0.05$, Figure 5). Based on the fact that a more depleted $\delta^{13}C$ indicates a relatively higher terrestrial carbon source, and an enriched $\delta^{13}C$ would indicate a relatively higher marine source (see section 1.4), the Oslofjord gulls were divided into two groups, supposed "marine feeders" and "terrestrial feeders". The cut-off was set at $\delta^{13}C$ between -23.5 and -22.5, this is about the middle of the range, making it a natural division, and there is a clear separation between the two clusters of individuals. The gulls on the right of this cut-off, the "marine" feeders, likely have more marine components incorporated in their diet, compared to the gulls on the left side of the cut-off, the "terrestrial" feeders.

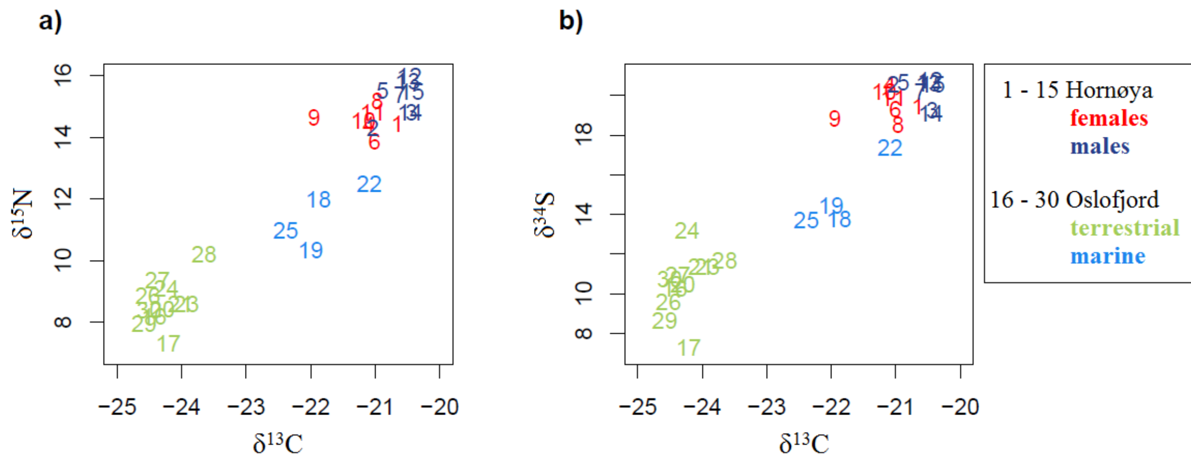

Figure 5. Stable isotope ratios of a) $\delta^{15}N$ (‰) plotted with $\delta^{13}C$ (‰), b) $\delta^{34}S$ (‰) plotted with $\delta^{13}C$ (‰). Individuals from Oslofjord (terrestrial n=11, marine n=4) and Hornøya (females n=7, and males n=8) plotted by individual ID (1-30).

The $\delta^{13}C$ signatures within Hornøya gulls showed the most enrichment for all samples, indicating the feeding of these birds is primarily from the marine environment (Figure 5). Four individuals from the Oslofjord considered to be "marine feeders" showed similar enrichment. However, $\delta^{13}C$ signature from "marine feeders" in the Oslofjord were statistically lower compared to gulls from Hornøya, indicating $\delta^{13}C$ within these birds may represent a mixed feeding signature of marine and terrestrial carbon sources. The marine feeders in the Oslofjord are most likely not feeding solely from the marine environment by represent more of a mixed diet of marine and terrestrial sources. This is supported by all isotope data, with the terrestrial feeders in the Oslofjord having the most depleted isotope ratios, and the Hornøya gulls the most enriched (Figure 5).

3.3 Contaminants

A total of 20 compounds are included and compared in this thesis (Table 2). Compounds not included (Table 3) were either below limit of detection i.e. not detected (n.d), not analysed (N.A) in one of the sample batches or not included (n.i) due to n.d or N.A in the other sample batch. Contaminant concentration mean ± SD, range and number of limit of detections (LODs) for all contaminants analysed, for either Oslofjord or Hornøya, is included in Appendix A.

Table 2. Mean ± standard deviation (SD) and range (Min – Max) contaminant concentrations (ng/g w.w.) in herring gulls, Oslofjord split into terrestrial (n=11) and marine (n=4) feeders, and Hornøya males and females pooled (n=15). The PCB homologs are included in parentheses, and indicates the number of chlorine substitutions on the biphenyl.

	Oslofjord		Hornøya	
Mean ± SD Min – Max	Terrestrial n=11	Marine n=4	Females n=7	Males n=8
D4	2.3 ± 0.39 2.0 – 3.0	2.1 ± 0.21 2.0 – 2.4	1.7 ± 0.44 1.5 – 2.5	1.9 ± 0.33 1.6 – 2.4
D5	2.8 ± 2.6 1.3 – 10	2.8 ± 1.2 1.7 – 4.0	1.6 ± 0 1.6 – 1.6	1.6 ± 0 1.6 – 1.6
D6	2.2 ± 0.59 1.1 – 2.5	2.0 ± 0.81 1.1 – 2.5	2.1 ± 0.11 2.0 – 2.3	2.2 ± 0.22 2.0 – 2.4
HCB	0.28 ± 0.32 0.063 – 1.2	0.32 ± 0.33 0.060 – 0.78	2.5 ± 0.32 2.2 – 3.8	2.7 ± 0.50 2.2 – 3.8
PCB 47 (tetra)	0.13 ± 0.093 0.021 – 0.35	1.2 ± 1.5 0.14 – 3.5	0.34 ± 0.12 0.21 – 0.54	0.38 ± 0.24 0.25 – 0.96
PCB 66 (tetra)	0.28 ± 0.26 0.037 – 0.99	2.7 ± 3.2 0.40 – 7.5	0.71 ± 0.22 0.47 – 1.0	0.88 ± 0.53 0.58 – 2.2
PCB 99 (penta)	0.82 ± 0.62 0.091 – 2.0	5.9 ± 7.1 0.78 – 16	2.5 ± 0.99 1.5 – 4.3	3.3 ± 2.4 2.0 – 9.1
PCB 105 (penta)	0.41 ± 0.32 0.039 – 1.0	3.4 ± 4.3 0.45 – 9.8	1.4 ± 0.35 0.93 – 2.0	2.1 ± 1.7 1.1 – 6.3
PCB 118 (penta)	1.4 ± 1.1 0.17 – 3.4	9.4 ± 12 1.4 – 27	4.6 ± 1.4 3.0 – 7.1	6.9 ± 5.5 4.0 – 20
PCB 128 (hexa)	0.32 ± 0.23 0.060 – 0.79	2.8 ± 3.9 0.32 – 8.6	1.6 ± 0.57 0.99 – 2.7	2.4 ± 2.2 1.4 – 7.7
PCB 138 (hexa)	2.6 ± 1.8 0.51 – 5.7	14 ± 16 2.7 – 38	9.9 ± 3.7 6.4 – 17	15 ± 912 8.7 – 43

PCB 153 (hexa)	3.7 ± 2.4 1.0 – 8.4	17 ± 17 3.3 – 42	13 ± 5.9 8.1 – 26	20 ± 16 11 – 58
PCB 180 (hepta)	0.74 ± 0.62 0.20 – 1.9	3.2 ± 3.2 0.39 – 7.9	4.3 ± 1.5 2.7 – 7.2	6.1 ± 5.0 2.8 – 18
PCB 183 (hepta)	0.35 ± 0.32 0.064 – 1.1	1.6 ± 1.9 0.28 – 4.4	2.1 ± 1.6 0.92 – 5.6	2.4 ± 1.1 1.2 – 4.3
PCB 187 (hepta)	0.95 ± 0.57 0.30 – 2.1	5.0 ± 5.8 1.0 – 14	0.87 ± 0.31 0.52 – 1.5	1.2 ± 0.95 0.64 – 3.5
PFOS	6.7 ± 10 1.5 – 37	25 ± 21 8.8 – 5	15 ± 11 5.3 – 38	21 ± 17 10 – 62
PBDE 47	0.16 ± 0.086 0.018 – 0.28	0.81 ± 0.85 0.21 – 2.1	1.05 ± 0.68 0.48 – 2.3	0.83 ± 0.41 0.45 – 1.7
PBDE 99	0.12 ± 0.097 0.0070 – 0.37	0.14 ± 0.078 0.065 – 0.25	0.60 ± 1.3 0.036 – 3.6	0.10 ± 0.079 0.039 – 0.27
PBDE 100	0.040 ± 0.023 0.0060 – 0.071	0.17 ± 0.17 0.051 – 0.43	0.36 ± 0.30 0.14 – 0.80	0.25 ± 0.12 0.12 – 0.47
PBDE 153	0.029 ± 0.017 0.0050 – 0.069	0.027 ± 0.016 0.010 – 0.047	0.19 ± 0.34 0.020 – 0.95	0.083 ± 0.092 0.026 – 0.30

Table 3. List of contaminants not analysed (N.A), not detected (n.d), and not included (n.i) in both Hornøya and Oslofjord samples.

Contaminant	Oslofjord	Hornøya	Contaminant	Oslofjord	Hornøya
6:2FTS	n.d	n.d	αHCH	n.d	n.i
PFOSA	n.d	n.d	βHCH	n.d	n.i
PFBS	n.d	n.d	γHCH	n.d	n.d
PFPS	n.d	n.d	δHCH	n.d	NA
PFHxS	n.i	n.d	PCB 28	n.d	n.i
PFHpS	n.d	n.d	PCB 52	n.d	n.i
brPFOS	NA	n.d	PCB 71	NA	n.i
PFNS	n.d	n.d	PCB 101	n.d	n.i
PFDcS	n.d	n.d	TBA	n.d	NA
PFHxA	n.d	n.d	PBDE 17	n.d	NA
PFHpA	n.d	n.d	PBDE 28	n.d	n.d
PFOA	n.d	n.d	PBDE 49	n.d	NA
PFNA	n.d	n.i	PBDE 66	n.d	NA
PFDcA	n.d	n.i	PBDE 71	n.d	NA
PFUnA	n.d	n.i	PBDE 77	n.d	NA
PFDoA	n.d	n.d	PBDE 85	n.d	NA
PFTriA	n.d	n.i	PBDE 119	n.d	NA
PFTeA	n.d	n.d	PBDE 126	n.d	NA
o,p-DDE	n.d	n.d	PBDE 138	n.d	n.d
p,p-DDE	n.d	n.i	PBDE 154	n.d	n.d
o, p DDD	n.d	n.d	PBDE 156	n.d	NA
p, p-DDD	n.d	n.d	PBDE 183	n.d	n.d
o,p-DDT	n.d	n.d	PBDE 184	n.d	NA
p,p- DDT	n.d	n.d	PBDE 191	n.d	NA
PeCB	n.d	n.i	PBDE 196	n.d	n.d
oxychlordane	NA	n.i	PBDE 197	n.d	n.d
trans-chlordane	NA	n.i	PBDE 202	n.d	NA
cis-chlordane	NA	n.i	PBDE 206	n.d	n.d
trans-nonachlor	NA	n.i	PBDE 207	n.d	n.d
cis-nonachlor	NA	n.i	PBDE 209	n.d	n.d
mirex	NA	n.i	Mercury	NA	n.i

3.3.1 Concentrations

A principal component analysis (PCA) was conducted to analyse the variation in contaminant concentrations and association with explanatory variables; *Location*, *BCI*, *DNA baseline*, *Lipid*, *Trophic Level* and *Sex*. The principal component (PC) 1, 2 and 3 explained 73%, 12% and 10% of total variation respectively. Inspection of the initial PCA indicated some contaminants were strongly positively correlated. In addition to grouping the polychlorinated biphenyl (PCB) homologs (tetra-, penta-, hexa-, hepta-), D4, D5 and D6 were grouped as cyclic volatile methylsiloxane (cVMS); polybrominateddiphenyl ether (PBDE) 99 and 153 were grouped; and PBDE 47 and 100 were grouped (Figure 6). PC1 was most strongly loaded by hexachlorobenzene (HCB), perfluorooctane sulfonate (PFOS), PBDE 47 and 100, and all the PCBs. PBDE 99 and 153 loaded the PC2, and HCB most strongly loaded the PC3. The initial PCA triplot with all contaminants, and triplots with PC3 are included in Appendix H. Significant explanatory variables were *Location* and *TL* (yellow box, RDA, $p<0.05$), explaining 9.4% and 18% of the variance.

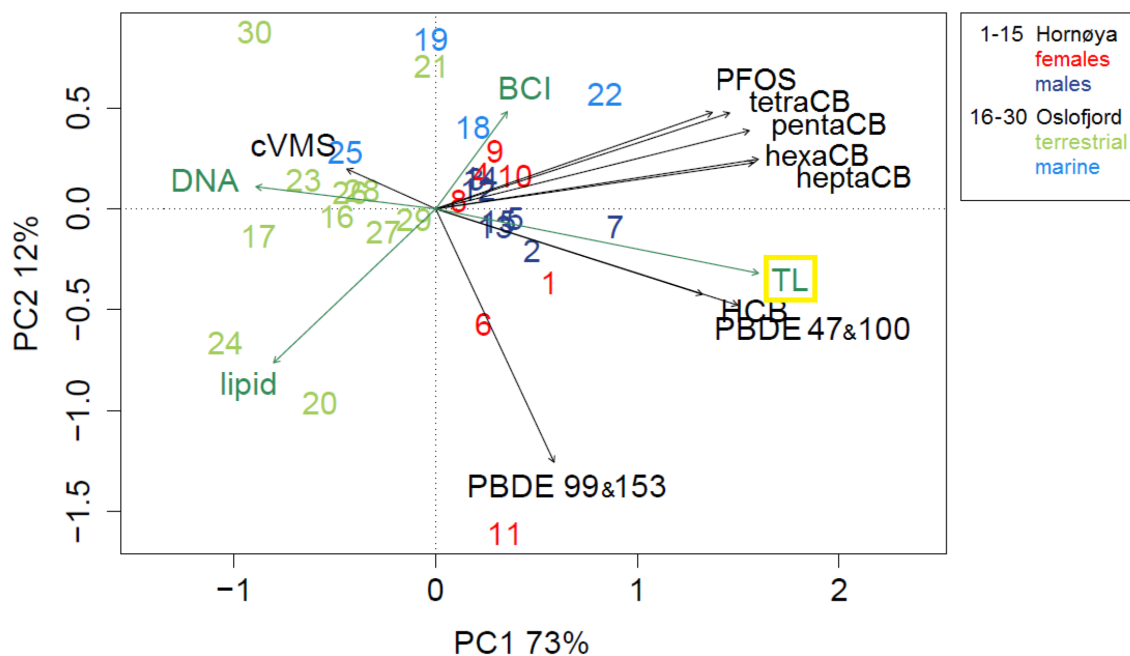

Figure 6. Principal component analysis (PCA) triplot showing environmental contaminant variables (log10 transformed contaminant concentrations, ng/g w.w.) and individual herring gulls (1-30) positioned according to each owns contaminant load (Hornøya females n=7, males n=8; Oslofjord terrestrial n=11, marine n=4). Explanatory variables are shown as passive vectors (green, significant explanatory variables are marked with a yellow box) not affecting the scoring of the samples (individual gulls) or the loadings of the responses (contaminants). The proportion of variance explained by PC1 and PC2 is shown on the x- and y-axis, respectively.

Analysis of covariance (ANCOVA) was conducted to determine difference between independent variable, *Location,* on dependent variables, contaminant groups, controlling for the covariate, *TL*.

For cVMS the location had an effect on the cVMS levels ($F_{1,22}=1.547$, $p>0.05$), while the TL does not affect the cVMS ($F_{1,22}=3.568$, $p=0.07$), the interaction between location and TL is also not significant ($F_{1,22}=0.394$, $p>0.05$), meaning the cVMS levels will depend only on the location regardless of herring gulls TL (Figure 7a).

For HCB, both location ($F_{1,26}=27.24$, $p<0.05$) and TL ($F_{1,26}=248.76$, $p<0.05$) affect the levels in the gulls. The interaction was insignificant ($F_{1,26}=0.00$, $p>0.05$), meaning the levels of HCB will depend in a similar manner on the TL at both locations (Figure 7b).

For ∑PBDE, the location does not affect the levels in gulls ($F_{1,26}=0.044$, $p>0.05$), but the TL has an effect ($F_{1,26}=6.481$, $p<0.05$). Again, the interaction is insignificant ($F_{1,26}=0.844$, $p>0.05$), and the ∑PBDE will depend on the TL, regardless of the location (Figure 7c).

In the level of PFOS, the TL has a significant effect ($F_{1,26}=4.705$, $p<0.05$), and location is barely significant ($F_{1,26}=3.910$, $p=0.0587$). The interaction was insignificant ($F_{1,26}=0.511$, $p>0.05$), meaning the level of PFOS will depend in a similar manner on the TL at both locations (Figure 7d).

The PCB homologs had similar patterns, and for hexaCB, only the location influenced the levels ($F_{1,26}=3.008$, $p<0.05$). The TL ($F_{1,26}=9.664$, $p>0.05$) and the interaction between TL and location ($F_{1,26}=0.276$, $p>0.05$) do not affect the PCB levels in the gulls (Figure 7e). Scatterplots with regression lines of tetra-, penta- and heptaCB are included in Appendix I.

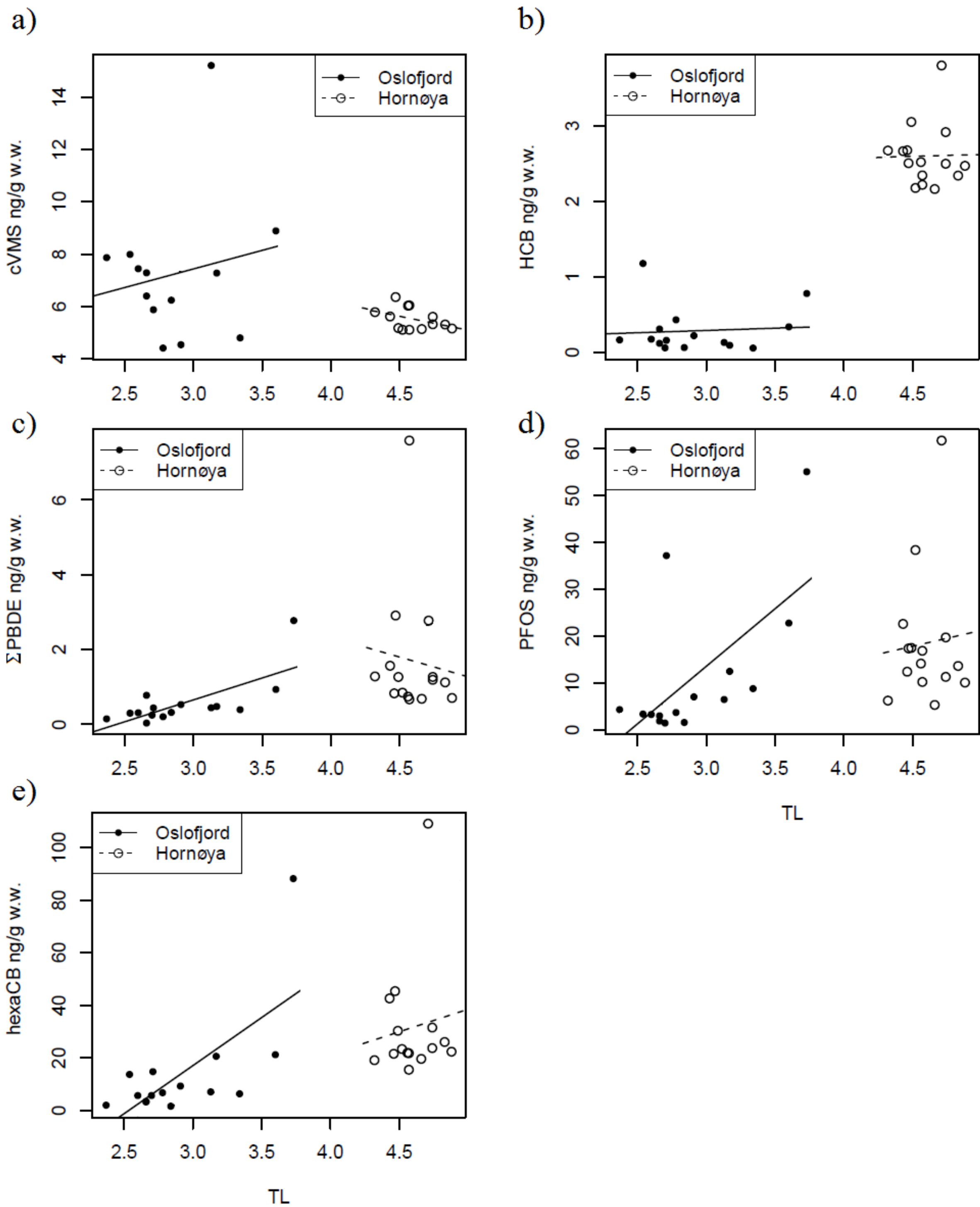

Figure 7. Trophic level (TL) and contaminant concentration (ng/g w.w.) in herring gulls separated into location, Hornøya (n=15) and Oslofjord (n=15). Regression lines are shown.

For the different PCB homologs, no difference was detected between the males and females from Hornøya; tetra-, penta- and hexaCB (Wilcoxon tests p>0.05, KS-tests p>0.05), heptaCB (t-test, p>0.05). Males and females were therefore pooled for location comparison. Between the terrestrial and marine feeders from Oslofjord, a significant difference in concentration was observed with marine feeders having higher levels of all homolog groups (t-test, p>0.05, Figure 8). When comparing the marine feeders of Oslofjord to the Hornøya gulls, no difference was found (Wilcoxon test p>0.05, KS-test p>0.05, Figure 8).

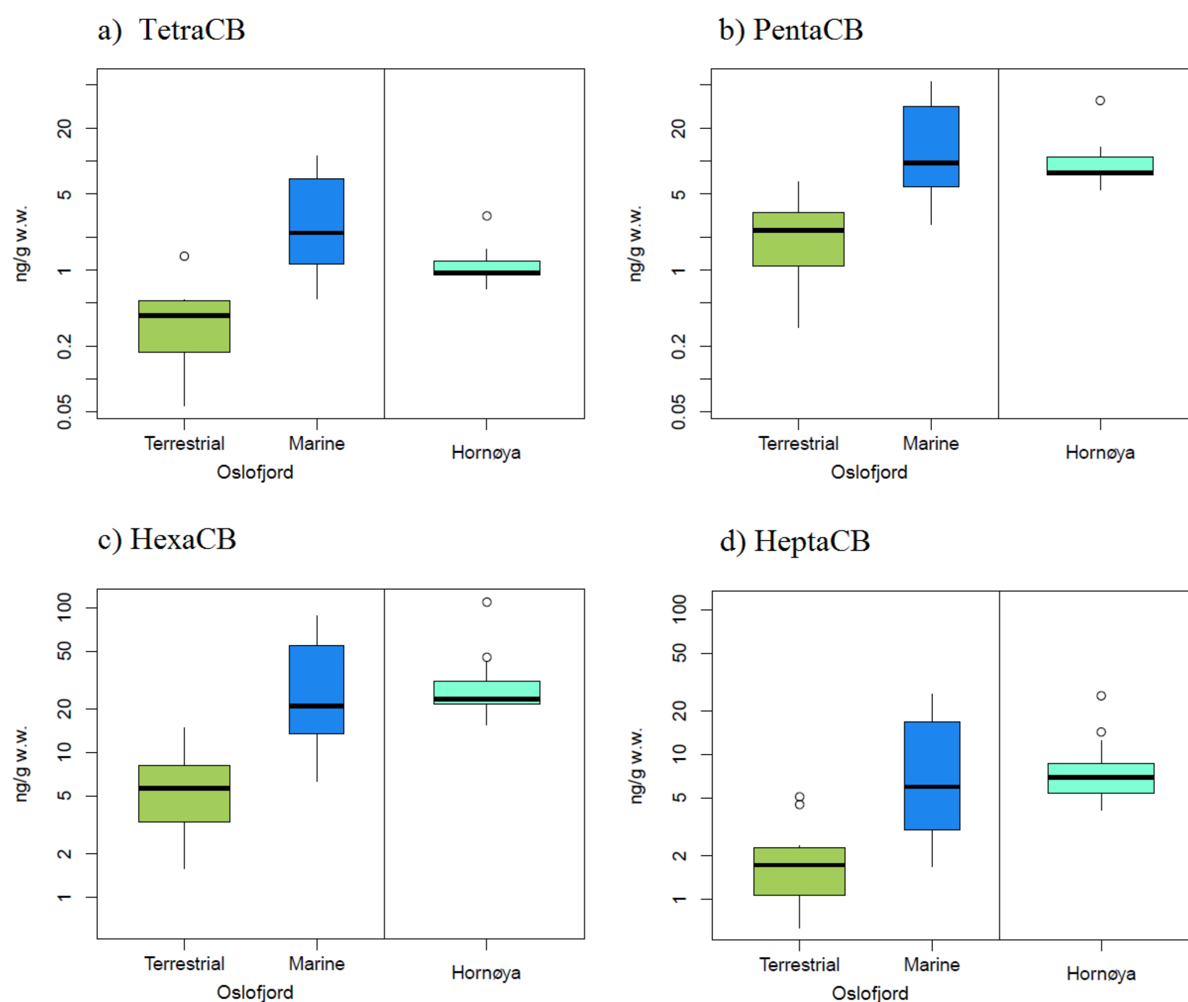

Figure 8. Concentrations (ng/g w.w.) of PCB homologs (tetra-, penta-, hexa-, hepta-) in Oslofjord terrestrial (n=11), marine (n=4), and the Hornøya gulls (n=15). The width of the boxes corresponds to the number of samples in each group. The boxes stretch from first to third quartile, with the horizontal line representing the measurement median. The whiskers extend to 1.5 times the interquartile range, and the points are extreme values, also defined as outliers.

For the cVMSs, there was no difference between Hornøya males and females (Wilcoxon test $p>0.05$, KS-test $p>0.05$), or between Oslofjord terrestrial and marine (Wilcoxon test $p>0.05$, KS-test $p>0.05$). Hornøya males and females, and Oslofjord terrestrial and marine were pooled by location for comparison, the Oslofjord individuals had higher levels than the Hornøya individuals (Wilcoxon test $p<0.05$, KS-test $p<0.05$, Figure 9a).

Similarly, for HCB there was no difference between Hornøya males and females (t-test, $p>0.05$), or Oslofjord terrestrial and marine (t-test, $p>0.05$). Pooling males and females, and terrestrial and marine, a higher concentration of HCB was found in Hornøya individuals (Welch test, $p<0.05$, Figure 9b).

For ∑PBDEs there was no difference between males and females on Hornøya (t-test $p>0.05$), but there was significantly more ∑PBDE in marine feeders, than in terrestrial feeders in Oslofjord gulls (t-test, $p<0.05$). When comparing the Hornøya males and females to the marine feeders, no difference was detected (t-test, $p>0.05$, Figure 9c).

The PFOS levels were not different in Hornøya males and females (t-test, $p>0.05$), but higher levels were detected in marine feeders when compared to terrestrial feeders in Oslofjord gulls (t-test, $p<0.05$). There was no difference when comparing the marine feeders to the Hornøya males and females (t-test, $p>0.05$, Figure 9d).

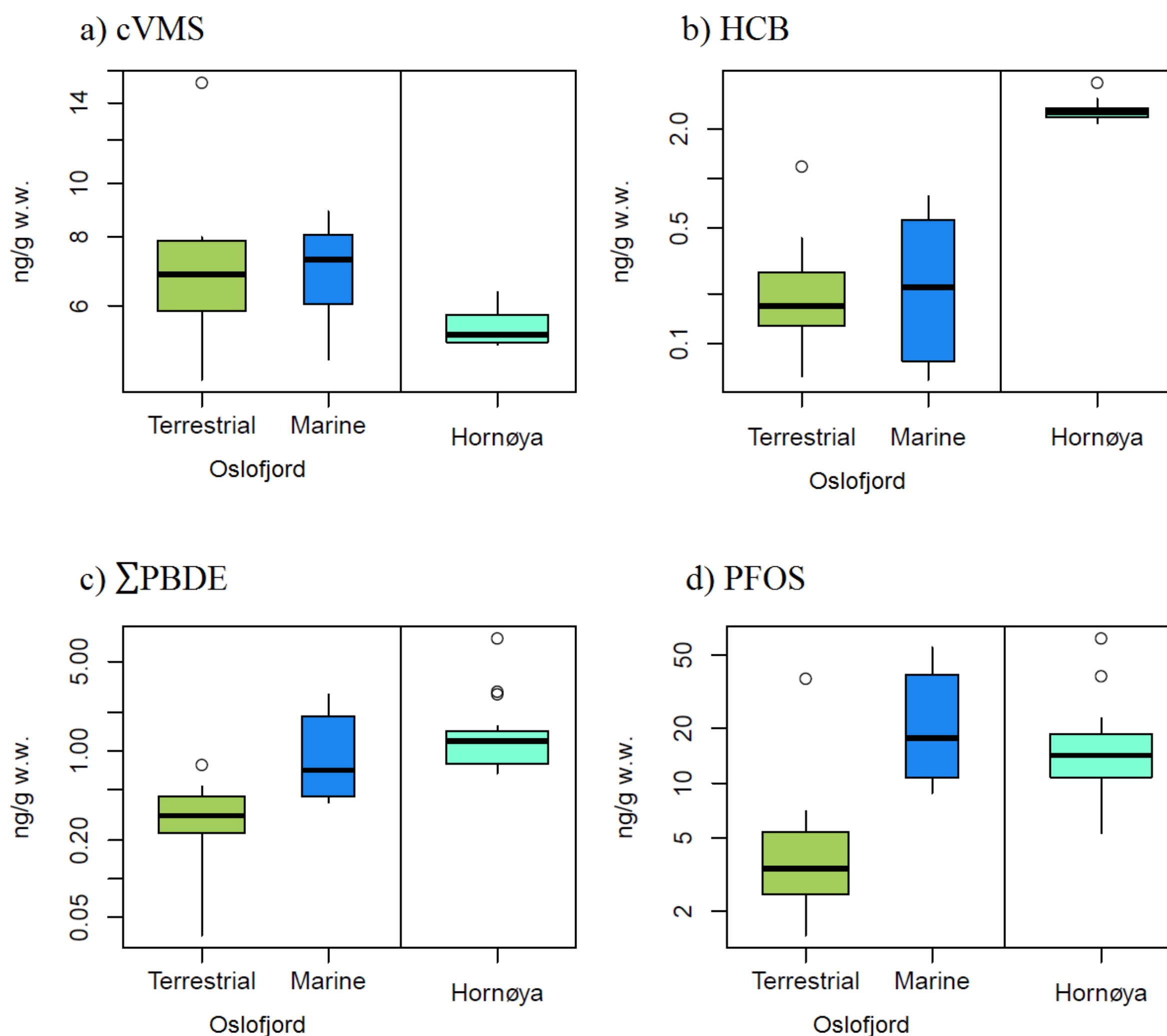

Figure 9. Concentrations (ng/g w.w.) of a) cVMSs, b) HCB, c) ∑PBDE, and d) PFOS in Hornøya herring gulls (n=15), and Oslofjord terrestrial (n=11) and marine (n=4) feeders. The width of the boxes corresponds to the number of samples in each group. The boxes stretch from first to third quartile, with the horizontal line representing the measurement median. The whiskers extend to 1.5 times the interquartile range, and the points are extreme values, also defined as outliers.

3.3.2 Patterns

Relative proportion of the contaminants (Figure 10) show the ∑PCB dominated in both areas, followed by PFOS, with similar proportions at both locations. The ∑cVMS are third most present in the Oslofjord gulls, while HCB is more prominent in Hornøya gulls. In Oslofjord gulls, HCB constituted the lowest proportion of contaminants, while in Hornøya gulls, the ∑PBDE constituted the lowest proportion.

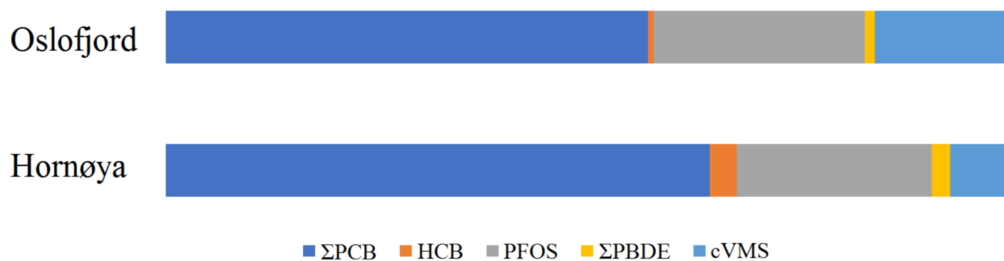

Figure 10. Relative proportions of the contaminant groups in the herring gulls divided into each location, Hornøya (n=15), Oslofjord (n=15). ΣPCB: PCB -47, -66, -99, -105, -118, -128, -138, -153, -180, -183, -187. ΣPBDE: PBDE -47, -99, -100, -153. ΣcVMS: D4, D5, D6.

PBDE 47 was the dominating congener among the PBDEs, followed by PBDE 99 at both locations (Figure 11a). The D5 and D6 only differed slightly between areas, with more D5 in Oslofjord gulls, and more D6 in Hornøya gulls (Figure 11b). The results for the cVMSs are likely influenced by background contributions, and the reliability of these data is discussed further in section 4.2. The Hornøya gulls had a higher proportion of PCB -153, -180 and -183, while the Oslofjord gulls had more of the PCB -66, -99, -105 and -187 (Figure 11c).

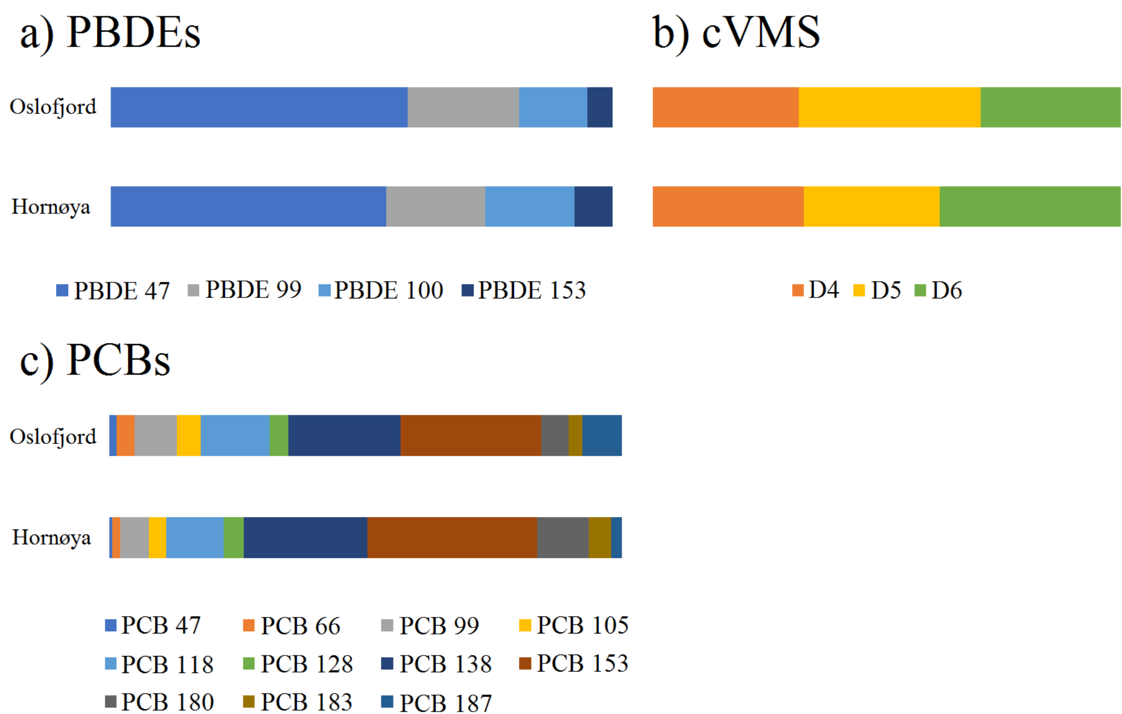

Figure 11. Relative proportions for each contaminant group (PBDEs, cVMS, PCBs) for both Oslofjord (n=15) and Hornøya (n=15).

3.4 DNA strand breaks

The results for DNA damage include test on human blood (my own) as a positive control for the two locations (Figure 12a), assessments of all treatments for both locations on WBCs, as well as assessments of any possible difference between the sexes. Measurements of DNA damage for each individual are presented in Appendix E. No results from the whole blood is presented or used in this thesis, due to overlapping tails in the assay because of high cell density, and almost complete disintegration of the individual cells, attempts at scoring cells revealed up to 90% damage.

3.4.1 Method quality assurance

Tail intensity of own blood treated with Phosphate-buffered saline with ethylenediamine-tetraacetic acid (PBS-EDTA) and with PBS without EDTA was tested to check for any effects of EDTA in the PBS-solution. PBS-EDTA on reference blood included one measurement from Hornøya and two measurements from the Oslofjord (n=3). No significant difference was detected between PBS and the PBS-EDTA in the reference (paired t-test, $p>0.05$, Figure 12a). For the Hornøya herring gulls, PBS-EDTA (n=2) and PBS (n=11), there was no significant difference between the samples treated with EDTA as opposed to the samples without EDTA (Wilcoxon test $p>0.05$, KS-test $p>0.05$, Figure 12b).

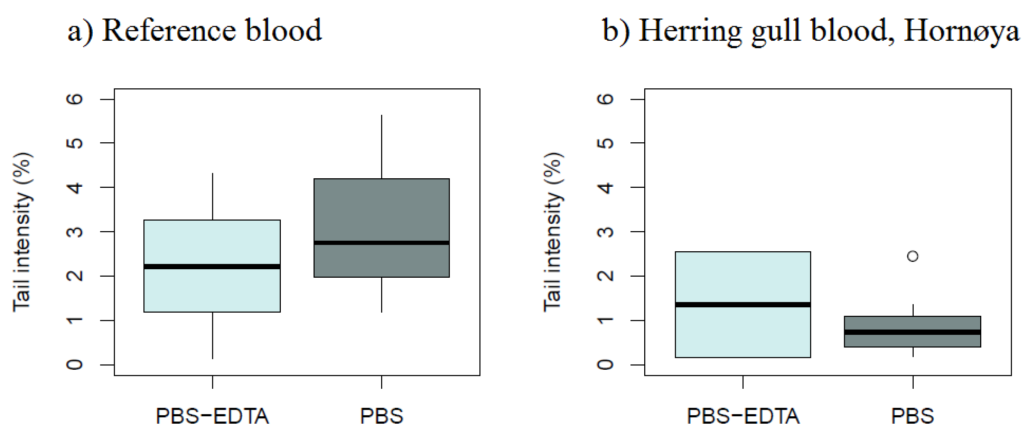

Figure 12. a) Tail intensity (%) comparison in reference blood (my own) between PBS-EDTA (n=3) and PBS (n=3) used for blood treatment. b) Tail intensity (%) comparison in herring gull WBCs from Hornøya between PBS-EDTA (n=2) and PBS (n=11). The boxes stretch from first to third quartile, with the horizontal line representing the measurement median. The whiskers extend to 1.5 times the interquartile range, and the points are extreme values, also defined as outliers.

An observed outlier in the data was one baseline film where the reference had a tail intensity of 27%, other reference baseline films only had between 0.10% and 5.6% tail intensity. The individual herring gulls on this film also had higher tail intensity compared to other herring gull from the same location (24% vs 0.17%). Consequently, the DNA damage of the two individuals on this film were excluded from any statistical analyses as the heightened tail intensity is assumed to be an error due to the handling of this particular film.

3.4.2 Location comparison

To account for possible differences between males and females in the Hornøya samples, all data points for baseline and H_2O_2 treatment were tested. The Hornøya samples (n=13) consisted of 6 males and 7 females. DNA baseline damage (t-test, $p>0.05$), H_2O_2 treatment (t-test, $p>0.05$). The recovery treatment (t-test, $p>0.05$). Overall no difference was detected between the sexes of the Hornøya gulls (Figure 13), and in the following statistics they are grouped.

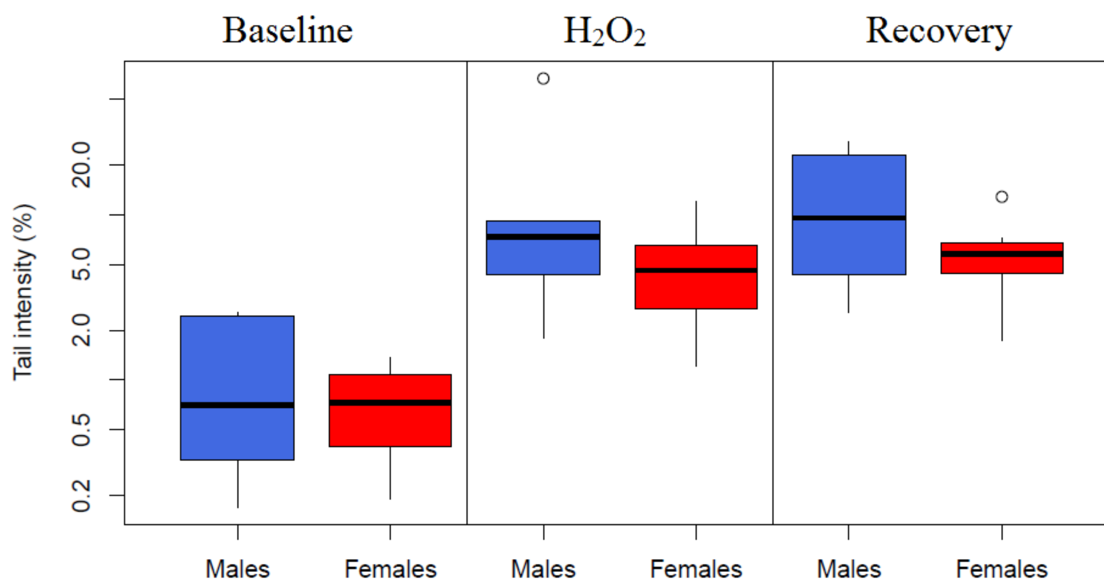

Figure 13. The baseline, H_2O_2-treatment and the recovery-treatment for the females (n=7) and males (n=8) from the Hornøya samples (n=15). The boxes stretch from first to third quartile, with the line representing the measurement median. The whiskers extend to 1.5 times the interquartile range, and the points are extreme values, also defined as outliers.

No statistical differences were found between terrestrial and marine feeders in the Oslofjord samples for baseline damage (t-test, p>0.05), H_2O_2 treatment (Welch test, p>0.05) or the recovery treatment (t-test, p>0.05). As no difference was detected between the terrestrial and marine Oslofjord gulls (Figure 14), they are grouped in the following statistics.

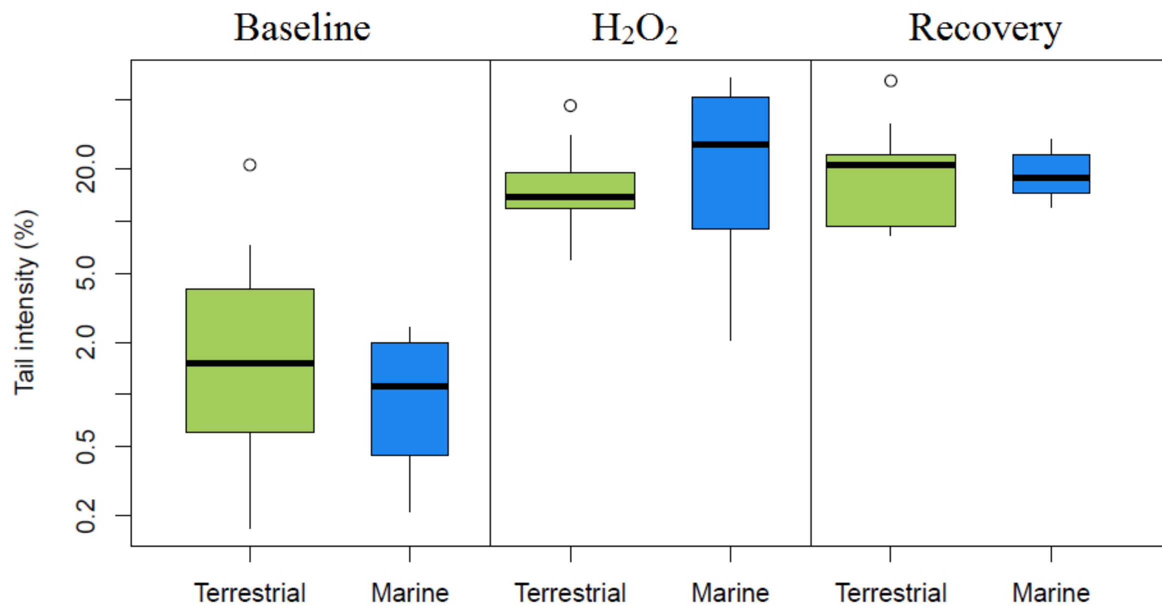

Figure 14. The baseline, H_2O_2-treatment and the recovery-treatment for the terrestrial (n=11) and marine (n=4) herring gulls from the Oslofjord samples (n=15). The width of the boxes corresponds to the number of samples in each group. The boxes stretch from first to third quartile, with the line representing the measurement median. The whiskers extend to 1.5 times the interquartile range, and the points are extreme values, also defined as outliers.

Statistical differences in DNA damage were found in Oslofjord samples for all treatments, i.e. baseline and H_2O_2 treatment (t-test, p<0.05), and recovery treatment (Welch test, p<0.05), Figure 15. To assess changes in DNA damage from baseline to peroxide, and from peroxide to recovery, paired two sample t-tests were performed. For both locations, there was a significant increase in DNA damage from baseline treatment to peroxide (paired t-test, p<0.05). But no improvement or deterioration was found after recovery (paired t-test, p>0.05, Figure 15).

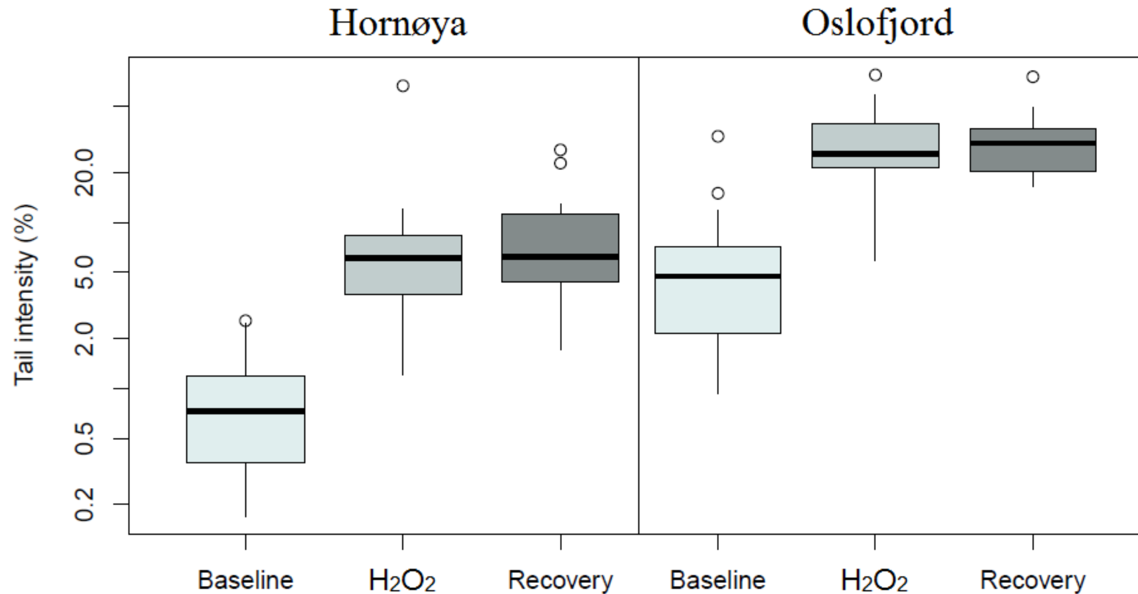

Figure 15. DNA damage (tail intensity) for both locations, Oslo (n=15), Hornøya (n=13) showing response to treatments. Baseline (films directly into lysis), H_2O_2 (films incubated in 5µM H_2O_2 for 15 min at 4°C, before being placed into lysis) and Recovery (films were incubated in PBS at room temperature for 4-8 hours after exposure to H_2O_2). The boxes stretch from first to third quartile, with the line representing the measurement median. The whiskers extend to 1.5 times the interquartile range, and the points are extreme values, also defined as outliers.

3.5 Association between DNA strand breaks and contaminants

In the PCA (Figure 6) the DNA baseline vector is negatively correlated to the contaminants. As observed in the univariate tests, the baseline is higher in Oslofjord individuals, and they are positioned closer to the vector. The DNA was added as a passive variable and did not affect the loading of the PCs in the triplot.

Using the PC1, PC2 and PC3 site scores (individual herring gulls) from the PCA (Figure 6), association with baseline DNA damage was tested.

For PC1 (73%), there was a significant negative relationship with the DNA damage (lm, R^2=0.46, $p<0.05$, Figure 16a). PC2 (12%) showed slight increase with DNA damage, but this was not significant (lm, R^2=0.020, $p>0.05$, Figure 16b). PC3 (10%) also had a slight negative relationship with DNA damage, but this was not significant (lm, R^2=0.10, $p>0.05$, Figure 16c).

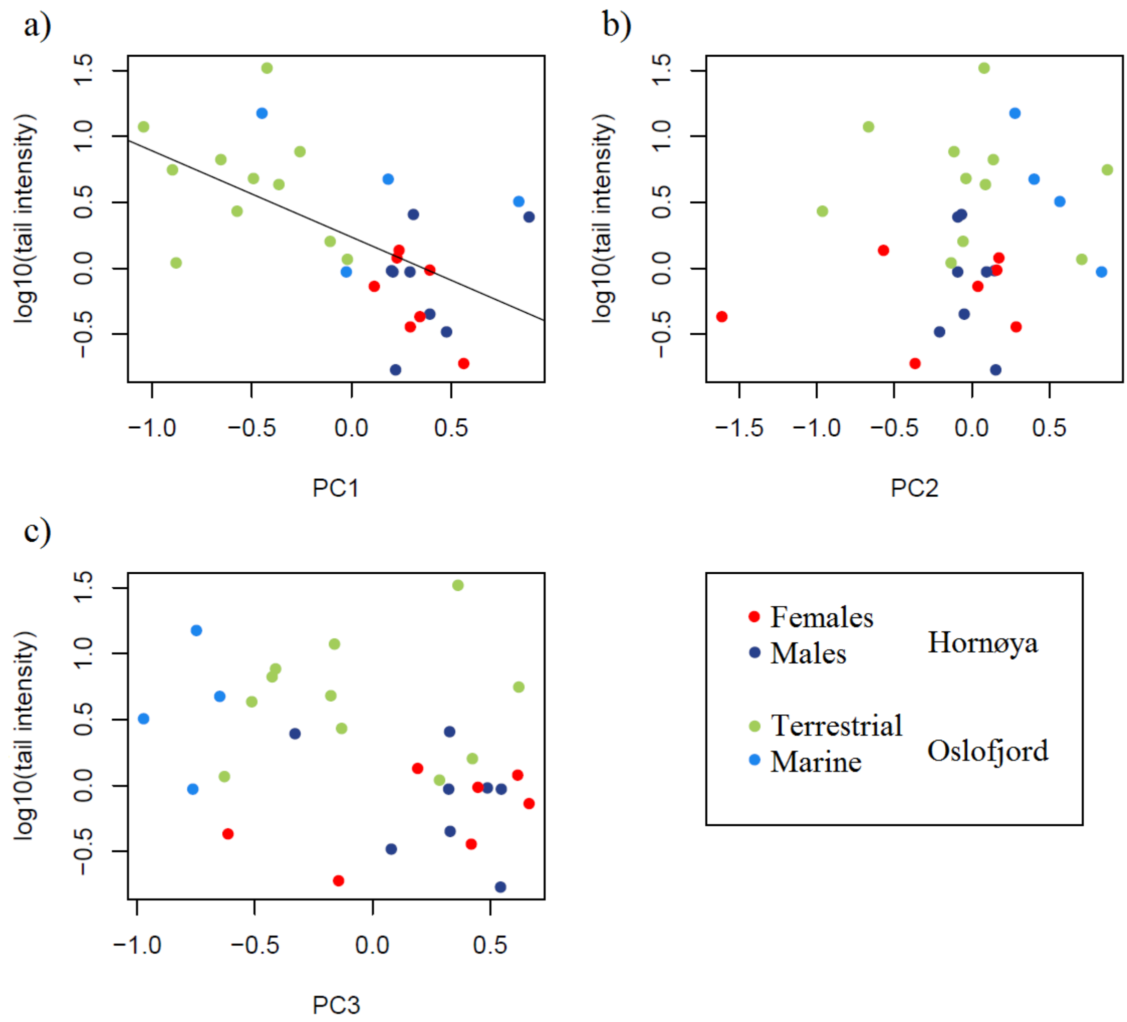

Figure 16. DNA damage (tail intensity, log10 transformed) plotted with the principal component 1-3 from the PCA. a) PC1, b) PC2 and c) PC3. Regression lines are shown.

4 Discussion

Due to differences in the stable isotope signatures, the Oslofjord population was divided into terrestrial and marine feeders. The marine feeders had similar concentration to the Hornøya gulls in many contaminants, and the level of POPs was higher in the rural population than in the urban population. DNA damage was higher in the urban areas, for both terrestrial and marine feeders. To my knowledge there are few studies assessing herring gull genotoxicity in natural populations, and linking it to contaminant load. The comet assay has only recently been optimised for use with avian white blood cells (WBC) (Haarr, 2016) and few studies have been conducted focusing on urban and rural differences in contaminant accumulation and genotoxicity.

4.1 Stable isotope signatures and condition

The division of the Oslofjord gulls into marine and terrestrial was based on the clear separation of the stable isotopes (SI) ratio of $\delta^{13}C$. Claiming terrestrial and marine is not entirely true, and a more precise definition would be “more terrestrial input” and “less terrestrial input” in the diet, and the Hornøya gulls would be defined as “minimal terrestrial input”. For simplicity, the Oslofjord herring gulls with more terrestrial input are referred to as “terrestrial feeder” and the herring gulls with less terrestrial input, and correspondingly more marine input are referred to as “marine feeders”. That being said, the terrestrial feeders may simply have a relatively smaller portion of marine-derived carbon in their diet, not necessarily feeding purely terrestrial. As this division of the Oslofjord gulls is reflected in the other isotope ratios, it is assumed to be an adequate cut-off.

The differences in stable isotopes indicate that the herring gulls from Hornøya and marine feeders in Oslofjord have very different feeding ecology from the terrestrial feeders. Assuming the $\delta^{13}C$ may be comparable between areas, the Hornøya herring gull population have a more marine-based diet, which was supported by examinations of the food fed to chicks during incubation, which contains mostly fish (Reiertsen 2017, pers.com.). The marine feeders have large proportions of their diet derived from marine sources, with some terrestrial input, and the terrestrial feeders have a relatively more terrestrial signal, indicating land-based food sources.

The $\delta^{15}N$ was higher in the Hornøya gulls, but, like the $\delta^{13}C$, the baseline appears to be inconsistent along the coast of Norway (Green et al., 2016). To counter confounding effects of

the different baselines, the trophic levels of the gulls were calculated using blue mussel (*Mytilus edulis*) $\delta^{15}N$ signatures as baseline (See section 2.11.5 for details). The differences in the TL were still substantial, placing the Hornøya gulls at about two levels over the Oslofjord gulls. This is a clear indication that the Oslofjord gulls feed less in the marine environment.

The size difference between the Hornøya females and the Oslofjord gulls might attribute to the difference in TL. A larger gull will more easily predate on fish, and be able to partake in the competition of scraps by fishing vessels (Nogales et al., 1995), both food sources leading to a higher level of $\delta^{15}N$. On Hornøya, herring gulls were observed predating on eggs and other seabirds, like black-legged kittiwakes (*Rissa tridactyla*), this will increase the $\delta^{15}N$ and $\delta^{13}C$ signatures. Field experiments with herring gulls and greater black-backed gulls (*Larus marinus*) on Hornøya have shown that they prefer fish to anthropogenic food items (Reiertsen 2016, pers.com.), indicating a weak urban influence on diet choice, as opposed to the Oslofjord gulls. In comparison to the rural herring gulls on Hornøya, the diet of gulls nesting close to human settlements shows a different preferred feeding strategy which includes earthworms, grass and insects (Götmark, 1984; Chudzik et al., 1994), and a tendency to feeding low on the marine food chain (Götmark, 1984; McCleery & Sibly, 1986). With the proximity to terrestrial and urban influences, these herring gulls have also adapted to feeding on anthropogenic waste (Chudzik et al., 1994; Auman et al., 2008). Examination of the Oslofjord gulls diet, by observing regurgitated food from the brooding parents, included bread, chicken fillets and various food items that have a clear anthropogenic origin (personal observations).

Auman et al. (2008) noted that gulls feeding on human waste tend to be larger, and appear to be in better condition, but the accessible, low-quality food appear to have a reproductive cost. In western gull (*Larus occidentalis*) reduced success in reproduction, with smaller clutch sizes, higher hatching failure, and shortened reproductive life-span, have been shown for individuals feeding largely on human waste. This reproductive remission was possibly due to the stress of poor nutrition in addition to reproductive stress (Annett & Pierotti, 1999). This could be linked to the elevated DNA damage in Oslofjord herring gulls, see below.

Both depleted $\delta^{13}C$ and enriched $\delta^{34}S$ have been linked to marine origin food. Knoff et al. (2001) showed a higher $\delta^{34}S$ in laughing gulls (*Leucophaeus atricilla*) depending on more marine origin food, than the gulls with more estuarine prey, the $\delta^{13}C$ was also more enriched in more marine food gulls. Lott et al. (2003) found enriched $\delta^{34}S$ in marine and coastal bird-eating raptors, when compared to inland feeders. According to Peterson and Howarth (1987), pelagic

plankton is more enriched in C, N and S, while inland plants were depleted in C, N and S. This tendency was evident in the Oslofjord gulls, where the marine group show the same enrichment in all stable isotope ratios compared to the terrestrial group. δ^{15}N and δ^{13}C in bald eagles (*Haliaeetus leucocephalus*) have been shown to be higher in off-shore marine areas, followed by inshore, estuarine and freshwater sites (Elliott et al., 2009). Morrissey et al. (2013) linked the depletion of δ^{34}S in Eurasian dipper (*Cinclus cinclus*) eggs to proximity to urban areas, but for the terrestrial feeders in this thesis, the δ^{34}S depletion is more likely linked to the more terrestrial inputs in the diet.

The baseline of δ^{13}C is needed to be able to precisely determine the carbon source, as depletion of δ^{13}C not necessarily means total terrestrial influence, but depends on the photosynthesis of the primary producers in the food web of the species examined. Different foodwebs across different marine areas will have different SI signatures (Peterson & Fry, 1987), the carbon and nitrogen baseline has been shown to be different along the coast of Norway (Green et al., 2016). To be able to fully compare isotope signatures between populations, a baseline should be quantified. The C ratio, for instance, depends on the photosynthesis pathways in primary producers in the food web, and also the CO_2 input from either seawater or atmosphere (Peterson & Howarth, 1987), a different phytoplankton composition may affect the observed levels. By examining baselines in the respective food webs of the study organisms, more precise and correct conclusions might be drawn.

Condition

The body condition (BCI) was shown to have little correlation with the concentrations of any of the contaminants. The strongest correlation is slightly positive with the PBCs and the PFOS. There are several different ways of estimating the condition of birds (Bustnes et al., 2002; Fox et al., 2007; Meillère et al., 2015), but no clear consensus as to which is the best suited for e.g. seabirds. The clear negative correlation with lipid content might be an indication of a less-than excellent fit of the BCI, but the estimation of lipid content from small blood samples, which have little lipid content, gives room for errors. A higher lipid content, as found in the urban gulls, have been linked to the foraging on human waste of these gulls (Auman et al., 2008), and any health benefits from this diet are up for discussion. Pierotti and Annett (1990) concluded the human waste diet was high in calories, but lacking in vital nutrients, for garbage-eating gulls.

4.2 Contaminant concentrations

The present thesis shared material and chemical analytical results with the Urban fjord monitoring programme (Ruus et al., 2016). As part of their time series on contaminants in the inner Oslofjord, certain contaminants were included in the analyses for the Oslofjord gulls, while others were excluded, and vice versa for Hornøya gulls. For example, several pesticides were excluded for the Oslofjord analyses and many of the polychlorinatedbiphenyl ethers (PBDEs) were not included in the Hornøya analyses.

Higher levels of persistent organic pollutants (POPs) have been linked to point sources, like the Great Lakes area in Canada (Norstrom et al., 2002; Gebbink et al., 2011), the inner Oslofjord (Berge et al., 2013) or highly urbanised areas (Morrissey et al., 2013). But high levels have also for a long time been detected in remote areas like the Arctic (Kelly et al., 2009; Muir & de Wit, 2010; AMAP, 2016). Due to legislation (Stockholm Convention, 2009), levels of legacy POPs (i.e. polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB)) are expected to exhibit downward trends in biota. PBDEs and perfluorooctane sulfonate (PFOS) are part of the more recent (2009) addition to the regulated POPs, and decreasing levels are expected, following the trend of the legacy POPs. The cyclic volatile methylsiloxanes (cVMSs) have yet to be restricted in a similar manner as the POPs, and high levels are still linked to point source e.g. industry, waste water treatment plants, so the presence of these compounds in the urban location was expected to be higher than the rural location. As the Hornøya location is expected to relatively clean, and is far from any influence of large cities, it was chosen to serve as a reference area for the urban population monitored in the Urban fjord programme in 2016.

Biological factors such as body condition, gender, relative trophic level (TL), carbon source and lipid content was thought to affect the concentrations of contaminants. TL was the only significant explanatory variable, in addition to location. The females are smaller than males, and tend to have a lower level of contaminants due to the “detox” effect of laying eggs (Verreault et al., 2006; Gebbink & Letcher, 2012). This was not observed in the Hornøya herring gulls, as no significant gender-related difference was detected for any contaminant. The lipid content appeared to be negatively correlated with the PCBs and PFOS, but the apparent differences were not significant. A negative association would have made sense, as increased contaminant levels could have been due to the mobilisation of lipid reserves (Henriksen et al., 1996). The herring gulls might migrate from as far south as England to Hornøya, and the additional reproductive stress of egg brooding might be a cause of the lower lipid content in the

rural herring gulls, due to starvation. The northern colony had higher body mass, but as noted by Barth (1968), the northern location could be the cause of this, as northern individuals have been noted to be generally bigger than their southern relatives.

The herring gulls tagged at Hornøya had a GLS (global location sensing unit) attached to one of the ID rings attached to their leg. The GLS are a low-cost method to get an approximate estimation of an individual's location by recording solar irradiation. The GLS from other herring gulls from the colony identify their overwintering areas as Tromsø, and the coast of Scotland (Reiertsen 2016, pers.com). Thus, there is no indication that the herring gulls are overwintering in Russia, which could be a potential source of high POP concentrations. However, Tromsø could be a point source, as Warner et al. (2014) found elevated concentrations of PCBs and cVMS in fish collected 30 km away from the city. For a better indication of the foraging strategies during overwintering, further studies, both observational and utilising stable isotopes in for instance feathers, to get a longer perspective on the SI ratios (Jaspers et al., 2009), should be conducted on herring gulls.

4.2.1 PCBs

The PCBs were divided into groups according to the number of chlorine substitutions. Oliver and Niimi (1988) showed an increase of the higher chlorinated biphenyls (penta-, hexa-, and hepta-) with increasing trophic level in aquatic systems. Lower chlorinated congeners are more readily metabolized, having a shorter half-life in biota than the higher chlorinated congeners. While the higher chlorinated congeners are the most bioaccumulative, they are also present at lower levels in the environment and additionally, they adsorb to particles and soil, making them less bioavailable to organisms (McFarland & Clarke, 1989). Half-life and assimilation efficiency has also been shown to decrease when the octanol-water partition coefficient (K_{ow}) gets sufficiently high ($logK_{ow}>7$), as is found for the hepta-, octa- and nonaCBs (Fisk et al., 1998). This explains the levels of hexaCB being higher than heptaCB in the herring gulls. This pattern of steady increase in concentration of the PCB homolog groups to a maximum at hexaCB, and then lower levels of the even higher chlorinated biphenyls is fairly consistent through several studies. Borgå et al. (2001) and Borgå et al. (2005) observed this in glaucous gulls (*Larus hyperboreus*), black-legged kittiwakes and in alcids, Borlakoglu and Walker (1989) noted the pattern in several piscivorous seabirds, and it has been observed in burbot (*Lota lota*) (Muir et al., 1990).

The levels of PCB 153 were 20 ng/g w.w. in males, and 13 ng/g w.w. in females, on Hornøya. The Oslofjord marine and terrestrial group had levels of 17 and 3.7 ng/g w.w. respectively. In comparison, PCB 153 measurements in whole blood from both male and female great black-backed gull from a colony in Northern Norway was about 37 ng/g w.w. (Helberg et al., 2005). Previous measurements of PCB 153 levels in Oslofjord herring gulls were 14 and 7.6 ng/g w.w. (Ruus et al., 2015; Ruus et al., 2016). The levels of tetraCB in the Hornøya herring gulls were average 0.58 ng/g w.w. The Oslofjord marine feeders had average levels of 1.0 ng/g w.w., while the terrestrial feeders measured a lower 0.21 ng/g w.w. Steffen et al. (2006) reported whole blood levels of PCBs (ng/g w.w.) in great black-backs from four locations along the Norwegian coast, including Hornøya. The overall levels of tetraCB in great black-backs were an average 0.98 ng/g w.w. (Hornøya 0.75 ng/g w.w.). The herring gulls form Hornøya had pentaCB levels of 3.5 ng/g w.w., while the marine feeders had higher levels at 6.2 ng/g w.w., and terrestrial feeders had levels of 0.88 ng/g w.w. The levels of pentaCB were average 5.6 ng/g w.w. (Hornøya 4.9 ng/g w.w.) in great black-backs samples (Steffen et al., 2006), higher than the Hornøya herring gulls. Hornøya herring gulls had hexaCB average levels of 11 ng/g w.w., while the marine feeders were 12 ng/g w.w., and terrestrial feeders were 2.2 ng/g w.w. In great black-backs samples the overall level was 20 ng/g w.w. (Hornøya 14 ng/g w.w.) for hexaCB (Steffen et al., 2006). The present levels of heptaCB in herring gulls were 2.9 ng/g w.w. on Hornøya, 3.3 ng/g w.w. in marine, and 0.68 w.w. in terrestrial individuals. In great black-backs, there was an average 5.9 ng/g w.w. overall (Hornøya 3.7 ng/g w.w.) for heptaCB (Steffen et al., 2006). The higher levels found in great black-backs could be explained by different food preferences, causing great black-backs to feed on a higher TL than herring gulls (Bustnes et al., 2008).

The Hornøya gulls and marine feeders from Oslofjord had similar levels of all PCBs, indicating the source of these contaminants are marine-derived food items, and the bioaccumulation is independent of the trophic level. Independence of TL contradicts earlier findings e.g. Muir et al. (1990); Borgå et al. (2001), where the accumulation increased with higher TL. A possible explanation for the high levels in marine feeders is the fact that PCBs are still present in the inner Oslofjord, due to suspected leaching from previously contaminated sediments (Breivik et al., 2004). This is supported by Savinov et al. (2003), as presence of higher chlorinated PCB indicates local sources while the lower chlorinated PCBs, which have higher vapor pressure than those with a higher number of chlorine substitutions (Dunnivant et al., 1992), are subject to long range transportation. Thus, the levels observed in marine feeders could be due to exposure to active point-sources via the marine diet, while the levels in Hornøya gulls are due

to legacy long-range diffusion. Savinov et al. (2003) noted that the presence of tri-, tetra-, and pentaCB in the Varangerfjord (close to Hornøya) was likely due to long range transportation in the atmosphere or by ocean currents.

4.2.2 PBDEs

The PBDE congener 47, 99, 100 and 153 are part of the commercial mixture PentaBDE, which has been banned and phased out (Stockholm Convention, 2009). The Hornøya herring gulls and the marine group have overall higher levels than the terrestrial groups. Levels of PBDE 47, for instance, were 1.1 and 0.83 ng/g w.w. for Hornøya females and males, respectively, and 0.81 and 0.16 ng/g w.w. for Oslofjord marine and terrestrial feeders, respectively. In comparison, the whole blood levels in glaucous gull males were 2.4, 0.3, 0.7 and 0.5 ng/g w.w. for BDE 47, -99, -100 and -153, respectively. And in females, the levels were 1.2, 0.2, 0.3 and 0.5 ng/g w.w. for BDE 47, -99, -100 and -153, respectively (Haarr, 2016). The higher levels in the glaucous gulls could be explained by biomagnification, as the glaucous gull occupies a high TL in the Arctic. In Oslofjord herring gulls, previous levels of BDE -47, -99, -100 and -153 were 0.72, 0.28, 0.20 and 0.092 ng/g w.w., respectively (Ruus et al., 2015). The year after the levels were 0.29, 0.15, 0.051 and 0.021 ng/g w.w., respectively (Ruus et al., 2016). These levels are consistent with the observed levels of the PBDEs in this year's Oslofjord terrestrial group. The Hornøya herring gulls and the marine group's overall higher levels, in the range of the glaucous gulls, is likely because of their higher TL. This indicates biomagnification of these compounds, as feeding on a higher TL causes higher overall levels of ∑PBDE. This is both supported and contradicted in previous studies. de Wit et al. (2010) noted bioaccumulation of PBDEs in Arctic biota and Johnson-Restrepo et al. (2005) found biomagnification in sharks and dolphins. On the contrary, Kelly et al. (2008) determined little or no bioaccumulation in marine food webs, linking higher levels of PBDEs with proximity to industrial and/or urbanized areas. Elliott et al. (2005) found greater amounts of PBDEs in great blue heron (*Ardea herodias*) eggs, as compared to double-crested cormorant, and explained this with a greater urban influence on the herons. Increased amount of PBDEs with greater urbanization is not consistent with the present findings in the herring gulls. One explanation is the indication of the terrestrial group that the diet has less marine components, resulting in lower levels, as lipid rich fishes might be enriched in the lipid-soluble POPs, perhaps because of long range transport on Hornøya and point sources in the Oslofjord. In the plasma of ring-billed gull (*Larus delawarensis*), a correlation between feeding in waste management facilities and lower levels of PentaBDE has been shown

(Gentes et al., 2015), indicating a diet of anthropogenic waste appear to cause less exposure to the legacy POPs (i.e. PentaBDEs), which are still found in aquatic food webs (Stapleton & Baker, 2003).

The overall low levels of the PBDEs is because the environmental persistence of the PBDEs are less than that of e.g. PCBs. The carbon-bromine bonds of the PBDEs are weaker in comparison to carbon-chlorine bonds (Hooper & McDonald, 2000), biota transform the PBDEs through debromination (Birnbaum & Staskal, 2004). The higher proportion of BDE 47 could be explained by the debromination process from BDE 99 to BDE 47 (Benedict et al., 2007).

4.2.3 HCB

Compared to other POPs, HCB has high volatility, and lower K_{ow} (Barber et al., 2005), meaning it can move around the environment through various compartments (i.e. air, water, sediment) through multiple volatilization and deposition steps, commonly referred to as the grasshopper effect (Wania & Mackay, 1996). This is evident, as various studies observed a south-north gradient with HCB, concentrations in both environment and biota increases with increasing latitude (Frank & Mackay, 1993; Barber et al., 2005), and various studies Even though levels of HCB have been shown to have a greater clearance rate and fluctuate (Bustnes, 2006), possibly due to the high volatility of this compound, Kelly et al. (2007) showed biomagnification in Arctic food webs, and higher levels have been detected in greater black-backed gulls, than lesser black-backed gulls (*Larus fuscus*) (Bustnes et al., 2006), due to the latter feeding on a lower trophic level (Bustnes et al., 2008).

The levels of the Oslofjord herring gulls, between 0.28 and 0.32 ng/g w.w. are similar to the previous Oslofjord findings, while the Hornøya gulls have similar levels to the black-backed gulls also from Hornøya, at between 2.5 and 2.7 ng/g w.w. Previous levels of HCB in whole blood, in Oslofjord herring gulls were 0.58 and 0.49 ng/g w.w. (Ruus et al., 2015; Ruus et al., 2016). In great black-backed gulls, Steffen et al. (2006) reported average whole blood levels of 3.0 ng/g w.w. (3.0 ng/g w.w. Hornøya), and Helberg et al. (2005) reported 5.5 ng/g w.w. in male, and 6.0 ng/g w.w. in female whole blood.

The present levels in the herring gulls, show a clear difference between the urban and rural location, most likely due to the overall higher levels of HCB in more northern areas (Hung et al., 2016). Hornøya also has an Artic climate, with colder temperatures possibly causing more

precipitation (Frank & Mackay, 1993). As there is no interaction between location and trophic level, but each exerts a significant effect, the indication is that the HCB levels tend to increase with increased TL, as well as with a more northern habitat.

4.2.4 PFOS

The PFASs are amphipathic, repelling both water and oil. Being proteinophilic, they tend to associate with blood proteins, leading to accumulation in liver and kidneys of exposed organisms (Haukås et al., 2007; Kelly et al., 2009). Trends of PFAS has shown that the PFOS often is the predominant PFAS found in various biota and tissues (Verreault et al., 2005; Haukås et al., 2007; Löfstrand et al., 2008). This is likely because PFOS highly bioaccumulative (Kelly et al., 2009), additionally, Tomy et al. (2004) showed in-vitro biotransformation (in rainbow trout *Onchorhynchus mykiss*) of N-ethyl perfluorooctanesulfonamide (N-EtPFOSA) and perfluorooctanesulfonamide (PFOSA) to PFOS, possibly explaining the presence of this less volatile compound in remote areas.

The levels in the terrestrial group are low, at 6.7 ng/g w.w., while the marine and Hornøya gulls all have levels between 15-25 ng/g w.w. Kannan et al. (2001b) reported levels in blood of ringed seal (*Phoca hispida*) of 156 and 9.1 ng/g w.w. in the Baltic and from Spitsbergen, respectively. In the Canadian Arctic, bearded seal (*Eriganthus barbatus*) and ringed seal had blood levels of 1.3 and 5.6 ng/g w.w., respectively (Powley et al., 2008). In the Canadian Great Lakes area, the whole blood levels were 125 and 63 ng/g w.w. in double-crested cormorant (*Phalacrocorax auritus*) and herring gull (Kannan et al., 2001a). These birds were reportedly feeding exclusively on fish, and presence of point sources near the area of sampling was included as a reason for the high levels. Previous levels of PFOS in Oslofjord herring gulls were 42 and 21 ng/g w.w. (Ruus et al., 2015; Ruus et al., 2016). The marine and Hornøya gulls have levels that are lower than the herring gulls from the Great Lakes area, and closer to that of the Arctic seals. Giesy and Kannan (2001) reported higher levels in urbanised locations, e.g. the Baltic or the Great Lakes, but this effect of urbanisation is not evident in the current data, as concentrations are similar between Oslofjord marine feeders and Hornøya. The trophic level, and to some extent the location, has significant effect on PFOS levels, indicating that biomagnification is an important source of the observed levels. This is confirmed by other studies, as mentioned above, and a more marine-based diet is likely the source.

4.2.5 cVMS

For the siloxane data, all results were either below or close to limit of quantification (LOQ). Warner et al. (2013) has previously shown that co-extracted matrix significantly contributes to background analytical variation at trace levels in cVMS analysis. This can result in the reporting of false positives, and to avoid this issue, the use of method detection limit (MDL, matrix defined) is recommended. Due to logistical issues in obtaining a matrix which is free of siloxanes and mimics the sample matrix (whole blood) under investigation, the limit of quantification (LOQ: average blank response + 10 × standard deviation in blank response, solvent defined) was used as a conservative detection limit to reduce the reporting of false positives. LOQ was found to be comparable or greater to MDL in the analysis of several environmental matrices (Krogseth et al., 2016) thus the LOQ (although not matrix defined) was considered appropriate to account for analytical variation introduced by the co-extracted matrix. However, concentrations approaching the LOQ will be more influenced by the background response. As the LOQ was found to be different for the individual siloxanes and between sampling areas, no certain conclusions should be drawn from the patterns and levels observed.

In the Hornøya individuals, elevated levels of D4 and D6 compared to D5 were observed. This trend is highly unusual, as higher emissions of D5 to the environment occur compared to D4 and D6 (Brooke et al., 2009a, 2009c, 2009b), and a higher proportion of D6 in samples has not been reported. For D4, 2 out of 14 Hornøya samples were below LOQ (1.5 ng/g w.w.), compared to 6 out of 13 below LOQ (2.0 ng/g w.w.) for Oslofjord samples. It is worth to note that all but three Hornøya samples were below the higher LOQ for Oslofjord as the LOQ differed between analyses. For D5, 14 out of 14 Hornøya samples were below LOQ (1.6 ng/g w.w.), compared to 1 out of 13 LOQ (1.3 ng/g w.w.) for Oslofjord samples. In this case, only 2 out of 13 Oslofjord samples were below the higher Hornøya LOQ. For D6, 8 out of 13 Hornøya samples were below LOQ (2.0 ng/g w.w.), compared to 12 out of 13 below LOQ (2.5 ng/g w.w.) for Oslofjord samples. None of the Hornøya samples would have been above the LOQ for Oslofjord samples. Previous levels of D4, D5 and D6 in Oslofjord herring gulls were 2.1, 7.1 and 2.5 ng/g w.w., respectively (Ruus et al., 2015), and 0.71, 4.5 and 2.0 ng/g w.w., respectively, the year after (Ruus et al., 2016).

Previous findings reported by Hanssen et al. (2013b) found higher proportion of D4 compared to D5 in human blood within a North Norwegian population. This could be attributed to the lower octanol-water partitioning coefficient (K_{ow}) of D4, resulting in greater partitioning from tissue to blood relative to D5. The Oslofjord gulls follow a more familiar pattern as the D5 is

usually the dominating siloxane in biota (Kaj et al., 2005; Warner et al., 2010; Whelan & Breivik, 2013). cVMS are used in silicone polymer production and in personal care products (PCPs). Followed by D6 and D4, D5 has the highest concentration in PCP, and dominates the use and consumption of cVMS in the EU (Wang et al., 2013).

D5 has a higher preference for fat, while D4 has a higher preference for blood (Andersen et al., 2001; Andersen et al., 2008), this has been confirmed in human plasma (Hanssen et al., 2013b) and could be expected in the herring gull blood as well. However, as the siloxanes are very volatile, they do not bioaccumulate to the same extent in air-respiring organisms, when compared to aquatic organisms with gills. The primary excretion route of cVMS is through respired air (Sarangapani et al., 2003; Tobin et al., 2008), which could explain the low levels observed in the herring gulls. However, this reasoning does not explain the higher levels reported for D6. The LOQ for D6 was found to be higher compared to D4 and D5. Concentrations reported close to the LOQ are more influenced by background concentrations, thus not too much interpretation should be made regarding D6 results.

Warner et al. (2010) linked exposure to cVMSs to proximity to human settlements, acting as point sources. The levels of cVMSs are reportedly higher in water, sediment and aquatic biota closer to urban areas (Schlabach et al., 2007; Sparham et al., 2008; Warner et al., 2010). This is somewhat consistent with the levels of cVMSs observed, as the Oslofjord herring gulls nest very close to the capital of Norway with more than 650 000 inhabitants (https://www.oslo.kommune.no/, accessed June 2017), while the town of Vardø houses about 2000 people (http://www.vardo.kommune.no/, accessed June 2017).

As mentioned above, all the data is close to or below the LOQ, so not too much interpretation should be made considering these results. That being said, the cVMS levels appear to be higher in the Oslofjord gulls, reflecting higher exposure due to proximity to point sources. This being in spite of the higher quantification limit for Oslofjord samples, resulting in a higher detection frequency. The unusual patterns observed is likely due to the influence of the variation introduced by the blood. To be able to quantify the introduced variation, a blood sample with little or no siloxanes should have been analysed as well. The detection limits could be adjusted with the incorporation of this kind of data. Warner et al. (2013) showed that incorporation matrix variation had a huge effect on the levels of positive detects in the analysis. Unfortunately, the quantification of the variation was not possible to conduct for this thesis, and the results may therefore reflect false levels of these contaminants in the herring gulls.

4.3 DNA strand breaks

In the present thesis, a negative relationship was discovered between the levels of contaminants and DNA strand breaks. Levels of DNA damage was similar in the Oslofjord colony between terrestrial and marine feeders, and higher than the males and females on Hornøya. Thus, the amount of DNA damage detected appear to not be affected by the levels of contaminants, as the marine feeders had higher contaminant levels than the terrestrial. The urban and rural colonies were sampled at roughly the same time in the breeding season, close to egg hatching, meaning there should be little difference between them when considering the reproductive stress.

An explanation to the apparent negative relationship between DNA damage and contaminants, is the likelihood that there are other contaminants not analysed, that cause the majority of genotoxicity, like PAH (Cachot et al., 2006) and heavy metals (Bolognesi et al., 1999). There is also the possibility of an unknown cocktail effect between POPs and/or other contaminants. Toxicity testing on one compound at the time rarely take into account the possible interactions of several of the POPs being present at the same time (Celander, 2011). He et al. (2009) showed that the mixture of PCB 153 and PBDE 47 had synergistic effects, causing more cytotoxicity when both are present. The biotransformation, or bioactivation, of e.g. aromatic compounds might create mutagenic or carcinogenic metabolites from otherwise harmless compounds (McFarland & Clarke, 1989).

Bourgeon et al. (2012) compared great skua (*Stercorarius skua*) from different locations, and found the colony with lowest concentration of POPs, had the lowest condition measured by oxidative damage, stress level and immunity biomarkers. Several confounding factors might be the reasons for this, from habitat choice, different kinds of contaminants, and food resources, to predation. In short, they concluded that other stressors caused more damage, and that the link between contaminants and biomarkers of health are context dependent. This could also be the case for the herring gulls, with factors like poor food quality and stress caused by the proximity to people in the urban environment, having bigger influence on the health of the birds, being evident in DNA damage detected.

Few studies have been conducted linking exposure to POPs with DNA damage in birds. Matson et al. (2004) determined the impact of mutagenic contaminants in common eider ducks, and concluded the damage detected was linked to the exposure to contaminants in the Baltic Sea. Østby et al. (2005) quantified DNA adducts in liver samples of glaucous gulls, although not

finding any significant correlation between DNA adducts and the examined PCB congeners, the conclusion was there are likely other genotoxic compounds influencing the amount of adducts. Krøkje et al. (2006) examined single and double strand breaks in glaucous gulls by use of agarose gel electrophoresis, but found no significant correlation between levels of POPs and DNA strand breaks.

According to Collins (2004), and also confirmed by Haarr (2016) white blood cells (WBC) are well suited for the comet assay, with low baseline damage at <10%. However, this only means that the WBCs are robust enough to endure the treatment for comet assay. The WBCs might not be best suited for detecting DNA damage caused by oxidative stress. Red blood cells (RBCs) have been suggested to function as oxidative sinks (Stier et al., 2015), absorbing the damage of the oxidative stress. Ideally, these cells should also have been examined in the comet assay, but the baseline damage of RBCs has been shown to be too high to be considered for this method (Haarr, 2016).

The induced oxidative damage was much more prominent in Oslofjord gulls than in Hornøya gulls. A possible reason for this is that the Oslofjord gulls, already having more DNA damage to repair, were less equipped to handle additional oxidative damage (Alonso-Alvarez et al., 2004). Another possibility is that the Hornøya gulls have a healthier diet that perhaps boosts cells' repair mechanisms, this could also explain why the DNA damage is lower in this colony as compared to the marine feeders in Oslofjord with similar levels of contaminants. No verification of this has been found, but Pierotti and Annett (1991) determined herring gulls feeding on intertidal species had higher number of eggs and fledglings than the herring gulls feeding on human waste or other seabirds, indicating better overall condition caused by this diet. A marine diet consisting of intertidal species and fish is considered healthy and rich in anti-oxidants (Hudthagosol et al., 2012), and could work as a buffer for oxidative damage on the DNA. Oxidative stress may also, as mentioned above, be the result of many other processes or stressors than contaminant exposure. The increased level of DNA strand breaks might be due to repair mechanisms by the cells, where the DNA strand is cleaved by the process of base- or nucleotide excision repair (Hook & Lee, 2004). If this was the case, then longer time in recovery medium might have resulted in lower levels of double strand breaks as the DNA was repaired. However, as no significant change was observed in any individuals even after recovery in phosphate buffered saline (PBS), this is not confirmed. The recovery medium was PBS, and any other observations of repair following H_2O_2 exposure have been after incubation in a nutrient rich medium. El-Bibany et al. (2014) re-suspended echinoderm cells in coelomic fluids,

Lorenzo et al. (2013) and Collins et al. (1995) incubated cells in a cell culture medium at 37°C. Thus, the recovery in PBS only, at room temperature likely does not represent the best recovery conditions for the WBCs. For future studies, measurements of base oxidation using formamidopyrimidine DNA glycosylase (FPG) and incubation in medium at 37°C would greatly improve the comet assay for detection of oxidative stress, and allow for optimal conditions for recovery from DNA strand breaks caused by endogenous factors. FPG is used to detect the major purine oxidation product 8-oxoguanine as well as other altered purines (Collins et al., 2004).

In previous studies reference human blood has been used as a control by addition to each film in the comet assay (Sareisian, 2014; Gilmore, 2015). The assumption is that the reference human blood (own) will be constant in-between tests, and any deviation of observed damage is due to the treatment of the films. In this thesis, the implementation of own human blood worked well and outliers assumed to be affected by the handling of the films were removed. The references were implemented to control for the effect of ethylenediamine-tetraacetic acid (EDTA) in PBS. No effect was found and all herring gull samples, apart from two individuals, were deemed suitable for comparison and statistical analyses. EDTA is used as an anticoagulant for haematological testing, and also allows good preservation of cellular components in blood cells (Banfi et al., 2007), and has likely been kept in the procedure to reduce aggregation of the cells examined.

5 Conclusion

In conclusion, urbanisation appear to have little effect on the concentrations of persistent organic pollutants (POPs) in herring gulls. Higher contaminant concentrations were detected in a supposedly cleaner, rural area with minimal anthropogenic influence. The explanation for this is long range transportation of POPs to northern areas, and biomagnification due to foraging on marine food that has bioaccumulated lipophilic POPs. The foraging strategies appear to be crucial for contaminant exposure in herring gulls, and turned out to be different within colonies. Urban herring gulls feeding predominantly marine had similar contaminant concentrations as the rural herring gulls, indicating that the contaminant source is through the diet.

Levels of DNA damage was expected to be higher in the herring gulls with higher contaminant concentrations, but the opposite was found. The herring gulls from the urban area had higher levels of DNA damage. This appeared to be regardless of the dietary inputs, as the urban herring gulls had very different contaminant concentrations. The most likely explanation for this is that the presence of other genotoxic contaminants or stressors in urban environments produce greater effects on oxidative stress and DNA damage. The health detriment of a diet influenced by human waste, as opposed to the healthier polyunsaturated fatty acids in a diet rich in fish, might also be a possible explanation for the differences in DNA damage. Further studies on herring gull diet and the nutritious value of marine versus terrestrial- and urban-influenced food items are required before any further conclusions can be drawn.

White blood cells from herring gulls with initially higher DNA damage deteriorated most after exposure to oxidative stressor, H_2O_2. This was likely because the already heightened oxidative damage in the cells caused a poorer response to the addition of more oxidants, as the anti-oxidant and repair systems were already fully "mobilised" in the cells. No recovery or repair of the induced damage was observed for either rural or urban herring gulls.

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Appendices

Appendix A: contaminant analyses for Oslofjord and Hornøya

Table A1: Contaminant concentration mean ± standard deviation (SD), range (Min – Max) and number of LODs for all contaminants analysed, for both Oslofjord (n=15) and Hornøya (n=15).

	Oslofjord			Hornøya		
ng/g w.w.	Mean ± SD	Range	#LOD/Q	Mean ± SD	Range	#LOD/Q
Hg	NA	NA	NA	209 ± 92	97 – 433	-
D4	2.3 ± 0.35	2.0 – 3.04	6	1.8 ± 0.34	1.5 – 2.5	2
D5	2.8 ± 2.3	1.3 – 10	1	1.6 ± 0	1.6	14
D6	2.2 ± 0.61	1.1 – 2.5	11	2.1 ± 0.18	2.0 – 2.4	8
o,p-DDE	-	-	15	-	-	15
p,p-DDE	5.1 ± 4.9	1.9 – 15	9	13 ± 7.3	6.2 – 35	-
o, p DDD	-	-	15	-	-	15
p, p-DDD	0.09	0.09	14	-	-	15
o,p-DDT	-	-	15	0.65±0.04	0.63 – 0.68	13
p,p- DDT	-	-	15	0.05±0.02	0.03 – 0.08	-
PeCB	0.04	0.04	14	0.09±0.02	0.07 – 0.16	-
aHCH	-	-	15	<0.01 ± <0.01	<0.01 – <0.01	-
bHCH	0.04 ± <0.01	0.04 – 0.04	13	0.11 ± 0.07	0.05 – 0.32	-
gHCH	-	-	15	<0.01 ± <0.01	<0.01 – <0.01	11
dHCH	-	-	15	NA	NA	NA
HCB	0.34 ± 0.33	0.07 – 1.2	3	2.6 ± 0.42	2.2 – 3.8	-
Oxychlordane	NA	NA	NA	1.5 ± 1.1	0.46 - 4.3	-
trans-chlordane	NA	NA	NA	0.01 ± 0.01	<0.01 – 0.03	-
cis-chlordane	NA	NA	NA	0.12 ± 0.06	0.04 – 0.25	-
trans-nonachlor	NA	NA	NA	0.85 ± 0.60	0.37 – 2.5	-
cis-nonachlor	NA	NA	NA	0.32 ± 0.08	0.21 – 0.51	-
mirex	NA	NA	NA	0.72 ± 0.44	0.38 – 2.1	-
PCB 28	0.27 ± 0.31	0.06 – 0.63	12	0.19 ± 0.68	0.11 – 0.41	-
PCB 47	0.43 ± 0.87	0.02 – 3.5	1	0.36 ± 0.19	0.21 – 0.96	-
PCB 52	0.24 ± 0.1	0.14 – 0.38	11	0.11 ± 0.10	0.03 – 0.43	-
PCB 66	0.93 ± 1.9	0.04 – 7.5	1	0.80 ± 0.41	0.47 – 2.17	-
PCB 71	NA	NA	NA	0.52 ± 0.23	0.36 – 1.3	-
PCB 99	2.17 ± 4.1	0.09 – 16	-	2.9 ± 1.9	1.6 – 9.14	-
PCB 101	0.84 ± 0.7	0.19 – 1.6	11	0.09 ± 0.10	0.02 – 0.41	1
PCB 105	1.2 ± 2.4	0.04 – 9.8	-	1.7 ± 1.3	0.93 – 6.26	-
PCB 118	3.6 ± 6.6	0.17 – 27	-	5.8 ± 4.2	3.0 – 20	-
PCB 128	0.97 ± 2.1	0.06 – 8.6	-	2.0 ± 1.6	0.99 – 7.7	-
PCB 138	5.7 ± 9.3	0.51 – 38	-	13 ± 9.0	6.4 – 43	-
PCB 153	7.2 ± 10	1.0 – 42	-	17 ± 12	8.12 – 58	-

PCB 180	1.4 ± 2.0	0.20 – 7.9	-	5.3 ± 3.8	2.7 – 18	-
PCB 183	0.69 ± 1.09	0.06 – 4.4	-	2.3 ± 1.3	0.92 – 5.6	-
PCB 187	2.0 ± 3.3	0.30 – 14	-	1.1 ± 0.73	0.52 – 3.5	-
6:2FTS	-	-	15	-	-	15
PFOSA	0.25 ± 0.07	0.20 – 0.30	14	-	-	15
PFBS	-	-	15	-	-	15
PFPS	-	-	15	-	-	15
PFHxS	0.35 ± 0.19	0.10 – 0.70	-	0.43 ± 0.23	0.25 – 0.69	12
PFHpS	0.2	0.2	14	-	-	15
brPFOS	NA	NA	NA	-	-	15
PFOS	12 ± 15	1.5 – 55	-	19 ± 14	5.3 – 62	
PFNS	-	-	15	-	-	15
PFDcS	0.33 ± 0.15	0.20 – 0.50	12	-	-	15
PFHxA	-	-	15	-	-	15
PFHpA	-	-	15	-	-	15
PFOA	2.3 ± 0.14	2.2 – 2.4	13	-	-	15
PFNA	0.9 ± 0.37	0.50 – 1.6	6	1.32 ± 0.61	0.49 – 2.34	1
PFDcA	0.86 ± 0.5	0.45 – 1.5	11	1.08 ± 0.57	0.36 – 2.80	-
PFUnA	1.1 ± 0.86	0.40 – 2.3	10	5.8 ± 2.6	2.5 – 14	-
PFDoA	0.64 ± 0.23	0.40 – 1.0	10	0.80	-	14
PFTriA	0.58 ± 0.27	0.35 – 1.0	10	1.7 ± 0.85	0.57 – 4.3	-
PFTeA	0.37 ± 0.03	0.35 – 0.40	12	0.29 ± 0.07	0.22 – 0.34	12
TBA	-	-	15	NA	NA	NA
PBDE 17	-	-	15	NA	NA	NA
PBDE 28	0.01	0.01	14	-	-	15
PBDE 47	0.37 ± 0.52	0.11 – 2.1	2	0.93 ± 0.54	0.45 – 2.28	
PBDE 49	0.02	0.02	14	NA	NA	NA
PBDE 66	0.02	0.02	14	NA	NA	NA
PBDE 71	-	-	15	NA	NA	NA
PBDE 77	0.002	0.002	14	NA	NA	NA
PBDE 85	0.01 ± 0.01	0.01 – 0.01	13	NA	NA	NA
PBDE 99	0.13 ± 0.09	0.05 – 0.25	1	0.34 ± 0.90	0.04 – 3.6	
PBDE 100	0.09 ± 0.11	0.03 – 0.43	2	0.30 ± 0.22	0.12 – 0.80	
PBDE 119	0.03 ± 0.02	0.01 – 0.04	13	NA	NA	NA
PBDE 126	-	-	15	NA	NA	NA
PBDE 138	-	-	15	-	-	15
PBDE 153	0.03 ± 0.02	0.01 – 0.07	2	0.13 ± 0.24	0.02 – 0.96	
PBDE 154	0.02 ± 0.01	0.01 – 0.06	6	0.31 ± 0.72	0.05 – 2.9	
PBDE 156	-	-	15	NA	NA	NA
PBDE 183	0.01 ± <0.01	0.01 – 0.02	6	0.13 ± 0.15	0.034 – 0.40	10
PBDE 184	-	-	15	NA	NA	NA
PBDE 191	-	-	15	NA	NA	NA

PBDE 196	0.03 ± <0.01	0.02 – 0.03	13	-	-	15
PBDE 197	0.03 ± 0.01	0.02 – 0.04	10	-	-	15
PBDE 202	-	-	15	NA	NA	NA
PBDE 206	0.13 ± 0.06	0.08 – 0.20	12	0.33 ± 0.61	0.01 – 1.6	9
PBDE 207	0.10 ± 0.08	0.03 – 0.23	4	0.30 ± 0.55	0.01 – 1.1	11
PBDE 209	2.1 ± 1.4	0.61 – 4.2	8	0.63 ± 0.73	0.20 – 2.0	9

Appendix B: table of biometric data

Table B1: Table of biometric data for all herring gulls included in this thesis, including body condition index (BCI) and trophic level (TL).

Location	ID	Sex	Wing (mm)	Weight (g)	Head (mm)	Lipid (%)	BCI	TL
Hornøya	1	f	431	1020	128	0.8	14	4.5
Hornøya	2	m	NA	1220	135	0.6	NA	4.4
Hornøya	3	m	450	1340	131	0.6	63	4.6
Hornøya	4	f	415	990	120	0.6	-35	4.5
Hornøya	5	m	437	1250	135	0.7	-31	4.7
Hornøya	6	f	420	1020	123	0.5	1.1	4.3
Hornøya	7	m	474	1280	142	0.5	6.0	4.7
Hornøya	8	f	430	1020	123	1.1	15	4.7
Hornøya	9	f	420	1070	116	0.8	54	4.5
Hornøya	10	f	429	960	119	0.9	-45	4.5
Hornøya	11	f	436	990	118	0.9	-4.7	4.6
Hornøya	12	m	449	1240	134	0.5	-38	4.9
Hornøya	13	m	454	1320	135	0.9	43	4.8
Hornøya	14	m	445	1280	131	0.7	1.5	4.6
Hornøya	15	m	467	1230	131	0.6	-44	4.7
Oslo	16	f	412	910	120	1.5	-17	2.6
Oslo	17	f	416	950	120	0.4	15	2.4
Oslo	18	f	850	424	112	1.0	-27	3.6
Oslo	19	f	426	940	116	0.6	22	3.2
Oslo	20	f	426	930	121	4.2	-33	2.7
Oslo	21	f	411	910	114	0.6	40	2.7
Oslo	22	f	448	1010	115	1.5	59	3.7
Oslo	23	f	423	810	113	0.8	-74	2.7
Oslo	24	f	437	970	120	2.7	-5.1	2.8
Oslo	25	f	446	930	117	1.3	-35	3.3
Oslo	26	f	427	920	118	14.8	-18	2.8
Oslo	27	f	435	950	118	0.8	-3.0	2.9
Oslo	28	f	426	975	117	0.9	48	3.1
Oslo	29	f	NA	NA	NA	1.6	NA	2.5
Oslo	30	f	416	935	117	0.8	28	2.7

Appendix C: table of stable isotope ratios

Table C1: Individual isotope ratios (‰) of C, N and S for each individual herring gull.

Location	ID	Ringnr	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Hornøya	1	JU675	-20.66	14.44	19.44
Hornøya	2	JU676	-21.04	14.29	20.57
Hornøya	3	JU689	-20.44	14.81	19.23
Hornøya	4	JU666	-21.10	14.40	20.55
Hornøya	5	JU688	-20.89	15.49	20.66
Hornøya	6	JU683	-21.01	13.87	19.29
Hornøya	7	JU687	-20.62	15.36	20.19
Hornøya	8	JU694	-20.97	15.16	18.51
Hornøya	9	JU680	-21.95	14.62	18.81
Hornøya	10	JU678	-21.18	14.52	20.18
Hornøya	11	JX511	-21.04	14.81	19.87
Hornøya	12	JX951	-20.47	16.00	20.76
Hornøya	13	JX510	-20.50	15.81	20.58
Hornøya	14	JX512	-20.46	14.80	19.07
Hornøya	15	JX513	-20.44	15.47	20.52
Oslo	16	JCH22	-24.43	8.184	10.23
Oslo	17	JCH23	-24.21	7.302	7.274
Oslo	18	JCH25	-21.88	11.99	13.78
Oslo	19	JCH27	-22.00	10.34	14.41
Oslo	20	JCL13	-24.30	8.423	10.45
Oslo	21	JCL43	-24.02	8.593	11.35
Oslo	22	JCL48	-21.09	12.49	17.36
Oslo	23	JCL51	-23.92	8.569	11.34
Oslo	24	JCL52	-24.23	9.100	13.21
Oslo	25	JCU50	-22.39	10.98	13.71
Oslo	26	JCX20	-24.52	8.861	9.597
Oslo	27	JCX95	-24.37	9.363	10.97
Oslo	28	JCX96	-23.65	10.20	11.66
Oslo	29	JCX00	-24.58	7.948	8.610
Oslo	30	JCH28	-24.50	8.392	10.73

Table C2. Mean ± standard deviation (SD) and range (Min – Max) measures of stable isotope ratios (‰) in the herring gull of the Oslofjord (n=15), split into terrestrial (n=11) and marine feeders (n=4), and Hornøya (n=15) split into females (n=7) and males (n=8).

	Oslofjord		Hornøya	
Mean ± SD Min – Max	Terrestrial n=11	Marine n=4	Females n=7	Males n=8
$\delta^{15}N$ (‰)	8.63 ± 0.763 7.30 – 10.2	11.5 ± 0.971 10.3 – 12.5	14.5 ± 0.394 13.9 – 15.2	15.3 ± 0.574 14.3 – 16.0
$\delta^{13}C$ (‰)	-24.2 ± 0.286 -24.6 – -23.7	-21.8 ± 0.544 -22.4 – -21.1	-21.1 ± 0.396 -21.9 – -20.7	-20.6 ± 0.232 -21.0 – -20.4
$\delta^{32}S$ (‰)	10.5 ± 1.59 7.27 – 13.2	14.8 ± 1.73 13.7 – 17.4	19.5 ± 0.731 18.5 – 20.6	20.2 ± 0.668 19.1 – 20.8

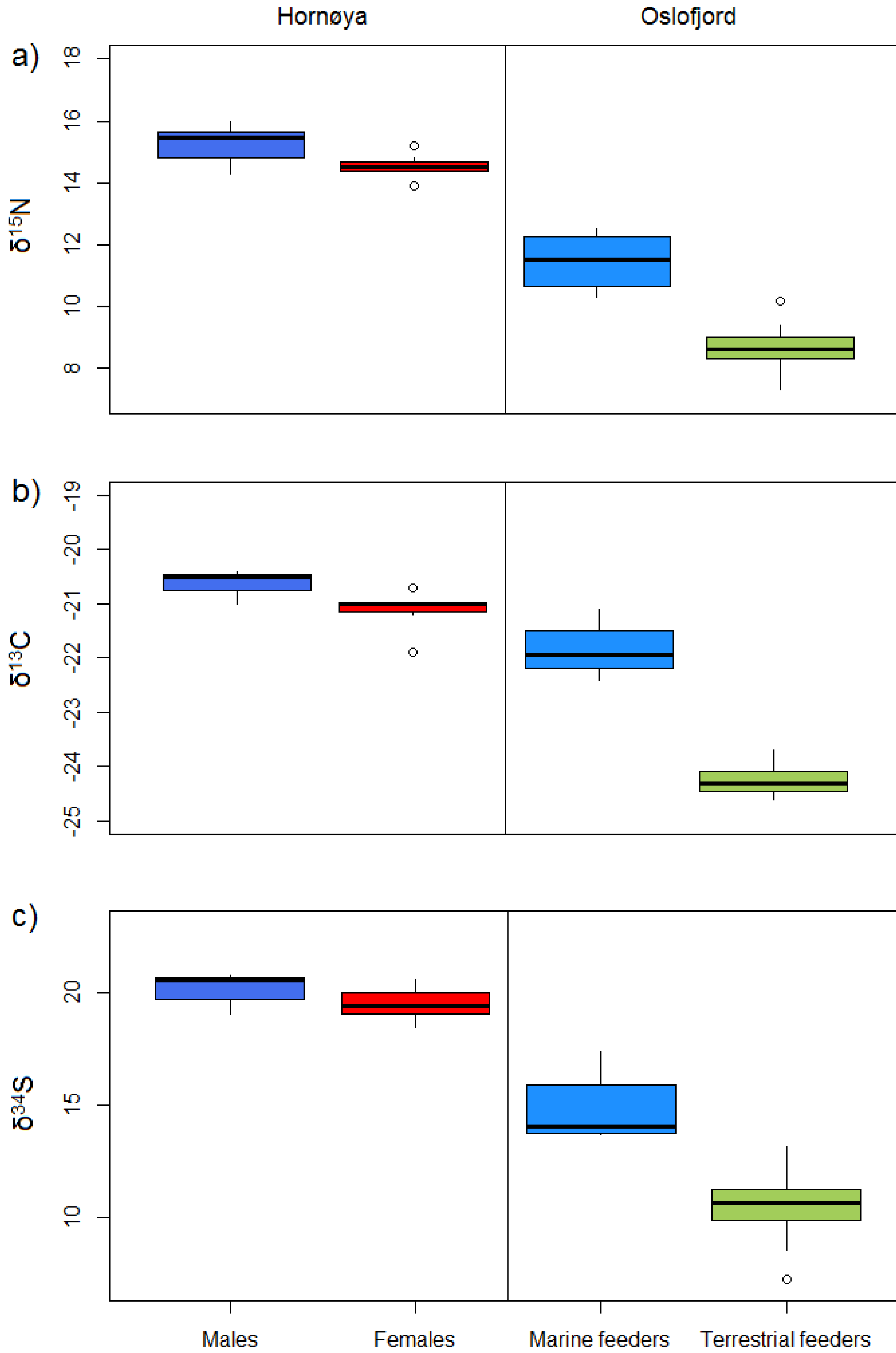

Figure C1. Isotope levels of N (‰), C (‰) and S (‰), Hornøya males (n=8), females (n=7). Oslofjord divided into marine (n=4) and terrestrial feeders (n=11). The boxes stretch from first to third quartile, with the horizontal line representing the measurement median. The whiskers extend to 1.5 times the interquartile range, and the points are extreme values, also defined as outliers.

Appendix D: ISTD and RSTD for Hornøya samples, NILU

	ISTD	RSTD
POPs	POPI (01.16)1:10 diluted, 15.06.16	13C PCB 159 213 pg/µL, 11.05.20165
PFAS	0,1 ng/µL 13 PFC mix, 26.05.2016	0,102 ng/µL 3,7-dimet-PFOA, 08.05.2015
cVMS	13C siloxane STD, 18.11.2015	200 ng/mL M4T in hexane, 13.02.2016

Appendix E: tail intensity (%) of white blood cells

Table E1: DNA tail intensity (%) for each individual herring gull included in this thesis.

Location	ID	Ringnr	Baseline	H_2O_2	Recovery
Hornøya	1	JU675	0.19	4.59	5.81
Hornøya	2	JU676	0.33	4.37	11.15
Hornøya	3	JU689	0.96	9.18	22.73
Hornøya	4	JU666	1.20	12.00	12.80
Hornøya	5	JU688	0.45	8.41	2.58
Hornøya	6	JU683	1.37	1.22	6.22
Hornøya	7	JU687	2.45	6.32	4.36
Hornøya	8	JU694	0.73	3.66	1.72
Hornøya	9	JU680	0.36	1.76	4.80
Hornøya	10	JU678	0.97	6.89	7.19
Hornøya	11	JX511	0.43	6.14	3.96
Hornøya	12	JX951	21.50	11.83	14.97
Hornøya	13	JX510	28.14	11.88	22.27
Hornøya	14	JX512	0.17	1.80	7.96
Hornøya	15	JX513	2.56	66.42	27.33
Oslofjord	16	JCH22	4.80	77.21	28.24
Oslofjord	17	JCH23	1.10	5.91	29.93
Oslofjord	18	JCH25	4.74	34.30	17.82
Oslofjord	19	JCH27	0.94	21.10	18.36
Oslofjord	20	JCL13	2.71	29.29	21.88
Oslofjord	21	JCL43	1.17	27.02	35.41
Oslofjord	22	JCL48	3.21	13.09	26.56
Oslofjord	23	JCL51	6.67	51.23	42.57
Oslofjord	24	JCL52	11.82	23.27	16.45
Oslofjord	25	JCU50	14.99	43.78	37.64
Oslofjord	26	JCX20	32.97	16.76	17.25
Oslofjord	27	JCX95	7.67	58.99	32.94
Oslofjord	28	JCX96	4.32	25.92	34.45
Oslofjord	29	JCX00	1.60	21.73	75.25
Oslofjord	30	JCH28	5.58	24.06	48.98

Appendix F: table of LOD of contaminants for both areas

Table F1: the limit of detection (LOD) or quantification (LOQ, cVMS only) for each contaminant at each location. No value, means the LOD was not quantified.

	Hornøya	Oslofjord
D4 (LOQ)	1.50	2.00
D5 (LOQ)	1.60	1.30
D6 (LOQ)	2.00	2.15
o,p-DDE	0.0029	0.035
p,p-DDE	0.0140	2.0
o, p DDD	0.0058	0.019
p, p-DDD	0.0053	0.030
o,p-DDT	0.0072	0.051
p,p- DDT	0.0074	0.13
PeCB	0.0085	0.069
αHCH	0.0020	0.028
βHCH	0.0035	0.022
ɣHCH	0.0037	0.032
HCB	0.0027	0.15
PCB 28	0.0013	0.050
PCB 47	0.0033	0.041
PCB 52	0.0066	0.062
PCB 66	0.0029	0.08
PCB 99	0.020	-
PCB 101	0.0030	0.12
PCB 105	0.0036	-
PCB 118	0.021	-
PCB 128	0.010	-
PCB 138	0.038	-
PCB 153	0.092	-
PCB 180	0.044	-
PCB 183	0.017	-
PCB 187	0.012	-
6:2FTS	0.25	0.30
PFOSA	0.25	0.10
PFBS	0.25	0.10
PFPS	0.25	0.20
PFHxS	0.25	0.10
PFHpS	0.25	0.20
PFOS	0.25	0.20
PFNS	0.25	0.20
PFDcS	0.25	0.20
PFHxA	0.10	0.50
PFHpA	0.10	0.50
PFOA	0.10	0.50
PFNA	0.10	0.10
PFDcA	0.10	0.50
PFUnA	0.10	0.40
PFDoA	0.10	0.40
PFTriA	0.10	0.40
PFTeA	0.10	0.40
PBDE 28	0.014	0.0062
PBDE 47	0.0040	0.055
PBDE 99	0.006	0.011
PBDE 100	0.013	0.015
PBDE 138	0.0080	0.013
PBDE 153	0.0030	0.015
PBDE 154	0.0030	0.011
PBDE 183	0.0080	0.0091
PBDE 196	0.0060	0.024
PBDE 197	0.0060	0.020
PBDE 206	0.0050	0.054
PBDE 207	0.0060	0.034
PBDE 209	0.070	0.66

Appendix G: Mercury analysis

Mercury analysis was only conducted on the Hornøya samples, and done by staff at NILU (Kjeller).

About 0.3-0.6 grams of sample were weighed in TFM tubes and 5 ml of diluted supra pure nitric acid was added. The samples were digested by microwave-assisted mineralization using an UltraClave. The samples were submitted to a four-step program with 220°C as maximum temperature. After digestion, the samples were diluted using deionized water to a volume of 50 mL. The samples were then split in two aliquots; one goes untreated to Hg determination, the other with an addition of supra pure HCl. The metal and mercury content is determined using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700x). Indium is used as internal standard.

Appendix H: PCA plots

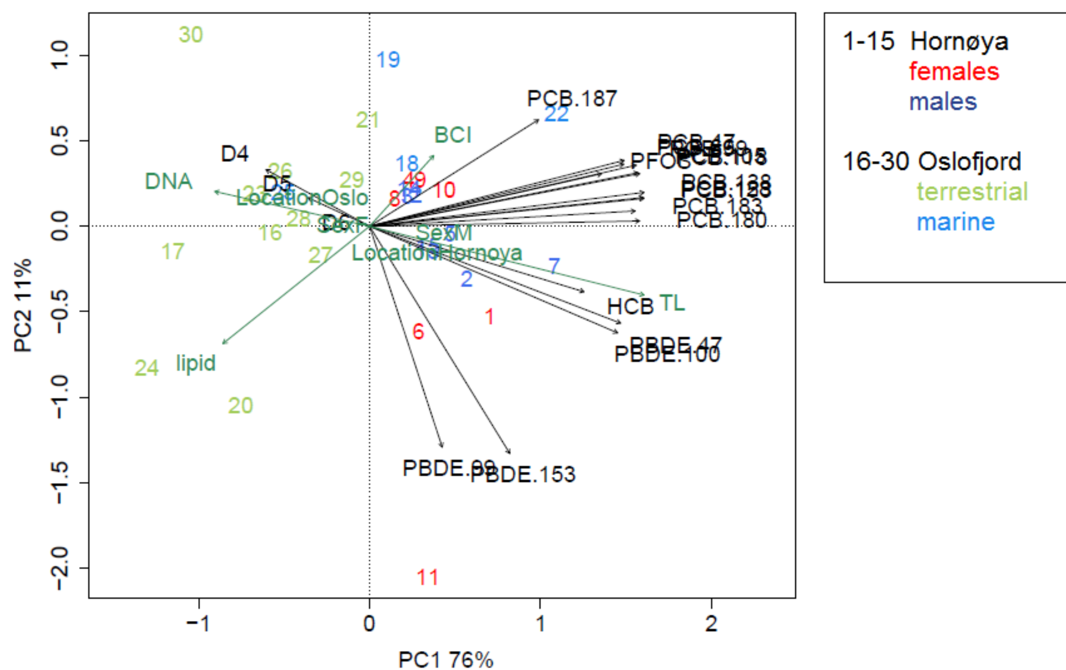

Figure H1. Principal component analysis (PCA) triplot with all contaminants (log10 transformed ng/g w.w.) and explanatory variables, individual herring gulls (1-30) positioned according to each owns contaminant load (Hornøya females n=7, males n=8; Oslofjord terrestrial n=11, marine n=4). Explanatory variables are shown as passive vectors (green) not affecting the scoring of the samples (individual gulls) or the loadings of the responses (contaminants). The proportion of variance explained by PC1 and PC2 is shown on the x- and y-axis, respectively.

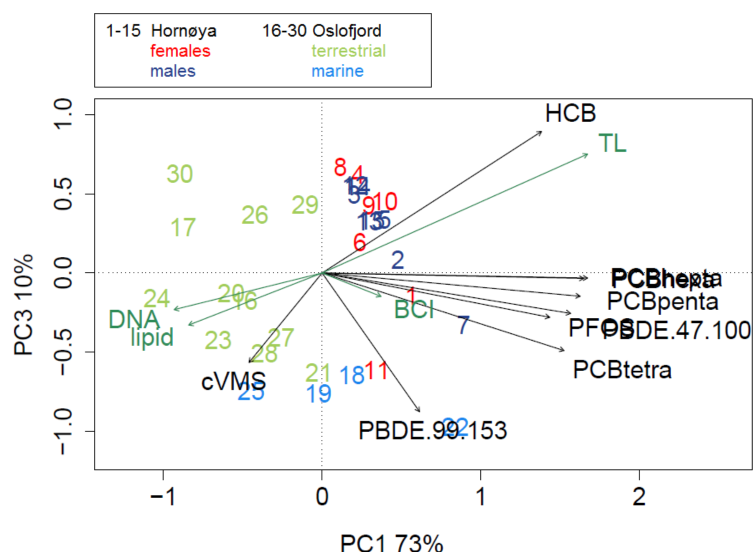

Figure H2. Principal component analysis (PCA) triplot with all contaminants (log10 transformed ng/g w.w.) and explanatory variables, individual herring gulls (1-30) positioned according to each owns contaminant load (Hornøya females n=7, males n=8; Oslofjord terrestrial n=11, marine n=4). Explanatory variables are shown as passive vectors (green) not affecting the scoring of the samples (individual gulls) or the loadings of the responses (contaminants). The proportion of variance explained by PC1 and PC3 is shown on the x- and y-axis, respectively.

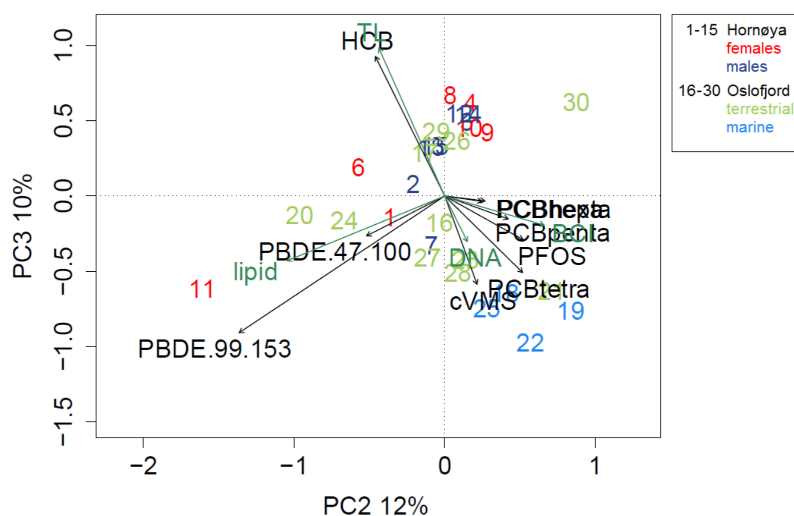

Figure H3. Principal component analysis (PCA) triplot with all contaminants (log10 transformed ng/g w.w.) and explanatory variables, individual herring gulls (1-30) positioned according to each owns contaminant load (Hornøya females n=7, males n=8; Oslofjord terrestrial n=11, marine n=4). Explanatory variables are shown as passive vectors (green) not affecting the scoring of the samples (individual gulls) or the loadings of the responses (contaminants). The proportion of variance explained by PC2 and PC3 is shown on the x- and y-axis, respectively.

Appendix I: PCB correlation plots

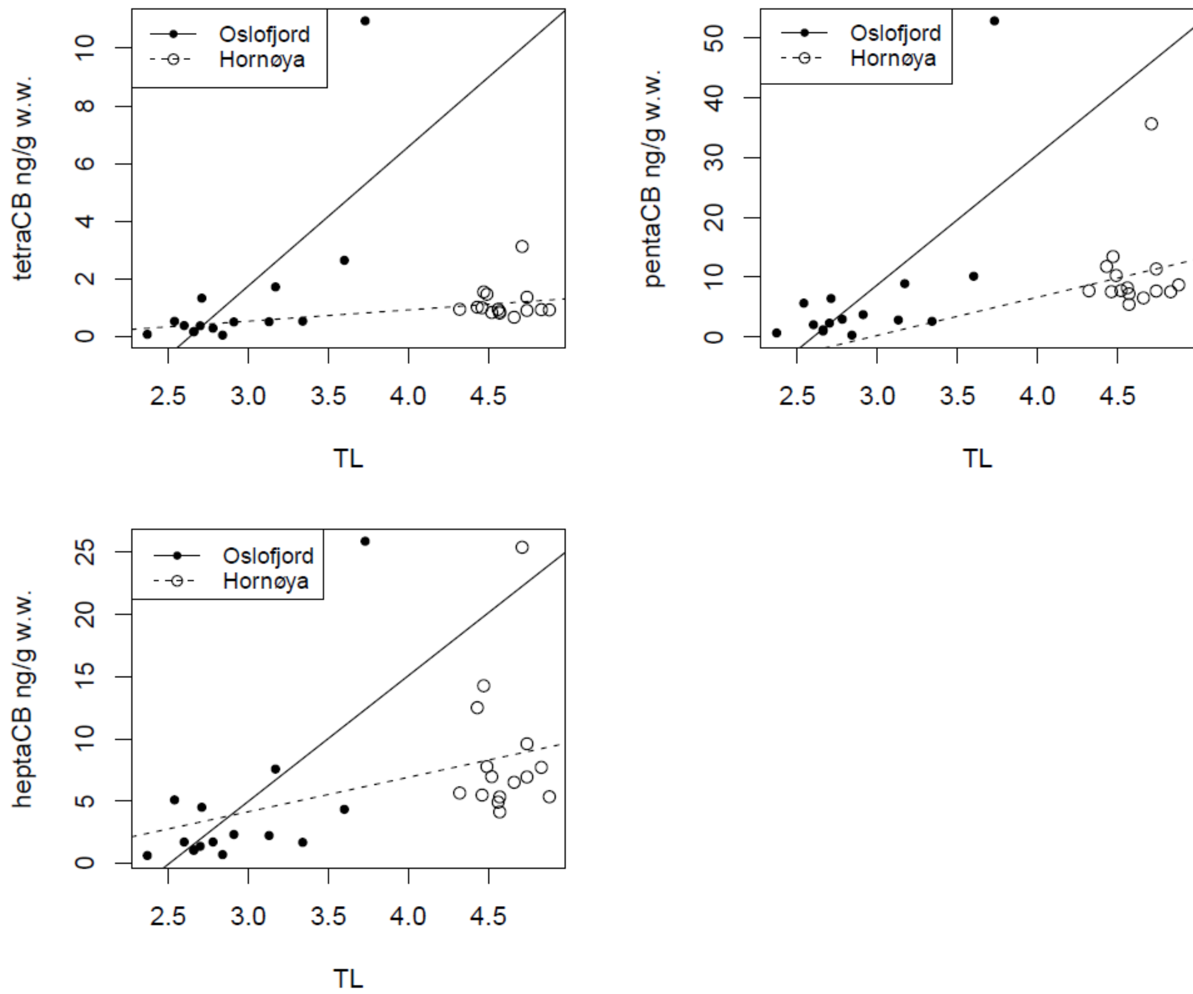

Figure I1: Figure 17. Trophic level (TL) and PCB concentration (ng/g w.w.) for tetraCB, pentaCB and heptaCB, in herring gulls separated into location, Hornøya (n=15) and Oslofjord (n=15). Regression lines are shown.

Appendix J: analytes

Table J1: List of analytes and abbreviation.

Group	Abbreviation	Analyte
Metal	Hg	Mercury
PFASs	6:2FTS	1H,1H,2H,2H-perfluorooctane sulfonic acid
	PFOSA	Perfluorooctane sulfonamide
	PFBS	Perfluorobutane sulfonate
	PFPS	Perflurorpentane sulfonate
	PFHxS	Perfluorohexane sulfonate
	PFHpS	Perfluoroheptane sulfonate
	brPFOS	Branched perfluorooctane sulfonate
	PFOS	Perfluorooctane sulfonate
	PFNS	Perfluorononanoic 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
Pesticides	o,p’-DDT	o,p’-dichlorodiphenyltrichloroethane
	p,p’- DDT	p,p’-dichlorodiphenyltrichloroethane
	o,p’ DDD	o,p’-dichlorodiphenyldichloroethane
	p,p’-DDD	p,p’-dichlorodiphenyldichloroethane
	o,p-DDE	o,p-dichlorodiphenyldichloroethylene
	p,p’-DDE	p,p’-dichlorodiphenyldichloroethylene
	α-HCH	α-hexachlorocyclohexane
	β-HCH	β-hexachlorocyclohexane
	ɣ-HCH	ɣ-hexachlorocyclohexane
	PeCB	Pentachlorobenzene
	HCB	Hexachlorobenzene
	trans-chlordane	
	cis-chlordane	
	oxy-chlordane	
	trans-nonachlor	
	cis-nonachlor	
	Mirex	
PCBs	PCB 28	2,4,4'-trichlorobiphenyl
	PCB 47	2,2',4,4'-tetrachlorobiphenyl
	PCB 52	2,2',5,5'-tetrachlorobiphenyl
	PCB 66	2,3',4,4'-tetrachlorobiphenyl

	PCB 71	2,3',4',6-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 128	2,2',3,3',4,4'-hexachlorobiphenyl
	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
cVMS	D4	Octamethylcyclotetrasiloxane
	D5	Decamethylcyclopentasiloxane
	D6	Dodecamethylcyclohexasiloxane
PBDEs	TBA	2,4,6-tribromoanisole
	PBDE 17	2,2',4-tribromodiphenyl ether
	PBDE 28	2,4,4'-tribromodiphenyl ether
	PBDE 47	2,2',4,4'-tetrabromodiphenyl ether
	PBDE 49	2,2',4,5'-tetrabromodiphenyl ether
	PBDE 66	2,3',4,4'-tetrabromodiphenyl ether
	PBDE 71	2,3',4',6-tetrabromodiphenyl ether
	PBDE 77	3,3',4,4'-tetrabromodiphenyl ether
	PBDE 85	2,2',3,4,4'-pentabromodiphenyl ether
	PBDE 99	2,2',4,4',5-pentabromodiphenyl ether
	PBDE 100	2,2',4,4',6-pentabromodiphenyl ether
	PBDE 119	2,3',4,4',6-pentabromodiphenyl ether
	PBDE 126	3,3',4,4',5-pentabromodiphenyl ether
	PBDE 138	2,2',3,4,4',5'-hexabromodiphenyl ether
	PBDE 153	2,2',4,4',5,5'-hexabromodiphenyl ether
	PBDE 154	2,2',4,4',5,6'-hexabromodiphenyl ether
	PBDE 156	2,3,3',4,4',5-hexabromodiphenyl ether
	PBDE 183	2,2',3,4,4',5',6-heptabromodiphenyl ether
	PBDE 184	2,2',3,4,4',6,6'-heptabromodiphenyl ether
	PBDE 191	2,3,3',4,4',5',6-heptabromodiphenyl ether
	PBDE 196	2,2′,3,3′,4,4′,5,6′-octabromodiphenyl ether
	PBDE 197	2,2′,3,3′,4,4′,6,6′-octabromodiphenyl ether
	PBDE 202	2,2′,3,3′,5,5′,6,6′-octabromodiphenyl ether
	PBDE 206	2,2',3,3',4,4',5,5',6-nonabromodiphenyl ether
	PBDE 207	2,2′,3,3′,4,4′,5,6,6′-nonabromodiphenyl ether
	PBDE 209	2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether