Environmental contaminants in herring gull (*Larus argentatus*) and maternal transfer in two colonies in the Oslofjord

Nina Cathrine Knudtzon

Master thesis in Toxicology

Institute of Biosciences
Faculty of Mathematics and Natural Sciences

UNIVERSITY OF OSLO

1st June 2019

2019

Environmental contaminants in herring gull (*Larus argentatus*) and maternal transfer in two colonies in the Oslofjord

Nina Cathrine Knudtzon

http://www.duo.uio.no

Trykk: Reprosentralen, Universitetet i Oslo

The project was a collaboration between the Department of Biosciences at the University of Oslo (UiO), the Norwegian Institute of Water Research (NIVA), and the Norwegian Institute of Air Research (NILU). The thesis is part of a larger project called “Environmental Contaminants in an Urban Fjord”, under contract by from Norwegian Environment Agency (Miljødirektoratet).

Acknowledgements

The presented thesis was conducted at the Department of Biosciences at the University of Oslo and is associated with the Environmental Contaminants in an Urban Fjord ("Urbanfjord") project. This project is a monitoring programme conducted over several years, which has focused on the levels and food web biomagnification of various contaminants in different species representing the Oslofjord community. Urbanfjord is a joint collaboration between the University of Oslo (UiO), the Norwegian Institute of Water Research (NIVA), the Norwegian Institute of Air Research (NILU), and is funded by the Norwegian Environment Agency.

I would first like to thank my supervisors, Katrine Borgå (UiO), Anders Ruus (NIVA/UiO) and Morten Helberg (UiO) for their help and support. Thanks to Anders for always being available when I needed advice and for answering all my questions. My research would not have been possible without Morten and Sindre Molværsmyr who handled and collected the herring gull samples in the field, as well as letting me take part in this process. I want to thank all the people at NILU for their guidance and help during the chemical analysis, especially Silje Eltvik Thomassen and Inger Christin Steen.

Especial thanks to Katrine for giving me this project and for all your comments, remarks and engagement through the learning process of this master thesis. Thank you for encouraging me to go to Svalbard, to present at conferences and to grow as a researcher. Thanks to Sabrina for trusting me enough to hire me as a research assistant and for translating a very important paper from German to English for me.

A big thanks to the great people at study room 4611. The last two years would not have been the same without you. Thank you to Emelie, Malin, Oda, Helene, Sofie and Clare for all the great support and for always being a positive element in a sometimes quite tedious everyday life.

Finally, I must express my gratitude to my friends and family for believing in me and always rooting for me. A special thanks to Johanne and Simon for proofreading my thesis and for the tremendous support during the course of this master thesis.

Abstract

A number of environmental contaminants bioaccumulate and biomagnify, and the accumulated levels are generally high in organisms at high trophic levels, such as seabirds. The herring gull has been used as an indicator species for the contaminant status of the inner Oslofjord. However, its role as an indicator of the overall contaminant levels in the fjord has been questioned, as the gull has been observed to search for food outside of the fjord.

This study aimed to assess the influence of food source and trophic niche, as well as biological variables, on the contaminant status in the herring gull and the maternal transfer to eggs. Blood and biometric measurements were collected from female herring gulls from one colony in the inner Oslofjord and one colony in the outer Oslofjord, as well as one egg from each nest. Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in both blood and eggs were quantified to determine the diet source and trophic niche of the two colonies during the breeding season.

A more marine signal and a higher trophic niche were found in the outer Oslofjord colony compared to the inner Oslofjord colony. The levels of lipid-soluble legacy POPs; polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and polybrominated diphenyl ethers (PBDEs), did not differ between the inner and outer fjord colony and were uncorrelated with $\delta^{13}C$ and $\delta^{15}N$. The level of the protein associated perfluoroalkyl substances (PFASs) was higher in the outer fjord colony, which is likely linked to local point source exposure from the use of fire-fighting foam related to a previously active fire drill area near the outer fjord colony. In both colonies, lipophilic contaminants were transferred from mother to egg to a higher degree than protein-associated contaminants and metals. PCBs dominated the contaminant load in the eggs from both colonies and were considerably higher than that observed in the mothers. The lipophilic legacy POPs in herring gull eggs were positively correlated with $\delta^{13}C$, suggesting increased maternal transfer of lipophilic POPs with a more marine diet. No differences in the levels of lipophilic legacy POPs were observed between the colonies, indicating similar transfer rate between the females of the two colonies, regardless of differences in diet.

Abbreviations

AIC	Akaike information criterion
ANCOVA	Analysis of covariance
Ag	Silver
As	Arsenic
BCI	Body condition index
BFR	Brominated flame retardant
C:N	Carbon-to-nitrogen ratio
CB	Chlorobiphenyl
Cd	Cadmium
CP	Chlorinated paraffins
Cu	Copper
Fe	Iron
GC	Gas chromatography
HCB	Hexachlorobenzene
Hg	Mercury
IFE	Institute for Energy Technology
ISTD	Internal standard
Kow	Octanol-water partition coefficient
lw	Lipid weight
LOD	Limit of detection
LOQ	Limit of quantification
Log	Logarithm
MS	Mass spectrometer
N_2	Nitrogen gas
NA	Not analysed
Na_2SO_4	Sodium sulphate
Ni	Nickel
NILU	Norwegian Institute for Water Research
NIVA	Norwegian Institute for Air Research
OHC	Organohalogen contaminant
Pb	Lead

PBDE	Polybrominated diphenyl ether
PBT	Persistent, bioaccumulative and toxic chemical
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCP	Personal care products
PFAS	Perfluorinated alkyl substance
PFCA	Perfluorinated carboxyl acid
PFDoA	Perfluorododecanoic acid
PFOS	Perfluorooctane sulfonate
PFSA	Perfluorinated sulfonic acid
POP	Persistent organic pollutant
RDA	Redundancy analysis
REACH	Registration, evaluation, authorisation and restriction of chemicals
SD	Standard deviation
SE	Standard error
SVHC	Substances of very high concern
TL	Trophic level
TMF	Trophic magnification factor
UiO	University of Oslo
cVMS	Cyclic volatile methylsiloxanes
ww	Wet weight
Zn	Zink
$\delta^{13}C$	Stable isotope ratio of carbon
$\delta^{13}N$	Stable isotope ratio of nitrogen

Table of contents

1 Introduction

1.1 Environmental contaminants

The industrial revolution marked a turning point in history by providing better living standards for the human population through the use of machines for manufacturing, as well as manmade chemicals for industrial and household purposes (Becker et al., 2012; Huber et al., 2015; KEMI, 2015). Although this has created better working and living conditions for humans, it has also led to the release of a wide range of contaminants into the environment, both manmade chemicals and naturally occurring elements (Azad et al., 2019; Huber et al., 2015; Ruus et al., 2019). Persistent organic pollutants (POPs) are one example of manmade chemicals. These contaminants have been produced both intentionally and unintentionally for a range of different purposes, such as in industry, agriculture and in consumer products (Letcher et al., 2010).

Some POPs have been investigated for decades. Several of these, such as dichlorodiphenyldichloroethylene (DDE), have had adverse effects in wildlife (Huber et al., 2015; Letcher et al., 2010). These are referred to as "legacy" POPs because their present-day contamination is a legacy of previous releases, as they are under current global regulation (Riget et al., 2010). The legacy POPs are persistent, bioaccumulative and toxic chemicals (PBTs; Lipnick et al., 2001; Riget et al., 2010). Being organic, many POPs are lipophilic compounds (Eljarrat & Barcelo, 2003), with typically a long half-life (Jones & de Voogt, 1999).

A large group of POPs are the organohalogen contaminants (OHCs). OHCs are compounds containing at least one halogen atom (fluorine, chlorine, bromine). They are toxic, either by themselves or by their biologically active metabolites created through degradation or *in vivo* biotransformation, with adverse effects on both humans and wildlife (Letcher et al., 2010). Some of the best known OHC groups are the polychlorinated biphenyls (PCBs) and the brominated flame retardants (BFRs), including the polybrominated diphenyl ethers (PBDEs). PCBs are highly lipophilic and were previously used in a variety of products, such as in sealants, plastics, flame-retardant plasticizers for paints, and for dielectric fluids in transformers and capacitors (Borgå et al., 2005; Lipnick et al., 2001). BFRs have been used in combustible materials, such as plastics, paper and textiles, in order to meet fire safety

regulations (de Wit, 2002). Both PCBs and BFRs are known to biomagnify in ecosystems, causing a potential risk to marine top predators (Borgå et al., 2005; de Wit et al., 2006; de Wit et al., 2010; Sørmo et al., 2011).

International regulations such as the UNEP Stockholm Convention, which came into force in 2004, have led to a decline in the emission of POPs globally (EC and EPA, 2009; Helgason et al., 2008). This treaty aims to protect human health and the environment from continued exposure to POPs (UNEP, 2001). Initially, twelve POPs, known as the "dirty dozen", were placed under restriction by the Stockholm Convention. These include pesticides such as dichlorodiphenyltrichloroethane (DDT) and hexachlorobenzene (HCB), and industrial chemicals such as PCBs. More recent POPs have also been added to the list and include pesticides and industrial chemicals such as PBDEs (i.e. tetra-, penta-, hexa-, and hepta-bromodiphenyl ether), short-chain chlorinated paraffins (SCCPs), and perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOS-F; UNEP, 2009).

Research focusing on chemicals of widespread, emerging concern, i.e. emerging contaminants, such as pharmaceuticals and personal care products (PPCPs), cyclic volatile methyl siloxanes (cVMS), pesticides, industrial compounds or by-products of these, and food additives, has not advanced as far as the research on legacy POPs (Lapworth et al., 2012). However, emerging contaminants have received increasing attention in recent years (Buck et al., 2011; Huber et al., 2015; Lapworth et al., 2012; Ruus et al., 2019). These emerging contaminants of concern are either newly introduced compounds and their metabolites, or compounds with previously unrecognized adverse effects on the ecosystem. Moreover, they are currently not regulated or included in any routine monitoring programs (Barceló & Petrovic, 2008). For example, cVMSs are still being used in personal care products (PCPs) and in silicone polymer production. Of the three most extensively used cVMSs (i.e., D4, D5 and D6), the levels of D5 dominate in PCPs and it is the most widely used cVMS in the European Union (EU; Wang et al., 2013). However, D5 was listed on the Norwegian priority list in 2006 due to its bioaccumulating, persistent and possible toxic properties, with the aim of reducing emissions and use by 2020 (Norwegian Environment Agency, 2017).

Unlike the lipophilic POPs, perfluoroalkyl substances (PFAS) are amphipathic and tend to bind to proteins (Jones et al., 2003). PFASs are chemicals with one or more C atoms on which all the substituents (present in the nonfluorinated analogues from which they are notionally

derived) have been replaced by F atoms, in such a manner that PFASs contain the perfluoroalkyl moiety C_nF_{2n+1} (Buck et al., 2011). The exact number of different PFAS compounds is not known. By May 2018, researchers had identified 4730 PFAS-related structures from patent filing and chemical registries, all of them possibly in commercial use (OECD, 2018). PFASs have properties that make them suitable for multiple applications, including surface-active agents, cosmetic products, plant protection products, textiles/leather/paper impregnation and foam-based fire extinguishing agents (KEMI, 2015). Perfluorooctane sulfonate (PFOS), one of the PFAS compounds, is classified as toxic for water living organisms and was banned in 2007 (UNEP, 2009). Long-chained perfluorinated carboxyl acids (PFCAs) containing 9 – 14 carbon atoms are identified as poorly biodegradable. They bioaccumulate in organisms, with long-term effects on health or environment. They are substances of very high concern (SVHC) and are on REACH's Candidate List, where REACH is the system for regulating chemicals in Europe (REACH, 2019).

In addition to the organic compounds, inorganic trace-elements can cause adverse effects even at very low levels of exposure. Metals are elements and thus non-biodegradable, and can be found throughout the environment (Davidson et al., 2007; Walker et al., 2012). Metals are either essential or non-essential to organisms. Essential metals are found in all living organisms and play a variety of roles, for example as structural elements or components of control mechanisms (e.g. in muscles; Davidson et al., 2007). A deficiency of these metals will result in impairment of biological functions. However, essential metals may be toxic to organisms when they exceed a certain level (Davidson et al., 2007). Non-essential metals such as nickel (Ni), arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg), have no intended function in organisms and may affect an organism by competing with the essential metals at active sites of important biological molecules (Walker et al., 2012).

The Agency for Toxic Substances and Disease Registry (ATSDR) and the US Environmental Protection Agency (EPA) are responsible for the Substance Priority List (SPL), which identifies substances posing the greatest threat to human health due to their known or suspected toxicity and potential for human exposure. Based on the SPL from 2017, As, Pb, Hg and Cd are ranked as the first, second, third and seventh compound in this list, respectively (ATSDR, 2017). Although these elements occur naturally in the environment, human activities such as smelting and combustion have led to increased release of these

elements into the environment, inducing acute or chronic toxicity to living organisms (Huber et al., 2015). Cd, As and Ni are carcinogenic metals that have been associated with DNA damage such as base pair mutation and deletion or formation of oxygen radicals (Hartwig, 2013; Landolph, 1994).

Several metals are bioaccumulative (Huber et al., 2015). Pb, used in many industrial applications, can interfere with the biosynthesis of heme, eventually leading to anaemia (Lubran, 1980). Cd is of high concern due to its accumulation in the environment and its long biological half-life, amplifying its toxicity, such as nephrotoxicity (toxicity in the kidneys; Goyer, 1989), in organisms (GESAMP, 1985; Järup et al., 1998). Cd accumulates in aquatic organisms (Dubois & Hare, 2009), and has been detected in several gull species such as glaucous gulls (*Larus hyperboreus*) and Iceland gull (*Larus glaucoides*; Dietz et al., 1996). The presence of Cd in the species, usually associated with the gulls' diet, arises mainly from anthropogenic sources, such as mining activities and fuel combustion (Johansen et al., 1991). Ni is able to bind to transferrin, one of the major proteins in cellular uptake of the essential metal iron (Fe), and thus interferes with Fe transport (Davidson et al., 2007).

Hg enters the biosphere from both natural and anthropogenic sources and is known to be potentially toxic (Dietz et al., 2013). International agreements such as the Minamata Convention has led to a reduction in the emissions of Hg, but discharges still occur. The anthropogenic sources of Hg account for roughly 30% of the Hg emissions into the air, while 60% is from reemissions of previously released Hg (Amos et al., 2013). Hg exists both in organic and inorganic forms: the methylated (organic) form is taken up more efficiently than the inorganic form (Walker et al., 2012). Methylmercury is the most toxic form of Hg. It acts as a neurotoxin and thereby causes harmful effects on organisms (Dietz et al., 2013). Female gulls have shown evidence of Hg transference to their feathers and eggs (Becker et al., 1989; Burger & Gochfeld, 1996). This makes the eggs, as well as adult gulls, good indicators of metal contamination. In marine ecosystems, organisms at the top of the food chain are of high risk of methylmercury exposure due to Hg's ability to biomagnify (Lehnherr, 2014).

1.2 Monitoring

International regulations such as the Stockholm Convention have contributed to reducing the emissions and use of POPs globally (EC and EPA, 2009; Helgason et al., 2008).

Unfortunately, several point sources remain, leading to reemissions of banned or restricted contaminants into the ambient air and waters (Berge et al., 2013). POPs have shown to bioaccumulate and biomagnify, making their levels evident in organisms high up in the food chain, such as marine mammals and seabirds (Furness & Camphuysen, 1997; Letcher et al., 2010).

In the late 1960s, elevated levels of organic contaminants were detected in herring gull (*Larus argentatus*) eggs from Lake Michigan. Moreover, the herring gull colony presented high embryonic mortality, eggshell thinning and low overall reproductive success (Keith, 1966). A few years later, the same adverse effects were found in herring gulls in the Canadian Great Lakes (Gilbertson & Hale, 1974; Gilbertson & Fox, 1977), which led to the inclusion of herring gull adults and eggs as a monitoring species in the Great Lake monitoring programme in Canada (Fox et al., 1978; Gilman et al., 1977). The Great Lake monitoring programme has found that contaminant levels in herring gull are linked with dietary uptake, and the programme uses this species as an indicator of temporal and spatial contaminant levels (EC and EPA, 2009; Ewins et al., 1994; Hebert et al., 2000).

1.3 Study species

The herring gull, being a prominent part of the aquatic bird community (Ewins et al., 1994), as well as on the basis of earlier studies (Keilen, 2017; Ruus et al., 2016), was chosen as study species for this thesis. The gull was used to examine the levels of several environmental contaminants in the females and the maternal transfer to eggs between two colonies in the Oslofjord.

The herring gull is a common species along the Norwegian coast, occupying a broad ecological niche (Huber et al., 2015) and a high trophic level (TL) in the food web, with an estimated value between 3 and 4 based on their marine diet (Sørmo et al., 2011). The gulls are opportunistic feeders, feeding on a variety of food items, ranging from marine invertebrates and fish, to terrestrial invertebrates, vegetation, other birds, and litter (Sørmo et al., 2011). Furthermore, levels of several contaminants in seagull eggs have been shown to reflect their maternal contaminant patterns (Verreault et al., 2006), and with the herring gull's wide distribution, their eggs as well as their blood are ideal for monitoring studies (Helgason et al., 2008; Huber et al., 2015; Ruus et al., 2016).

1.3.1 Ecological niche

Levels of POPs have been linked to trophic position and food source in several gull species (Borgå et al., 2001; Haukås et al., 2007; Ruus et al., 2002; Sørmo et al., 2011). The POP levels in seabirds are primarily determined by feeding habits and the seabirds' ability to biotransform these contaminants (Borgå et al., 2005).

Stable isotopes of carbon and nitrogen has been used extensively during the last two decades as dietary markers to describe feeding habits (e.g. Hobson & Clark, 1992; Ruus et al., 2017). Stable isotope analysis can be used to describe the trophic position ($\delta^{15}N$) and the dietary carbon source ($\delta^{13}C$) of the organism (Peterson & Fry, 1987). $\delta^{15}N$, which is equivalent to the ratio of ^{15}N to ^{14}N, is calculated based on the notion that the heavier ^{15}N isotope is retained in the body, while the lighter ^{14}N gets metabolised and excreted (Peterson & Fry, 1987). The enrichment of $\delta^{15}N$ with each TL is on average 2.4‰ for seabirds (Mizutani et al., 1991). The ratio of the two stable isotopes of carbon, ^{13}C and ^{12}C (i.e., $\delta^{13}C$), can be used to determine the diet's carbon source, as the carbon isotope composition of animals is comparable to that of their diet (Peterson & Fry, 1987). This ratio varies between primary producers due to different photosynthetic pathways in plants, i.e., C_3 and C_4 carbon fixation, where C_3 plants are more depleted in $\delta^{13}C$ than C_4 plants. Planktonic photosynthesis results in a more enriched $\delta^{13}C$ than for both C_3 and C_4 plants (Peterson & Fry, 1987). The isotope ratios from these plants are retained in consumers and predators, resulting in a lower $\delta^{13}C$ ratio for organisms with more C_3 plant intake compared to a more C_4 based diet (Vogel & van der Merwe, 1977). A lower $\delta^{13}C$ indicates a diet feeding on terrestrial or freshwater organisms, while an animal with a higher $\delta^{13}C$ is indicative of a marine-based, pelagic diet (Elliott et al., 2014).

The turnover rate, the time it takes for 50% of the stable isotopes in the tissue to be replaced by the stable isotopes in the diet, is mainly affected by the metabolic rate (Hobson & Clark, 1992; Tieszen et al., 1983). The turnover rate has been shown to vary widely among different tissues: the liver provides a few days' worth of isotopic information, whole blood a few weeks and bone collagen several months (Hobson & Clark, 1992).

Being opportunistic feeders, herring gulls may incorporate food items of anthropogenic origin when in proximity to urban areas (Burger et al., 1979). Measurements of stable isotopes of carbon and nitrogen in the herring gulls can indicate the marine signatures of their diet and trophic niche, respectively. As exposure and biomagnification of many contaminants are linked to dietary intake, the feeding preferences of herring gulls are essential to understanding the contaminant levels and patterns (Hebert et al., 2009).

1.4 The Oslofjord ecosystem

The city of Oslo is highly inhabited and herring gulls are often observed to be feeding on garbage and junk food in this area (Denny & Gaines, 2007). The Oslo metropolitan area has a population of 1.5 million people and is the fastest-growing major city in all of Europe, population-wise (World Population Review, 2019). Close to the city centre is the Oslofjord. This fjord is approximately 107 km long and stretches from Langesund in the south to Oslo in the north. The fjord has been shown to contain a range of contaminants, both inorganic and organic (Ruus et al., 2016). Environmental Contaminants in an Urban Fjord ("Urbanfjord") is a monitoring programme conducted for several years by the Norwegian Environment Agency. Urbanfjord has documented the levels of various contaminants in a range of species representing the Oslofjord community, and modelled food web biomagnification (Ruus et al., 2015; Ruus et al., 2016; Ruus et al., 2017; Ruus et al., 2019).

Keilen (2017) compared contaminant levels and stable isotopes in herring gulls from the urban inner Oslofjord to a more rural colony in northern Norway. The Oslofjord gulls were grouped into marine and terrestrial gulls based on carbon isotope ratios. The levels of lipophilic contaminants in the marine gulls and the northern Norway gulls were more similar than those between the marine and terrestrial gulls, indicating that these contaminant levels are dependent on the dietary signal and not the habitat (Keilen, 2017). Ruus et al. (2017) found lower isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in herring gull compared to other marine species in the Oslofjord. These findings suggest that the herring gull searches for food outside of the fjord, which may include urban areas, and that the inner Oslofjord gulls are not representative of the Oslofjord ecosystem. By investigating the contaminant levels in the herring gulls at a smaller scale, comparing the inner Oslofjord colony to one located in the outer fjord, the contaminant status for the fjord system can be revealed.

Egg laying is a major route of contaminant transfer for female seabirds (Hitchcock et al., in press; Russell et al., 1999). Much of the energy investment used by the mother during egg production is in the form of lipids (Astheimer & Grau, 1990). Lipophilic pollutants with long half-lives, such as PCBs, PBDEs and hexachlorobenzene (HCBs), often get eliminated from the mother to the offspring by this elimination route (Bjerk & Holt, 1971; Borgå et al., 2004; Herzke et al., 2009; Verreault et al., 2006). The levels of several compounds in seagull eggs reflect their maternal contaminant patterns (Verreault et al., 2006). Thus, by investigating both the adult herring gulls from the Oslofjord and their eggs, the amount and efficiency of contaminant transfer to these eggs can be determined.

1.5 Aims and objectives

The main aim of this master thesis was to determine the contaminant levels in herring gull females and their maternal transfer to eggs in two colonies in the Oslofjord: Søndre Skjælholmen in the inner fjord and Store Revlingen in the outer fjord. By comparing herring gull populations between the inner and outer Oslofjord, differences in both diet and contaminant levels between the stations are expected due to dissimilar dietary preferences. Contaminants with various properties (e.g., lipophilic, protein-associated) are included in this study, so a secondary aim was to investigate the transfer efficiency of these contaminants from mother to egg.

The objectives addressed were:

Objective 1) Investigate diet in the two colonies based on stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to evaluate whether the inner fjord's herring gulls are representative for the entire Oslofjord system.

- H_1: Due to a more marine diet in the gulls from the outer fjord (Revlingen), they have a higher value of $\delta^{13}C$ compared to the inner fjord (Skjælholmen) gulls.
- H_2: The outer fjord gulls have higher $\delta^{15}N$ due to a more marine diet than the inner fjord gulls, based on previous findings of low $\delta^{15}N$ in the inner fjord gulls.

Objective 2) Compare contaminant levels in adult herring gulls between the inner and outer Oslofjord to address whether the inner fjord is more contaminated due to its proximity to populated areas, or if the outer fjord colony shows higher contaminant levels due to bioaccumulation in a marine food web.

- H_3: The lipophilic legacy POPs that have been banned for many years show higher levels in the outer fjord colony. These contaminants bioaccumulate and biomagnify up the food web, leading to higher levels of lipophilic POPs with increasing $\delta^{15}N$. Moreover, the inner fjord colony show higher levels of the more recently banned or restricted lipophilic contaminants due to proximity to urban areas more influenced by these contaminants from building materials and consumer products.
- H_4: The inner fjord colony have higher levels of PFASs in the inner fjord colony due to the use of PFASs such as in consumer products and textiles in urban areas.
- H_5: The metals show higher or lower levels in the gulls from the inner or outer fjord colony based on their use and properties. Cd accumulates in aquatic organisms, leading to higher levels in the outer fjord colony. Pb, which is often used in building materials, will show higher levels in gulls from the inner fjord, closer to industrial areas. Hg levels will be higher in the outer fjord colony due to trophic magnification in the marine food chain.

Objective 3) Compare the levels of contaminants transferred from mother to eggs, as well as investigate the transfer efficiency of these contaminants and the association with dietary preferences of the mothers.

- H_6: The lipophilic contaminants are more efficiently transferred from mother to egg than protein associated PFAS and metals, due to much of the energy investment used by the mother during reproduction being in the form of lipids.
- H_7: Higher contaminant load of lipophilic contaminants is associated with a more marine diet (higher $\delta^{13}C$), due to more effective lipid-accumulation with a marine diet.

2 Materials and methods

2.1 Study sites

In May 2017, whole-blood samples from adult female herring gulls, as well as one egg from their respective nest, were sampled in two separate island colonies in the Oslofjord; Søndre Skjælholmen in the inner Oslofjord and Store Revlingen in the outer Oslofjord (Figure 1). The distance in a straight line between the two locations is approximately 51 kilometres.

The Oslofjord has several sills dividing the deeper habitats into interconnected basins. The main sill, with a water depth of 19.5 m, is located in Drøbaksundet. This sill separates the inner Oslofjord from the outer Oslofjord, making the inner fjord a fairly enclosed body of water with consequent restrictions of the water circulations. The inner Oslofjord covers approximately 193 km^2 and is located within the most densely populated area of Norway (Abdullah et al., 1982; Breivik et al., 2004; Powell et al., 2018).

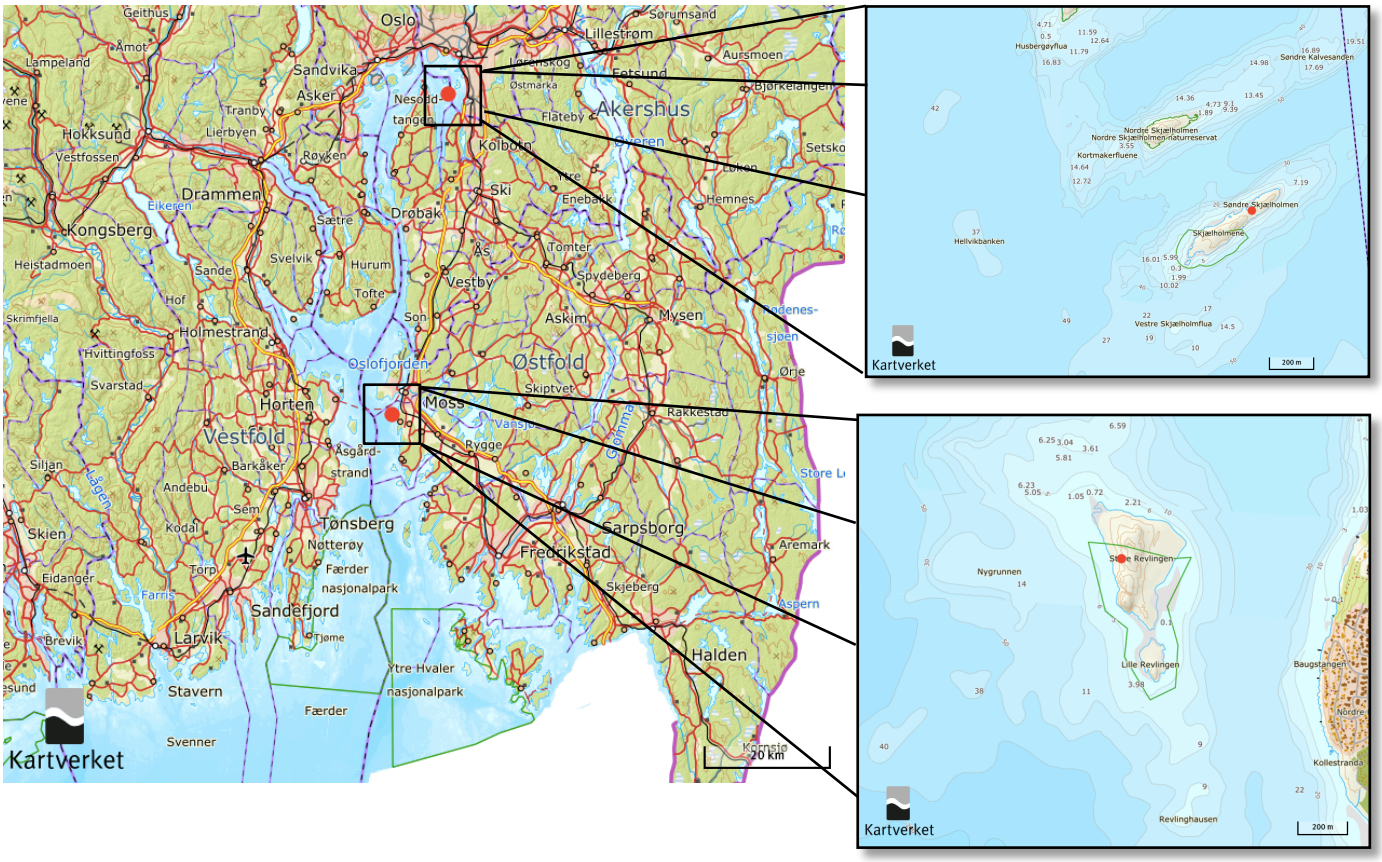

Figure 1. Map of the Oslofjord with sample areas highlighted: Søndre Skjælholmen (inner fjord) at the top and Store Revlingen (outer fjord) at the bottom. Map from Kartverket.no

2.1.1 Søndre Skjælholmen: the inner Oslofjord

Herring gull blood and eggs from Søndre Skjælholmen (Nesodden municipality, Akershus county; 59°51′14″N 010°43′48″E, hereinafter referred to as inner fjord) was sampled the 15th and 19th of May 2017. Parts of the island are a nature reserve and restricted to the public, while the rest of the island is open to the public during the entire year. Søndre Skjælholmen is located about 6 km from the Oslo city centre.

2.1.2 Store Revlingen: the outer Oslofjord

Sampling of herring gull blood and eggs from the outer Oslofjord was conducted the 21st of May 2017, on an island called Store Revlingen (hereinafter referred to as outer fjord), Rygge, Østfold, Norway (59°23′50″N 010°38′06″E). The southern part of this island is a nature reserve, and permission to take samples of the gulls was given by the County Governor of Østfold. Store Revlingen is located approximately 10 km from Rygge airport. The herring gull colony at Revlingen is in close proximity to numerous agricultural fields, and several of the birds nesting at Revlingen have been observed to be feeding on worms and other items on these fields (Ringmerking.no, 2019).

2.2 Field procedures

The fieldwork was coordinated as part of the Urbanfjord project and also included another MSc project (Thorstensen, 2019). Sampling of whole blood from the birds was done by Morten Helberg, University of Oslo (UiO), while the author assisted in the handling of the birds. Herring gulls incubating on the nest were caught using a walk-in trap, and then carried away from the nesting area to minimise disturbance to other breeding birds. One blood sample were taken from a vein under the wing, and the bird was then released as quickly as possible. One egg from each female gull was taken from each nest. Herring gull nest on the ground, making the eggs easy to collect. The total sample size of the present project included 30 blood and 30 egg samples from herring gulls, sampled from 15 different individuals from each colony. Approximately 5 mL blood were collected from the wing of each gull. For each adult female herring gull, body weight, head length, beak height and wing length were measured, and a metal ring were added on one tarsus, and an individual coded color-ring on the other. This last ring enables individual identification later in the project.

2.2.1 Sex determination

The sex of the herring gull was determined biometrically in the field. Based on previous sex determination studies in herring gulls using DNA, the gulls are treated as females if their head length is under 121 mm and as males if over 121 mm (Morten Helberg 2017, pers.com).

2.2.2 Stage determination of eggs

The eggs were divided into five different stages based on how developed they were, a system developed by Kine Bæk (NIVA) for previous Urbanfjord samples. The stages were; 1: without any indication of a foetus; 2: small signs of development, such as blood; 3: a clear indication of a foetus but no/small signs of feathers; 4: foetus with feathers but still a visible vitellus (egg yolk); 5: egg near hatching.

2.2.3 Ethical considerations

Permission to capture, handle, and take samples from the gulls followed the guidelines set out by the Norwegian Animal Welfare Act and were granted by the Norwegian Food Safety Authority (FOTS id 12394). In each location, eggs were only collected if the nest had two or more eggs. The whole-blood sample size of the females never exceeded 6 mL. Based on previous experience (Ruus et al., 2015; Ruus et al., 2016; Ruus et al., 2017), the amount of blood taken does not cause any harm to the investigated species.

Sampling was always performed in one area at the time to prevent stressing the birds longer than needed. The traps were never kept over the nests for more than 30 minutes without being checked to prevent the birds from being too hot or too stressed. The nests were monitored using binoculars. The camp where the sampling and measurements were performed was located in the shade to keep the birds away from the sun.

2.3 Homogenisation and sample splitting

Defrosting and homogenisation of the eggs and sample splitting were performed by the author and personnel at NIVA. The procedure was carried out in the open air on the roof of the building to prevent contamination of siloxanes, and staff involved made sure to wear siloxane-free care products at least 24 hours before handling the samples.

Each blood sample contained a volume of approximately 5 mL, while the eggs weighed approximately 50 g. The eggs were homogenised with a blender. After homogenising, the samples were divided into several subsamples for further analyses; 0.1 mL of blood and 0.1 – 0.2 g of egg to the Institute for Energy Technology (IFE) for stable isotope analysis; 1.0 mL of blood and 3.0 - 5.0 g of egg to NILU, Tromsø, for analysis of cyclic volatile methyl siloxanes (cVMS) and phosphorous flame retardants; 0.3 mL of blood and 1.0 – 2.0 g of egg to NIVA for analysis of PFASs, as well as 0.5 mL of blood and 3.0 – 5.0 g of egg for analysis of UV-chemicals. The rest, 1.5 – 3.0 mL of blood and 20.0-30.0 g of egg matrix was sent to NILU Forskningsparken, Kjeller, for analysis of lipid-soluble POPs and metals.

2.4 Analysis of stable isotope ratios of carbon and nitrogen

Stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were performed by staff at the Stabile Isotope Laboratory at the Institute for Energy Technology (IFE), Kjeller, Norway. The whole-blood and egg samples were dried overnight at 80°C and subsequently homogenized with an agate pestle and mortar. Approximately 1 mg of each sample was transferred into a tin capsule. The sample capsules were combusted at 1700°C in the presence of oxygen (O_2) and chromium (III) oxide (Cr_2O_3) in a Eurovector EA3028 elemental analyser. NO_x was reduced to nitrogen gas (N_2) at 650°C in the presence of copper (Cu). Residual H_2O was removed by a magnesium perchlorate $Mg(ClO_4)_2$ trap before N_2 and carbon dioxide (CO_2) were separated on a 2 m Poraplot Q gas chromatograph (GC) column. N_2 and CO_2 were transferred directly to a Horizon Isotope Ratio Mass Spectrometer (IRMS) from Nu-instruments to measure the ratios of heavy to light stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) values. Known standards of the chromatographic peak areas were used as a comparison to quantify the carbon-to-nitrogen elemental ratio (C:N ratio). Both carbon and nitrogen isotope ratios were expressed in δ notation, where the δ values are the isotope ratio of the sample relative to a standard and is expressed in parts per thousand (‰) (Equation 1 and 2).

$$\delta^{13}C = ([(^{13}C/^{12}C\text{-})_{sample}/(^{13}C/^{12}C\text{-})_{standard}] - 1) * 1000 \quad \text{(Equation 1)}$$

$$\delta^{15}N = ([(^{15}N/^{14}N\text{-})_{sample}/(^{15}N/^{14}N\text{-})_{standard}] - 1) * 1000 \quad \text{(Equation 2)}$$

The standards of $\delta^{13}C$ and $\delta^{15}N$ were the carbon- and nitrogen isotope ratios of PeeDee Belemnite (PDB) marine fossil limestone formation (Vienna) and atmospheric nitrogen (N_2), respectively. The bird tissues had negative values of $\delta^{13}C$ and positive values of $\delta^{15}N$, meaning that they had a lower $\delta^{13}C$ ratio than PDB and a higher $\delta^{15}N$ than N_2.

2.5 Homogenization and gravimetric lipid determination of eggs

The author homogenized the eggs, while the gravimetric lipid determination of the eggs was performed by Inger Christin Steen from NILU (Forskningsparken, Kjeller).

All egg samples were mixed with sodium sulphate (Na_2SO_4) in a blender to homogenize the sample. The samples were kept in a freezer at -20°C for an hour for the Na_2SO_4 to blend into the sample before they were run through the blender one more time. Gravimetric lipid determination was done by running two parallels of each sample, each containing 2 g of sample. A mixture of 3:1 cyclohexane/acetone solvent was added to each parallel. After a couple of days, everything but the lipids had evaporated from the sample, and lipid determination was done gravimetrically (Equation 3 and Equation 4). The mean of the two parallels was calculated to find the percentage of lipid in each egg sample.

$$Amount\ of\ lipid\ in\ sample\ (g) = Sample\ glass\ before\ evaporation\ (g) - Sample\ glass\ after\ evaporation\ (g) \quad \text{(Equation 3)}$$

$$Lipid\ \% = \frac{Amount\ of\ lipid\ in\ sample\ (g) \times 100}{Sample\ used\ for\ lipid\ determination\ (2\ g)} \quad \text{(Equation 4)}$$

2.6 Contaminant analyses

Analyses of contaminants were conducted at the Norwegian Institute of Air Research (NILU) at Kjeller, NILU at the Fram Centre, Tromsø, and the Norwegian Institute of Water Research (NIVA). The analyses were performed for both whole blood and eggs of the herring gulls. Samples were analysed in groups of 7 or 8 samples and a blank control. The author performed homogenisation, extraction and clean-up of the lipophilic contaminants in eggs. Lipid determination, sample preparations of PFASs and metals, instrumental analyses and quantification of the contaminants was conducted by the staff at NILU and NIVA.

2.6.1 Analysis of cyclic volatile methyl siloxanes

Octamethylcyclotetrasiloxane (D4), deecamethylcyclopentasiloxane (D5), dodecamethylcyclohexasiloxane (D6) and M3T (Ph) were analysed by staff at NILU as described by Ruus et al. (2019). Products containing cVMSs were avoided for at least 24 hours prior to collecting blood samples and eggs, to prevent contamination of the samples. State-of-the-art clean room and clean bench technologies were used to avoid background contaminants, as cVMS are applied in e.g. skin products.

Sample preparation and extraction

The herring gull samples were extracted using solid-liquid extraction with a biphasic (i.e. two-phase) solvent system of acetonitrile and hexane.

Analysis

The collected extracts were analysed using Concurrent solvent recondensation large volume injection gas chromatography mass spectrometry (CSR-LVI-GC-MS). CSR-LVI-GC-MS has shown to provide accurate and reproducible results when determining volatile compounds such as cVMS (Companioni-Damas et al., 2012).

Limits of detection

The limits of detection (LODs) and limits of quantification (LOQs) were calculated for each sample using the standard method, i.e. the average of the blanks plus 3 and 10 times the standard deviation (SD) of the blacks, respectively. Samples were extracted and analysed in batches with a minimum of 3 procedural blanks to account for background contamination and to calculate both the LOD and LOQ per batch. Procedural blanks were run before and after samples to make sure results were above the LOD and not due to background variation.

To investigate potential contamination coming from sample collection and preparation, field blanks were used. Each blank contained approximately 3 grams of XAD-2 sorbent in filter bags of polypropylene/cellulose. The XAD-2 sorbent was cleaned with a 1:1 mixture of hexane/dichloromethane and dried overnight in a clean cabinet. The clean cabinet was equipped with a High-Efficiency Particulate Absorber (HEPA) and charcoal filter to prevent contamination from indoor air. The filter bags surrounding the XAD-2 sorbent was cleaned by ultrasonic treatment (i.e. sonication). After 30 minutes, the hexane was removed and

substituted with clean dichloromethane and the field blanks sonicated once more for 30 minutes. The filter bags were then placed to dry in a clean cabinet overnight in the same way as with the XAD-2 sorbent. When the XAD-2 sorbent was dry, it was transferred to filter bags and sealed in polypropylene containers to be used for sampling purposes. These were used to determine the reference concentrations before sampling. The field blanks were exposed to field and sample preparation conditions.

2.6.2 Analysis of per- and polyfluoroalkyl substances

PFASs were analysed by NIVA (Forskningsparken, Oslo) during Autumn of 2017 (Ruus et al., 2019).

Extraction

The biota samples were homogenized, and 6 ng of each of the isotopic labelled internal standards was added before extraction. 7 mL of the extraction solvent acetonitrile was added to the sample and placed in an ultrasonic bath for 30 minutes, breaking down and extracting the sample. PFAS compounds are amphipathic, showing both lipophilic and hydrophilic properties (Jones et al., 2003), and acetonitrile, a polar solvent, has shown to provide good recoveries when used as an extraction solvent on these compounds (DeWitt, 2015). The sample was then centrifuged and the supernatant extract removed, before the extraction procedure was repeated using 5.0 mL of acetonitrile.

Clean-up

The PFAS extracts were cleaned up using graphitized carbon as an absorbent together with acetic acid. 0.4 mL of the extract and 0.2 mL of ammonium acetate were added together. The final extract was centrifuged, and a clear supernatant was transferred to an autoinjector vial.

Instrument analysis

To separate, identify and analyse the different PFAS compounds in the extract, an Acquity Ultra-Performance Liquid Chromatography (UPLC) system (Waters Corporation, Milford, MA, USA) was used. 7 mL of extract was injected onto a Waters Acquity BEH C18 reversed phase column (100 x 2.1 mm, 1.8 μm particles). This column consists of C18-bonded stationary phases which are used to separate the target compounds when the sample mixture and the mobile phase pass through the particle bed. The compounds in the mixture were

separated at a flow rate of 0.5 mL/min using acetonitrile and 5.2 mM ammonium acetate (NH_4OAc) in water. A binary gradient was applied as follows: 0-1.5 min, 12% of acetonitrile; 1.5-11 min, linear change to 99% of acetonitrile; 11-13 min, 99% of acetonitrile. The separated mixture was then transferred to a detector which sent an electrical signal to a computer data station where it was recorded as a peak.

The Xevo G2-S Q-ToF-HRMS instrument (Waters Corporation, Milford, MA, USA), which stands for Quadrupole Time-of-Flight High-Resolution Mass Spectrometry, is designed to identify, quantify and confirm a range of compounds. Mass spectrometers convert the target molecules to a charged (ionized) state, which can be analysed on the basis of their mass to charge ratio (m/z) (Pitt, 2009). The instrument uses a negative ion electrospray ionization mode (ESI negative mode) as an ion source. Mass spectra are registered in full scan mode (mass range *m*/*z* 150-1100). Optimized parameters used are described in Appendix A. Quantitative analysis was performed employing extracted mass chromatograms from full scan recording using the *m*/*z* (typical mass tolerance of 0.03 u) for the different analytes. LODs and LOQs were calculated for each sample using 3 times and 9 times the signal/noise ratio (z/n), respectively.

2.6.3 Analysis of lipid-soluble persistent organic pollutants

The laboratory analysis on the herring gull blood was done by personnel at NILU, while the analysis on the herring gull eggs was done by the author with the help of Inger Christin Steen and Silje Eltvik Thomassen from NILU (Forskningsparken, Kjeller). The target compounds were short-chained chlorinated paraffins (SCCP), medium-chained chlorinated paraffins (MCCP) and decabromodiphenyl ethane (DBDPE), as well as a range of PCBs and PBDEs (Ruus et al., 2019).

Extraction

A mixture of isotope labelled internal standards were added before extraction of the sample, for quantification purposes. The exact amount of each internal standard added can be found in Appendix B. Internal standards will show up as a chromatographic peak close to the target analytes, and by adding a known amount of the internal standard and comparing this to a recovery standard it is possible to report the amount of sample lost during the extraction. Based on the lipid determination, 4.0 g of each homogenized egg sample from the inner Oslofjord was used during the extraction. This method targets a range of persistent organic

pollutants (POPs) and uses a 3:1 cyclohexane/acetone mixture as a solvent to extract them. The 30 egg samples were divided into 4 groups, where each group consisted of 7 or 8 egg samples. One negative control (blank) was included for each group to monitor contamination during the analytical process. For each sample, a glass column was filled with a piece of clean cotton and cleaned with 6.0 – 8.0 mL of 3:1 cyclohexane/acetone mixture. Based on Equation 5, an exact amount of the homogenized sample was added to the glass column.

$$Homogenized\ mass\ for\ extraction\ = \frac{Total\ homogenized\ mass\ (g) \times 4g\ (analysis\ amount)}{12g\ (Raw\ egg\ sample\ \ amount)} \qquad \text{(Equation 5)}$$

Glass vials were placed under the glass columns to collect the extracted sample. The internal standards were introduced to the columns before the addition of 150.0 mL of solvent (3:1 mixture of cyclohexane/acetone), flushing the sample out of the bottom of the column. The glass columns with the samples were left to run overnight. The following day the sample extract, now in a glass vial, was concentrated to a volume of about 0.5 mL using the TurboVap 500 (Sotax AG (previously Zymark), Aesch, Switzerland) with an endpoint detection technology. The TurboVap consists of a heat bath with nitrogen-flow to concentrate the samples. The solvent was changed to n-hexane as a solvent for further elution using a silica-column.

Clean-up

The clean-up procedure was done to remove lipids and other unwanted compounds, which started with silica column chromatography. This method uses the polarity of the molecules to separate the different compounds. Glass columns were packed with one clean cotton frit in the tip. 4.0 g of activated silica (SiO_2) was added on top of the cotton frit, which adsorbed the unwanted chemicals, followed by about 1 cm Na_2SO_4. The column was then cleaned with 30.0 mL of diethyl ether hexane ($C_{10}H_{24}O$, 10% diethyl ether and 90% n-hexane) before adding the concentrated sample followed by 60.0 mL of diethyl ether hexane. After the solvent had run through the silica column, the sample was concentrated in the TurboVap, in a similar procedure as explained above.

To further clean up the samples, they were treated with sulphuric acid (H_2SO_4). This treatment contributes to the removal of lipids and unwanted compounds. The samples went through four rounds of sulphuric acid treatment. N-hexane was added to each of the 0.5 mL concentrated samples, to create a total volume of about 2.0 mL. 2.0 – 6.0 mL of sulphuric acid

was added, and the samples were left overnight for separation. The following day, the upper layer (the hexane fraction) were transferred to a new glass vial and another 2.0 – 6.0 mL of sulphuric acid was added and mixed on a Whirlmixer before it was left for one hour for separation. The samples were then cleaned a third time, repeating the previous step, and the samples were left for 30 minutes for separation. The last sulphuric acid treatment (round four) was done in the same way as the third step. After 30 minutes, the n-hexane fraction was transferred to new glass vials and concentrated to 0.5 mL on the TurboVap.

A new round of silica column chromatography was repeated in the same way as before, only with 6.0 g of activated silica instead of 4.0 g in the glass column and 30.0 mL diethylether hexane instead of 60.0 mL as a solvent. The samples were transferred to the TurboVap and concentrated to a volume of 0.5 mL. About 2.0 – 6.0 mL of isooctane was added to the concentrated sample to change the solvent, before the sample was concentrated to a volume of 0.5 mL. The samples were then concentrated to a volume of 100.0 µl using nitrogen gas, and 20.0 µl recovery standard was added to each sample. The compounds were quantified on GC-HRMS (Waters Autospec) by the staff at NILU.

Limit of detection:

The LOD and LOQ were calculated for each sample, using the accepted standard method, i.e. the average of blanks plus 3 and 10 times the SD for blanks, for LOD and LOQ, respectively.

2.6.4 Analysis of metals

Metal analysis was performed by NILU Kjeller as described in the Urbanfjord report for 2017 (Ruus et al., 2019).

Sample preparation

Samples of herring gull blood and eggs were added to supra pure nitric acid and digested at high pressure and temperature in a microwave-based digestion unit (UltraClave, Milestone, Italy). A minimum of two blanks were included with each digestion. Reference material (traceable to NIST) was digested with the samples.

Instrumental analysis

Concentrations of Ni, Cd, Hg, Pb, silver (Ag) and Cu were determined using an Agilent 7700x Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Internal standards were

added to all samples, standards and blanks prior to analysis. In addition, chromium (Cr), zinc (Zn), As and antimony (Sb) were determined.

Limits of Detection

The LOD and LOQ were calculated from 3 times and 10 times the SD of blanks, respectively.

2.7 Data treatment and statistical analysis

2.7.1 Data below limit of detection

Several of the values from contaminants in both eggs and blood of the herring gulls were quantified below the limit of detection (LOD), i.e., non-detects. Non-detects are low-level concentrations of pollutants, where the values are between 0 and the LOD. They are often reported to be under the analytical threshold (e.g. < 0.01), where the exact value is unknown (Helsel, 2005, 2006).

To determine which compounds to include for further statistical analysis, the non-detects were counted and summed up, and by investigating the pattern of non-detects (Appendix C), the contaminants quantified above LOD in more than 75% of the sample population was included. Based on this, 45 out of the 131 contaminants and metals were included in the final data set (Table 1). The same cut off value, where each compound had values with LOD > 75%, was chosen for both whole-blood and eggs to be able to compare these matrices to each other.

Table 1. Abbreviations and full names of the 45 compounds included in the data analysis. The number in brackets behind each PCB congener indicates the number of chlorine substitutions.

Group	**Abbreviation (group)**	**Analyte**
PCBs	PCB-47 (Tetra)	2,2′,4,4′-Tetrachlorobiphenyl
	PCB-66 (Tetra)	2,3′,4,4′-Tetrachlorobiphenyl
	PCB-74 (Tetra)	2,4,4′,5-Tetrachlorobiphenyl
	PCB-99 (Penta)	2,2′,4,4′,5-Pentachlorobiphenyl
	PCB-105 (Penta)	2,3,3′,4,4′-Pentachlorobiphenyl
	PCB-118 (Penta)	2,3′,4,4′,5-Pentachlorobiphenyl
	PCB-128 (Hexa)	2,2′,3,3′,4,4′-Hexachlorobiphenyl
	PCB-138 (Hexa)	2,2′3,4,4′,5′-Hexachlorobiphenyl
	PCB-153 (Hexa)	2,2′,4,4′,5,5′-Hexachlorobiphenyl
	PCB-156 Hexa)	2,3,3′,4,4′,5-Hexachlorobiphenyl
	PCB-157 Hexa)	2,3,3′,4,4′5′-Hexachlorobiphenyl
	PCB-167 (Hexa)	2,3′,4,4′5,5′-Hexachlorobiphenyl
	PCB-170 (Hepta)	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl
	PCB-180 (Hepta)	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl
	PCB-183 (Hepta)	2,2′,3,4,4′,5′,6-Heptachlorobiphenyl
	PCB-187 (Hepta)	2,2′,3,4′,5,5′,6′-Heptachlorobiphenyl
	PCB-189 (Hepta)	2,3,3′,4,4′,5,5′-Heptachlorobiphenyl
	PCB-194 (Octa)	2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl
	PCB-206 (Nona)	2,2′,3,3′,4,4′,5,5′,6-Nonachlorobiphenyl
	PCB-209 (Deca)	Decachlorobiphenyl
PBDEs	PBDE-47	2,2′,4,4′-Tetrabromodiphenyl ether
	PBDE-99	2,2′,4,4′,5′-Pentabromodiphenyl ether
	PBDE-100	2,2′,4,4′,6-Penta-bromodiphenyl ether
	PBDE-153	2,2′,4,4′,5,5′-Hexabromobiphenyl ether
	PBDE-154	2,2′,4,4′,5,6′-Hexabromodiphenyl ether
	PBDE-207	2,2′,3,3′,4,4′,5,6,6′-Nonabromodiphenyl

Table 1 continued. Abbreviations and full names of the 45 compounds included in the data analysis. The number in brackets behind each PCB congener indicates the number of chlorine substitutions.

Group	Abbreviation (group)	Analyte
PFAS	PFUdA (PFCA)	Perfluoroundecanoic acid
	PFDoA (PFCA)	Perfluorododecanoic acid
	PFTrDA (PFCA)	Perfluorotridecanoic acid
	PFTedA (PFCA)	Perfluorotetradecanoic acid
	PFOS (PFSA)	Perfluorooctane sulfonate
Metals	Fe	Iron
	Ni	Nickel
	Cu	Cobber
	Zn	Zink
	As	Arsenic
	Ag	Silver
	Cd	Cadmium
	Pb	Lead
	Hg	Mercury
CPs	SCCP	Short-chained chlorinated paraffin
	MCCP	Medium-chained chlorinated paraffin
cVMSs	D5	Decamethylcyclopentasiloxane
	D6	Dodecamethylcyclohexasiloxane
HCBs	HCB	Hexachlorobenzene

There are several methods of dealing with non-detects in the compounds included further in the statistical analyses. The simplest way is through deletion, where data below the LOD are replaced by NA. Although these entries do not provide an exact value, they still indicate that the value lies somewhere between 0 and the LOD, so deleting them would result in an aberrant pattern and also an overestimation of the remaining values (Baccarelli et al., 2005). The most common method used today is to substitute the values under the LOD with one-half or one over the square root of two times the detection limit (Helsel, 2010). However, this method does not consider the distribution of the data, which could result in a different pattern than the original data pattern (Helsel, 2010). Kaplan-Meyer analysis is another method to treat censored data, which is a non-parametric method and does not assume a specific distribution of the data (Helsel, 2010). Kaplan-Meyer has shown to be a useful method for substituting

non-detect values, but since it is a distribution-free procedure, it will not work well on data having the same LOD-value. For the PFAS compounds analysed, the values under the LOD for each compound were the same, excluding Kaplan Meyer as a procedure to deal with substituting the non-detects. Distributional methods, on the other hand, assume that the data arises from a specified parametric distribution (Baccarelli et al., 2005).

The method chosen to replace the values under the LOD in this thesis was distribution-based multiple imputation, which fits a parametric distribution to the data and uses this distribution to "draw" a value for each non-detect until a complete dataset is created. To account for the uncertainty of replacing the value, the method replaces each non-detect with several values, thereby creating multiple datasets containing parameter estimates and covariances. These are then combined, and a total variance is computed (Baccarelli et al., 2005). Imputation under the normality assumption has become a more frequently used approach during the last two decades. These model assumptions are quite restrictive, so by using distributions with other symmetry behaviours in the imputation process, one might be able to handle data that is not normally distributed, which is the case for the herring gull contaminant data presented here.

The beta distribution, a continuous probability distribution, can take on a variety of distributional shapes depending on the shape parameters chosen (Demirtas & Hedeker, 2008). The two positive shape parameters, α and β, control the shape of the distribution, which is bound on the interval [0,1] (Crawley, 2002). β was set to 1 in the imputation based on the shape of the pollutant data in the dataset. By creating an imputation formula in R using different shape parameters for α and running the imputation 1000 times, the strength of the different α values can be tested and the value causing the least variation can be chosen. Based on this, α was set to 5.

The level of significance (α) was set to $p = 0.05$ during all statistical analyses, which indicates that there is a 5% risk (or 1 out of 20) that the difference found is completely attributable to chance and not to actual differences in the data (Whitlock & Schluter, 2015).

2.7.3 Univariate analysis

The statistical programme, R studio (version 3.3.3, the R project for statistical computing) was used for statistical analysis. The Shapiro Wilk's test was used to check for normally distributed data, while the Fligner-Killeen test was used to test for homogeneity of variance as it works on both normally distributed and non-normally distributed data. When normality assumptions and assumptions of equal variance were met for a compound, Two-Sample t-tests were performed. When only normality assumptions and not assumptions of equal variance were met, Welch's t-test was used. When investigating paired samples with normal distribution, a paired t-test was performed. This test makes no assumption about the homogeneity of variance of the two measurements and can therefore be used on samples with both equal and unequal variance as long as the two measurements are normally distributed (Whitlock & Schluter, 2015). When the normality assumption was not met, the Wilcoxon ranked sum test was performed when comparing sample means between females or between eggs, and a paired Mann-Whitney U test when comparing blood and egg samples from the same colony.

Analysis of covariance (ANCOVA) was performed to determine a difference between the independent variables $\delta^{15}N$ in blood and Location (i.e. colony), on the dependent variable $\delta^{15}N$ – eggs. The same was performed for isotopic values of carbon, with the independent variables $\delta^{13}C$ – blood and Location, on the dependent variable $\delta^{13}C$ – eggs.

Log ratios between egg and blood were calculated to determine the transfer efficiency of contaminants from mother to egg transferred during reproduction. Log ratios of lipophilic contaminants were investigated in lipid weight (lw) due to lipid differences, while PFASs and metals were investigated in wet weight (ww). A log ratio of 0 indicates a 1:1 relationship between the contaminant levels in females and their eggs.

The 20 PCB congeners included in the statistical analysis were grouped by homologue group based on the number of chlorine substitutions per molecule. The three highest chlorinated PCBs (from eight to ten chlorine substitutions) were combined to one group (higherCB) in this thesis. Thus, the PCB congeners were grouped into 5 groups; tetraCB (4): PCB-47, -66, -74; pentaCB (5): PCB-99, -105, -118; hexaCB (6): PCB-128, -138, -153, -156-, 157, -167; heptaCB (7): PCB-170, -180, -183, -187, -189; higherCB: PCB-194, 206, -209. The PFASs

were grouped into perfluorocarboxylic acids (PFCAs): PFDoA, PFTrDA, PFTeDA and PFUdA; and perfluoroalkane sulfonates (PFSAs): PFOS. When investigating the levels of PFASs, the different compounds were grouped based on their carbon chain length (Table 2).

Table 2. Group names, names and acronyms, names, and carbon chain length of the different PFAS compounds analysed in herring gull females and their eggs. Based on values from ITRC (2018) and Wellington Laboratories (2012).

PFAS group	**PFAS contaminants**	**Chain length (No. of fluorinated carbons)**
PFCAs (acids)	Perfluoroundecanoic acid (PFUdA)	C_{11} (C_{10})
	Perfluorododecanoic acid (PFDoA)	C_{12} (C_{11})
	Perfluorotridecanoic acid (PFTrDA)	C_{13} (C_{12})
	Perfluorotetradecanoic acid (PFTeDA)	C_{14} (C_{13})
PFSAs (sulfonates)	Perfluorooctane sulfonate (PFOS)	C_8 (C_8)

Correlation tests

Correlations of the contaminant levels in the female and her egg were investigated so examine whether a higher contaminant load in the mother resulted in a higher contaminant load in the egg. Shapiro-Wilk normality test were performed to test for normally distributed groups. When the contaminants in each of the groups investigated where normally distributed, Pearson's correlation test were run. When data was not normally distributed, Spearman's rank correlation test, a non-parametric correlation test, was used to investigate the correlation between the mother and her egg. Spearman's test estimates a rank-based measure of both variables to calculate a measure of correlation. The Greek letter ρ (rho) is used to represent the correlation between the two variables. ρ lies between -1 and 1 and has no dimension. A negative correlation (towards -1) means that one variable decreases as the other increases, whereas a positive correlation (towards 1) indicates that both variables increase or decrease together. 0 indicates zero correlation (Whitlock & Schluter, 2015).

2.7.5 Body condition index of adult gulls

Body condition is often used to provide an estimate of an individual's physiological state (Jakob et al., 1996). A body condition index (BCI) was calculated for each of the adult herring gulls using a multiple linear regression model. The multiple linear regression model uses multivariate data to explain one or more continuous response variables and uses explanatory variables to predict the response (Greenacre & Primicerio, 2013). This index can be used to control for body mass and body size. Body mass was used as the dependent variable and wing length, bill height and head length (body size) as independent variables for each individual, based on Fox et al., 2007. The BCI was defined by the residuals from the regression line (Jakob et al., 1996). Pearson's correlation test or Kendall's correlation test were performed on the biometric data to find which parameters correlated the most with weight (wing, head or bill height). The correlated variables (wing, head) were used as independent variables against the dependent variable, weight, to run the regression. Both wing length and head length explained the herring gull weight ($R^2 = 0.32$, $p = 0.0007$ and $R^2 = 0.17$, $p = 0.01$, respectively). A negative BCI indicates a body condition below average, while a positive BCI indicates a body condition above average.

2.7.6 Multivariate statistics

Datasets used in ecotoxicological studies are often large and contain multiple contaminants with intercorrelated response variables for each individual. Multivariate analyses can be used to examine the intercorrelations among several variables at the same time, by condensing the variables to a new set of variables while still retaining the majority of the variance in the original dataset. Ordination works to recreate the complex pattern of all the variables in a few dimensions. Ordination allows us to visualise the relationship between our variables much more easily (Sparks et al., 1999).

Cook's distance test was performed to find influential outliers and based on the results, one egg from the inner Oslofjord (JJP18) and one blood sample from the outer Oslofjord (JJP39) were removed before performing both PCA and RDA. One gull from the outer Oslofjord (JJP23) did not have any recorded measurements for BCI and was omitted when investigating multivariate statistics. BCI was used as an explanatory variable in the associated eggs, so the egg collected from the same female (JPP23) was also omitted when performing multivariate statistics.

Principal component analysis

Principal component analysis (PCA) was performed on absolute contaminant concentrations to investigate correlations and interrelationship to dietary descriptors, location, egg stage, egg weight, and BCI. PCA is a multivariate technique that reduces the dimensions in the original data set to new uncorrelated (orthogonal) variables, while still retaining as much as possible the variation in the original data set. These variables are called principal components (PCs; Bro & Smilde, 2014). The first PC (PC1) explains most of the variance in the data set, and the following PCs will explain successively less of the total amount of variation (Sparks et al., 1999).

With PCA, indirect interpretations about the influence of the explanatory variables can be made, which provides an overview of the explained variation in the samples. However, PCA does not specify which explanatory variables are contributing to the variation observed in the samples (Van Wijngaarden et al., 1995). This can be performed with an RDA.

Redundancy analysis

Redundancy analysis (RDA) is a constrained ordination technique. RDA has the same assumptions as PCA, as it identifies orthogonal axes to explain as much of the variation as possible (Palmer et al., 2008), but is different in the way that the ordination scales are forced to be functions of the observed explanatory variables (Greenacre & Primicerio, 2013). The axes are constrained to be linear combinations of the explanatory variables and each axis' eigenvalue is a measure of this variation (Palmer et al., 2008). RDA, followed by post hoc tests (permutation tests), was performed to identify significant associations between the response variables (contaminants) as functions of the explanatory variables ($\delta^{13}C$, $\delta^{15}N$, location, egg stage, egg weight, BCI).

Due to different lipophilicity of the compounds, lipid % was used as a covariable in both the PCA and RDA when examining contaminant levels (ng/g ww). All contaminants were $\log_{10}$+1-transformed in both the multivariate analyses. The "vegan" package in R was used when conducting PCA and RDA (Oksanen et al., 2019).

Post hoc analysis

Significant relationships between the response variables and the explanatory variables were identified using a forward model selection with the *ordistep*-function in R (R package "stats"). In the *ordistep* function, one first provides a "null" model, i.e. a model without any explanatory variables, and global test containing all explanatory variables. A forward model selection was then performed, where explanatory variables were added one by one based on the results from a Monte Carlo permutation test. The Monte Carlo permutation test checks whether the explanatory variables significantly explain the variation in contaminant levels, and also if the explanatory variables explain more of the variation than the same number of randomly generated explanatory variables. Forward selection uses the AIC criteria (Akaike information criterion) and p-values from the Monte Carlo permutation test (set to 999) when comparing the variables. The model with the lowest AIC-value was chosen.

3 Results

3.1 Biometric data

Individual herring gull measurements of the biological variables are shown in Appendix D. The biometric parameters, BCI, and lipid (%) in females, as well as egg weight and lipid (%) in eggs, did not differ between the inner and outer fjord colonies (Table 3, Two Sample t-tests and Wilcoxon rank sum tests, $p > 0.05$). The outer fjord eggs were slightly more developed than in the inner fjord eggs, albeit insignificant (Welch's two-sample t-test, $p = 0.44$).

Table 3. Biological variables in adult herring gulls (*Larus argentatus*) and eggs from the inner and outer Oslofjord. Data is presented as mean ± standard deviation (SD) and range (min – max). Hyphen indicates values not recorded.

Mean ± SD **Min - Max**	**Inner Oslofjord**		**Outer Oslofjord**	
	Females (n = 15)	**Eggs (n = 15)**	**Females (n = 15)**	**Eggs (n = 15)**
Body mass (g)	896.7 ± 54.8 770 – 990	-	913.6 ± 68.2 820 – 1050	-
Bill height (mm)	17.4 ± 0.8 16.3 – 18.9	-	17.78 ± 0.8 16.1 – 19.8	-
Wing length (mm)	429.1 ± 7.0 415 – 438	-	432.5 ± 12.7 412 – 457	-
Head length (mm)	117.7 ± 1.8 113.7 – 120.7	-	117.5 ± 2.8 110.9 – 120.9	-
Body condition index (BCI)	-59.2 ± 42.3 -73.8 – 91.8	-	63.1 ± 41.6* -60.6 – 99.4	-
Lipid (%)	2.28 ± 1.52 0.60 – 5.30	8.13 ± 1.21 5.92 – 9.97	2.27 ± 1.06 1.10 – 4.30	7.09 ± 1.63 3.98 – 9.30
Egg stage	-	3.3 ± 1.1 1 – 5	-	3.6 ± 1.2 1 – 5
Egg weight (g)	-	78.54 ± 10.60 61.80 – 97.78	-	82.34 ± 7.37 67 – 90.78
$\delta^{13}C$	-24.45 ± 0.35 -25.18 – -24.02	-25.79 ± 0.75 -26.94 – -24.67	-23.79 ± 1.01 -24.96 – -21.85	-24.98 ± 1.15 -26.61 – -22.97
$\delta^{15}N$	8.43 ± 0.73 7.39 – 10.17	8.92 ± 1.08 6.92 – 10.82	10.01 ± 1.14 8.55 – 12.69	10.89 ± 1.37 8.83 – 14.37
C:N ratio	4.10 ± 0.60 3.41 – 5.30	7.43 ± 1.33 4.85 – 9.10	3.40 ± 0.08 3.25 – 3.52	6.861 ± 1.16 4.81 – 8.33

*One individual from Store Revlingen did not have measurements for body mass, wing length and head length and was excluded for these variables, resulting in n = 14 for these measurements. Three gulls from Revlingen did not have bill height measurements, resulting in n = 12 for these measurements.

3.2 Stable isotopes

Individual measurements of the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are shown in Appendix D. The outer fjord females had higher $\delta^{13}C$ values than the inner fjord females. The same trends were observed in eggs, with higher $\delta^{13}C$ values in eggs from the outer fjord compared to the inner fjord eggs (Figure 2, Table 3) (Female: Welch Two Sample t-test, $p = 0.03$ / Egg: Two Sample t-test, $p = 0.03$). Within each colony, the adult female gulls had higher $\delta^{13}C$ values than the eggs (Paired t-test, $p < 0.01$).

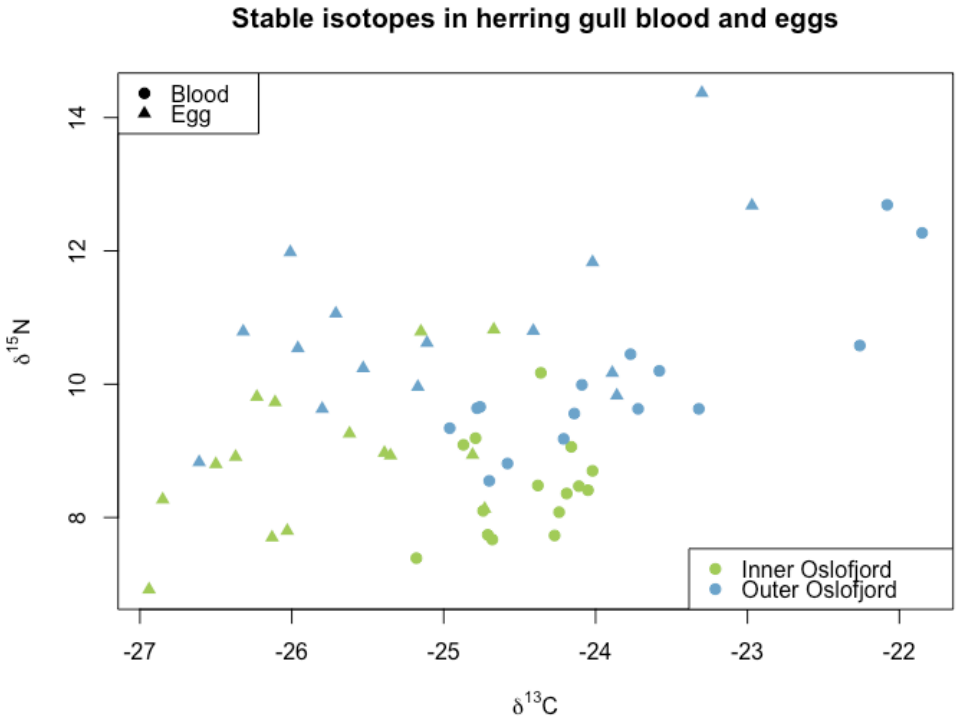

Figure 2. Relationship between stable isotope ratios of carbon ($\delta^{13}C$) on the x-axis and nitrogen ($\delta^{15}N$) on the y-axis. Herring gulls (*Larus argentatus*) from the inner Oslofjord (n = 30) are represented in green, and the outer Oslofjord (n = 30) gulls in blue. Whole-blood samples from adult herring gulls are represented as circles and eggs as triangles.

The $\delta^{15}N$ values were higher in the outer fjord colony for both females and eggs (Wilcoxon rank sum test, $p < 0.01$ and Two Sample t-test, $p < 0.01$, respectively). The $\delta^{15}N$ values between blood and eggs in the inner and outer fjord colony showed higher values in eggs compared to the females (Wilcoxon signed ranked test and paired t-test, $p < 0.01$, respectively).

The C:N ratio was higher in the inner fjord females compared to the outer fjord females, while no difference was observed in the eggs (Welch's two sample t-test, $p < 0.01$ and $p = 0.08$, respectively). Furthermore, the ratio was higher in the females compared to the eggs for both the inner and outer fjord colony (Paired t-tests, $p < 0.01$).

The eggs were sampled from the nest of each adult herring gull which is reflected by a positive correlation between the females and eggs in values of both $\delta^{15}N$ (Figure 3, $p < 0.01$, $R^2 = 0.66$) and $\delta^{13}C$ ($p < 0.01$, $R^2 = 0.49$). Analysis of covariance (ANCOVA) was conducted to determine whether the $\delta^{13}C$ and $\delta^{15}N$ in females and in eggs differed between the locations (colonies), where the results showed that location did not affect the correlation between the stable isotope ratios of blood and eggs ($p > 0.05$).

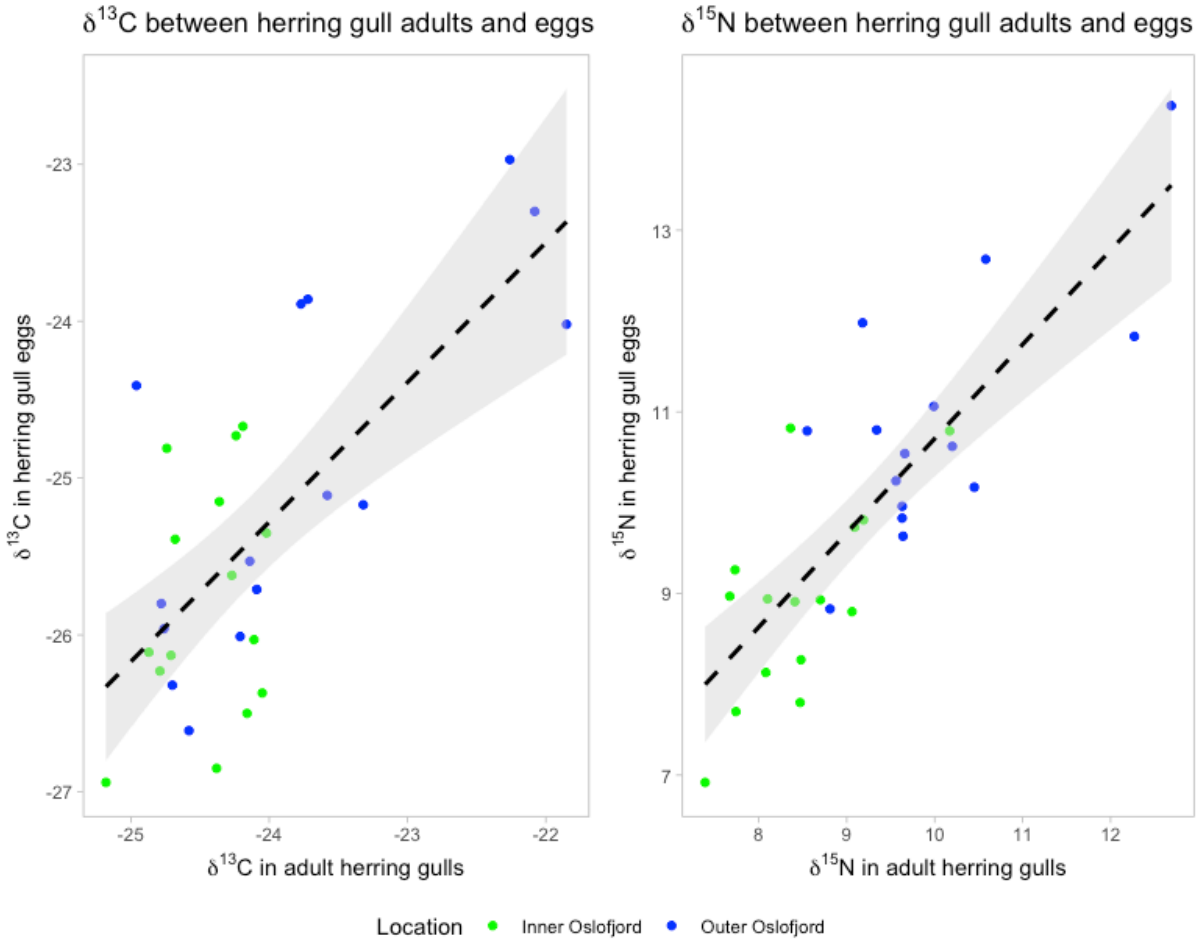

Figure 3. Stable isotope values of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) in herring gull (*Larus argentatus*) females (n = 30) and eggs (n = 30) from the inner and the outer Oslofjord. Regression lines are shown as dotted lines and confidence intervals as grey areas following the regression line.

3.3 Contaminant levels

Of the 131 individual compounds analysed, 45 were quantified above the LOD and 86 were found in less than 75% of the samples. 9 of the 45 compounds included in the statistical analysis were metals, while the resulting 36 were OHCs. The OHCs included 20 PCBs, HCB, 2 cVMSs, 2 CPs, 6 PBDEs, and 5 PFASs (Table 4). Individual contaminants (ng/g ww) of PCBs, PBDEs and PFASs detected above the LOD are presented in Appendix F. The metals included Fe, Ni, Cu, Zn, As, Ag, Cd, Pb, Hg (Table 5).

The highest OHC levels were observed in the outer fjord and inner fjord eggs, constituting 41% and 37% of the total OHC load. The mean ΣOHC was 3.85 and 3.32 times higher in eggs than in females from the inner and outer fjord (Wilcoxon signed rank tests, $p < 0.01$), respectively. The levels of OHCs were 1.3 times higher in the females from the outer fjord compared to the inner fjord females (Wilcoxon rank sum test, $p = 0.03$), while only slightly higher levels of OHCs were observed in the outer fjord eggs compared to the inner fjord eggs (Wilcoxon rank sum test, $p = 0.40$).

Table 4. Organic contaminant levels (ng/g wet weight), quantified in herring gull (*Larus argentatus*) whole blood from females and in their eggs from the inner and outer Oslofjord in 2017. Contaminant levels are reported as mean ± standard deviation (SD) and range (min – max).

Mean ± SD **Min – Max**	**Inner Oslofjord**		**Outer Oslofjord**	
	Females (n = 15)	**Egg (n = 15)**	**Females (n = 15)**	**Egg (n = 15)**
SCCP	50.3 ± 32.4 14 – 108	35.0 ± 24.9 13 - 91	30.3 ± 49.0 5 - 200	42.0 ± 46.3 18 - 178
MCCP	28.3 ± 23.8 8 – 76	29.1 ± 19.8 6 - 68	38.9 ± 64.6 6 – 200	69.6 ± 160 3 - 630
HCB	0.423 ± 0.313 < LOD – 1.27	3.66 ± 2.61 0.720 – 9.35	0.794 ± 2.13 < LOD – 8.49	3.03 ± 1.67 0.848 – 6.66
∑TetraCB	1.54 ± 1.64 0.220 – 6.18	15.8 ± 19.6 0.610 – 65.4	5.77 ± 17.9 0.206 – 70.4	10.0 ± 10.1 1.37 – 36.2
∑PentaCB	3.71 ± 2.88 0.658 – 10.2	53.0 ± 53.9 3.60 – 213	11.2 ± 34.5 0.579 – 136	37.8 ± 32.5 7.04 – 111
∑HexaCB	10.4 ± 9.49 2.69 – 40.8	132 ± 101 16.6 – 385	22.2 ± 57.4 1.74 – 229	115 ± 86.6 25.7 – 317
∑HeptaCB	2.93 ± 2.08 0.984 – 9.27	49.0 ± 30.5 8.94 – 120	9.83 ± 29.1 0.561 – 1145	37.0 ± 25.6 9.57 – 95.3
∑HigherCB	0.359 ± 0.326 0.104 – 1.30	6.07 ± 4.29 1.35 – 16.1	1.00 ± 3.03 0.0531 – 11.9	3.47 ± 1.98 0.950 – 8.04
∑PBDE	0.892 ± 1.01 0.287 – 4.07	19.0 ± 34.3 1.59 – 129	1.10 ± 2.27 0.146 – 9.24	6.77 ± 3.54 1.66 – 12.6
D5	1.09 ± 0.765 < LOD – 2.42	56.1 ± 69.2 < LOD – 206	0.493 ± 0.322 < LOD – 1.21	111 ± 167 13.0 – 695
D6	0.594 ± 0.558 < LOD – 2.01	11.9 ± 17.9 < LOD – 65.5	1.72 ± 0.713 0.706 – 3.56	8.96 ± 4.52 3.89 – 19.7
∑PFCA	2.64 ± 1.41 1.46 – 6.42	3.49 ± 2.78 1.26 – 12.3	5.23 ± 2.25 1.89 – 10.8	5.11 ± 4.67 1.15 – 20.7
PFSA (PFOS)	11.2 ± 7.14 2.72 – 6.43	25.6 ± 42.3 4.17 – 172	18.7 ± 11.5 6.45 – 51.4	38.5 ± 32.2 4.39 – 126

Table 5. Levels of Fe, Ni, Cu, Zn, As, Ag, Pb (μg/g wet weight) and Hg (ng/g wet weight), quantified in herring gull (*Larus argentatus*) whole blood from females and in their eggs from the inner and outer Oslofjord in 2017. Contaminant levels are reported as mean ± standard deviation (SD) and range (min – max).

Mean ± SD **Min – Max**	**Inner Oslofjord**		**Outer Oslofjord**	
	Females (n = 15)	**Egg (n = 15)**	**Females (n = 15)**	**Egg (n = 15)**
Fe	572 ± 76.8 458 – 717	35.1 ± 6.55 24.3 – 45.6	566 ± 132 403 – 884	33.3 ± 5.49 23.5 – 44.3
Ni	0.0584 ± 0.192 < LOD – 0.109	0.0309 ± 0.0338 0.00888 – 0.144	0.0492 ± 0.0162 0.0194 – 0.0734	0.0403 ± 0.0275 0.00132 – 0.127
Cu	0.502 ± 0.0826 0.387 – 0.676	0.814 ± 0.108 0.608 – 0.967	0.501 ± 0.0716 0.416 – 0.638	0.785 ± 0.112 0.642 – 1.07
Zn	6.27 ± 0.985 4.95 – 8.22	15.2 ± 2.55 11.9 – 20.1	5.67 ± 1.00 4.10 – 7.35	15.7 ± 3.93 10.0 – 25.7
As	0.0797 ± 0.097 0.0109 – 0.328	0.0541 ± 0.0467 0.00611 – 0.13	0.121 ± 0.176 0.0147 – 0.550	0.124 ± 0.0483 0.0336 – 0.251
Ag	0.000476 ± 0.000720 1.21×10^{-5} – 0.00300	0.000555 ± 0.0004 6.96×10^{-5} – 0.00128	0.000401 ± 0.000577 0.000148 – 0.00244	0.000845 ± 0.000474 0.000355 – 0.00191
Cd	0.000938 ± 0.000349 5.69×10^{-5} – 0.00170	0.000139 ± 0.0000565 6.98×10^{-5} – 0.000253	0.00149 ± 0.000657 0.000499 – 0.00230	0.000173 ± 0.0000746 6.50×10^{-5} – 0.000406
Pb	0.0912 ± 0.0487 0.0254 – 0.193	0.0117 ± 0.0959 0.00194 – 0.0337	0.0500 ± 0.0279 0.0218 – 0.120	0.00652 ± 0.00259 0.00306 – 0.0119
Hg	88.6 ± 67.1 17.1 – 289	62.7 ± 40.6 9.79 – 167	112 ± 67.2 25.7 – 288	84.5 ± 67.5 25.2 – 226

3.3.1 Organohalogen contaminants and biological variables in females and eggs

OHC levels and biological variables in females (blood) and eggs were investigated in a principal component analysis (PCA) (Figure 4). The two first principal components (PC1 and PC2) extracted from the PCA explained 60% of the total variation. Significant explanatory variables were Matrix ($p < 0.01$), $\delta^{15}N$ ($p < 0.01$) and Location ($p = 0.04$) (RDA, permutation tests, $p < 0.05$). These variables cumulatively explained 17.5% of the constrained variation in the dataset. In the RDA, Matrix, $\delta^{15}N$ and Location each explained 13.4%, 4.3% and 2.5% of the variation, respectively. Given that matrix was a significant explanatory variable, the levels in each matrix (whole-blood or eggs) were investigated separately below.

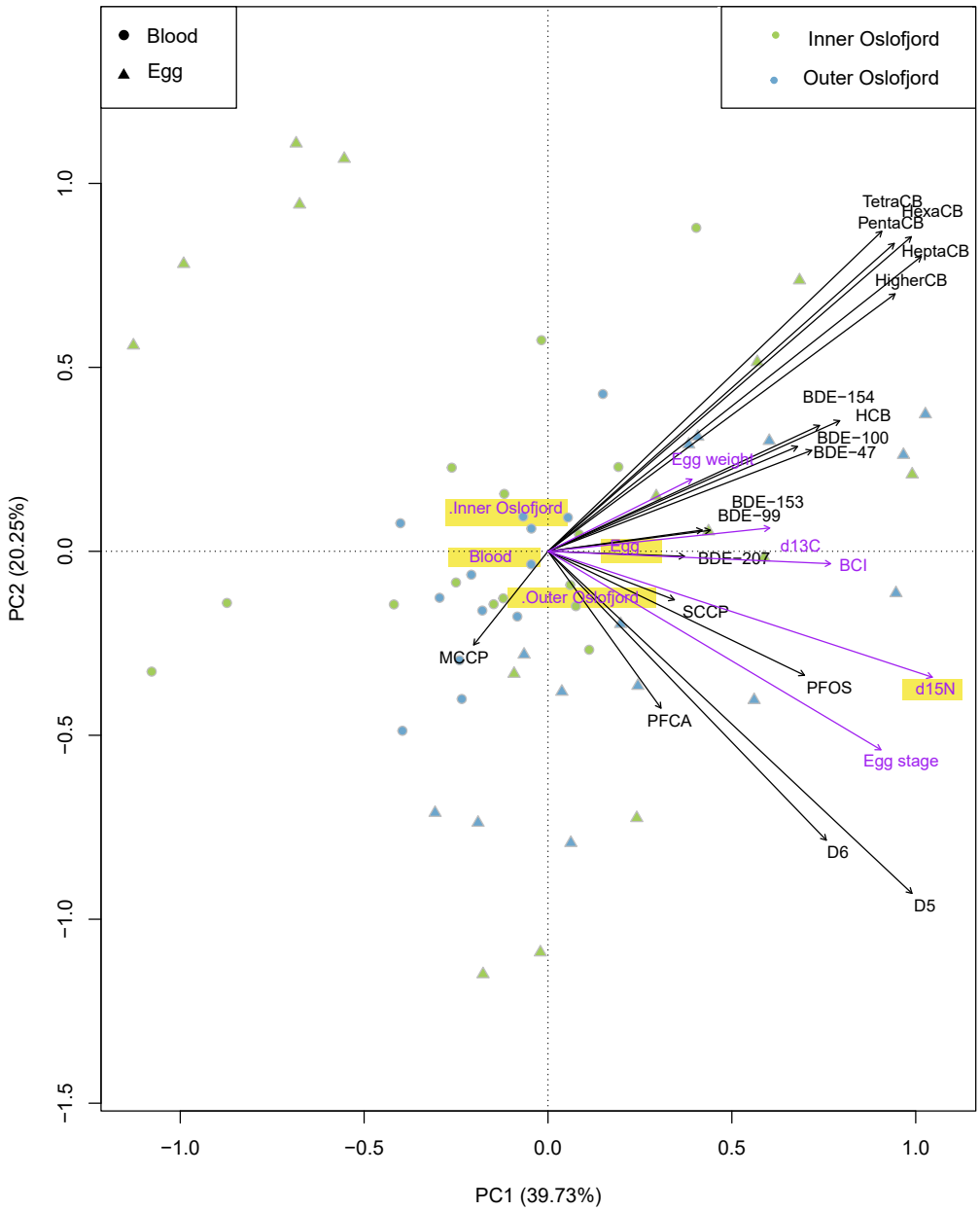

Figure 4. Principal component analysis biplot showing the organohalogen contaminant (OHC) concentrations (log-transformed contaminant concentrations ng/g ww) in herring gull (*Larus argentatus*) whole-blood from females (inner fjord n=15, outer fjord n=13) and eggs (inner fjord n=15, outer fjord n=14) positioned according to their contaminant load. Lipid is used as a covariable. The individual females and eggs from each location are represented as points in the plot. Response loadings (contaminants) are represented as black arrows, and explanatory passive vectors as purple arrows and boxes (centroids). Significant explanatory variables are marked with a yellow box. Being passive, the explanatory variables did not influence the sample scores or the response variables. The direction and the length of the arrows indicate increasing concentration and amount of variation, respectively. The degree of correlation is indicated by the cosine of the angle between the arrows, where the smaller the cosine, the higher correlation there is between the variables. Arrows pointing in the same direction are positively correlated, arrows pointing in the opposite direction are negatively correlated, and arrows orthogonal to each other are uncorrelated. The amount of variance explained by PC1 and PC2 is shown on the x- and y-axis, respectively.

3.3.2 Organohalogen contaminants and biological variables in females

Multivariate analysis was conducted to investigate the variation in the levels of OHCs and the association with the explanatory variables; $\delta^{13}C$, $\delta^{15}N$, BCI, Egg weight, Egg stage, and Location. For the female herring gulls, the two first principal components (PC1 and PC2) extracted from the PCA of the OHCs explained 57% of the total variation in contaminant levels (Figure 5).

All five groups of PCBs, HCB, and the PBDEs BDE-47, -100 and -154 were positively correlated, and were separated from the chlorinated paraffins (CPs) and D5 along PC2. The CPs and D5 were positively correlated with each other, and negatively correlated with the dietary markers $\delta^{13}C$ and $\delta^{15}N$. The PFASs, D6 and BDE-207 were positively correlated with both dietary markers and egg stage. The placement of the sample scores indicate higher contaminant load of the CPs and D5 in the females from the inner fjord, and of PFASs, BDE-207 and D6 in the outer fjord females. Levels of D5, and the CPs were higher in the inner fjord females, the PFASs, D6 and PBDE-209 were higher in the outer fjord females, while the other contaminants were uncorrelated to the explanatory variable Location.

Significant explanatory variables were Location ($p = 0.005$) and $\delta^{15}N$ ($p = 0.04$) (RDA, permutation tests, $p < 0.05$). These significant variables cumulatively explained 17.7% of the constrained variation in the dataset. In the RDA, Location and $\delta^{15}N$ each explained 15.8% and 8.5% of the variation, respectively.

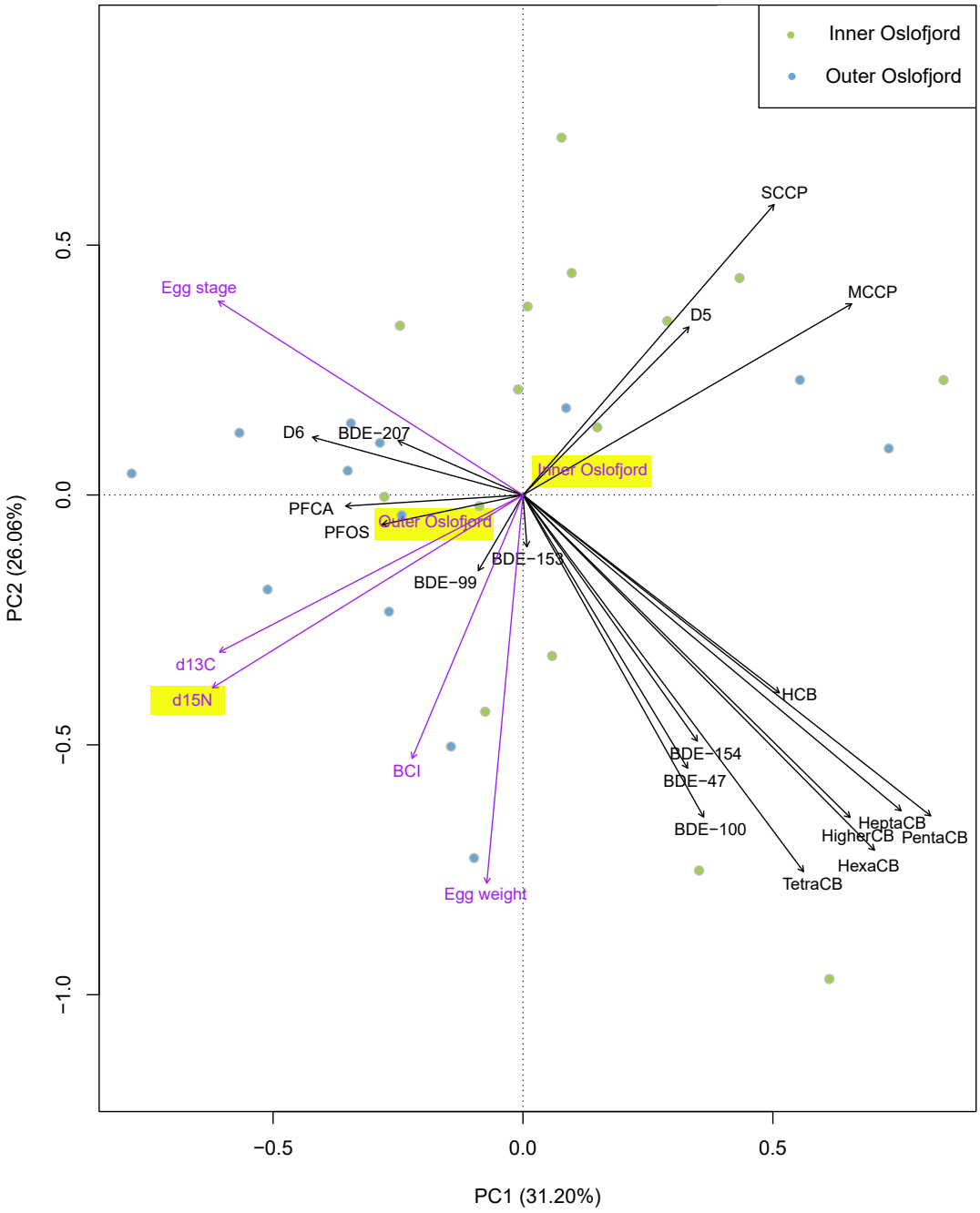

Figure 5. Principal component analysis biplot of the organohalogen contaminant (OHC) levels (log-transformed contaminant concentrations ng/g ww) in herring gull (*Larus argentatus*) whole-blood from females (inner fjord n=15, outer fjord n=13). Lipid is used as a covariable. The herring gull individuals are represented as points in the plot. Response loadings (contaminants) are represented as black arrows, and explanatory passive vectors as purple arrows. Significant explanatory variables are marked with a yellow box. Being passive, the explanatory variables did not influence the sample scores or the response variables. The direction and the length of the arrows indicate increasing concentration and amount of variation, respectively. The degree of correlation is indicated by the cosine of the angle between the arrows, where the smaller the cosine, the higher correlation there is between the variables. Arrows pointing in the same direction are positively correlated, arrows pointing in the opposite direction are negatively correlated, and arrows orthogonal to each other are uncorrelated. The amount of variance explained by PC1 and PC2 is shown on the x- and y-axis, respectively.

Based on the PCA biplot of OHC levels and the grouping of the different contaminants, linear regression analysis was performed to illustrate the interrelationship between the contaminants SCCP, PCB-153 and PFOS, and the explanatory variables ($\delta^{13}C$ and $\delta^{15}N$) within each colony. $\delta^{13}C$ was not a significant explanatory variable in herring gull females but was included in the interrelationship tests to get an indication of whether the contaminants differed based on dietary preferences. Analysis of covariance (ANCOVA) was performed to determine a difference between the independent variables $\delta^{13}C$ or $\delta^{15}N$ and Location, on the dependent variable SCCP, PCB-153 or PFOS. The test showed no difference between the contaminant levels of SCCP, PCB-153 and PFOS and the dietary markers in the females between the inner and outer Oslofjord ($p > 0.05$).

Correlation tests were performed to test whether contaminant levels showed similar increases or decreases in the two colonies (Figure 6). All significance values of the different correlations are depicted in Figure 6. The differences in female herring gulls between colonies for SCCP and PCB-153 were not of significance, while there was an increase in $\delta^{13}C$ with increasing PFOS in the female gulls from the inner fjord. ANCOVA was performed using location as a covariable to investigate differences between colonies for the increase or decrease of contaminant levels in female herring gulls. No differences in the increase or decrease contaminant levels of SCCP, PCB-153 and PFOS between the two colonies were found.

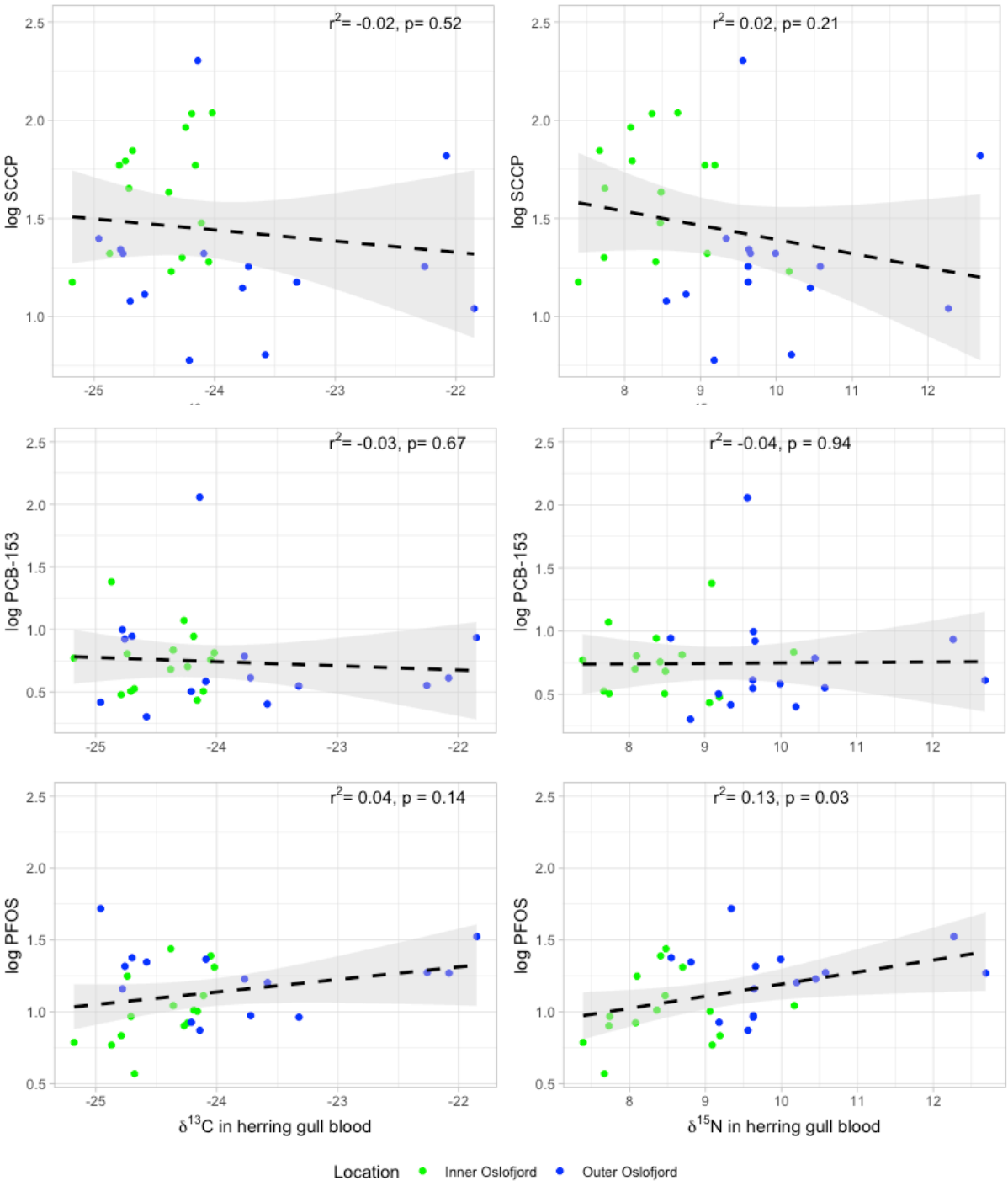

Figure 6. Linear regression scatter plots illustrating relationships between the contaminant levels of SCCP, PCB-153 and PFOS (log-transformed ng/g ww), and explanatory variables $\delta^{13}C$ and $\delta^{15}N$ in female herring gulls (*Larus argentatus*). Significance level and regression coefficient is shown for each plot. 95% confidence is indicated by the grey area surrounding the dotted black lines.

Organohalogen contaminants levels in females between the colonies

Differences in the levels of contaminants in females between the inner and outer fjord were investigated using t-tests. Differences in levels of the lipophilic contaminants were calculated in ng/g lw, while differences in PFAS levels were calculated in ng/g ww. Boxplots illustrating

levels of PentaCB, HeptaCB, and HigherCB, as well as the individual contaminants not shown in Figure 7, can be found in Appendix F.

No differences in levels of PCBs, both when grouped based on their homologue groups (TetraCB, PentaCB, HexaCB, HeptaCB or HigherCB) or when investigated as individual congeners (n = 20) were observed in females between the inner and outer fjord (Wilcoxon rank sum tests, $p > 0.05$). The same trends were observed for the levels of HCB and the individual PBDEs, with no differences between the colonies (Wilcoxon rank sum tests, $p > 0.05$).

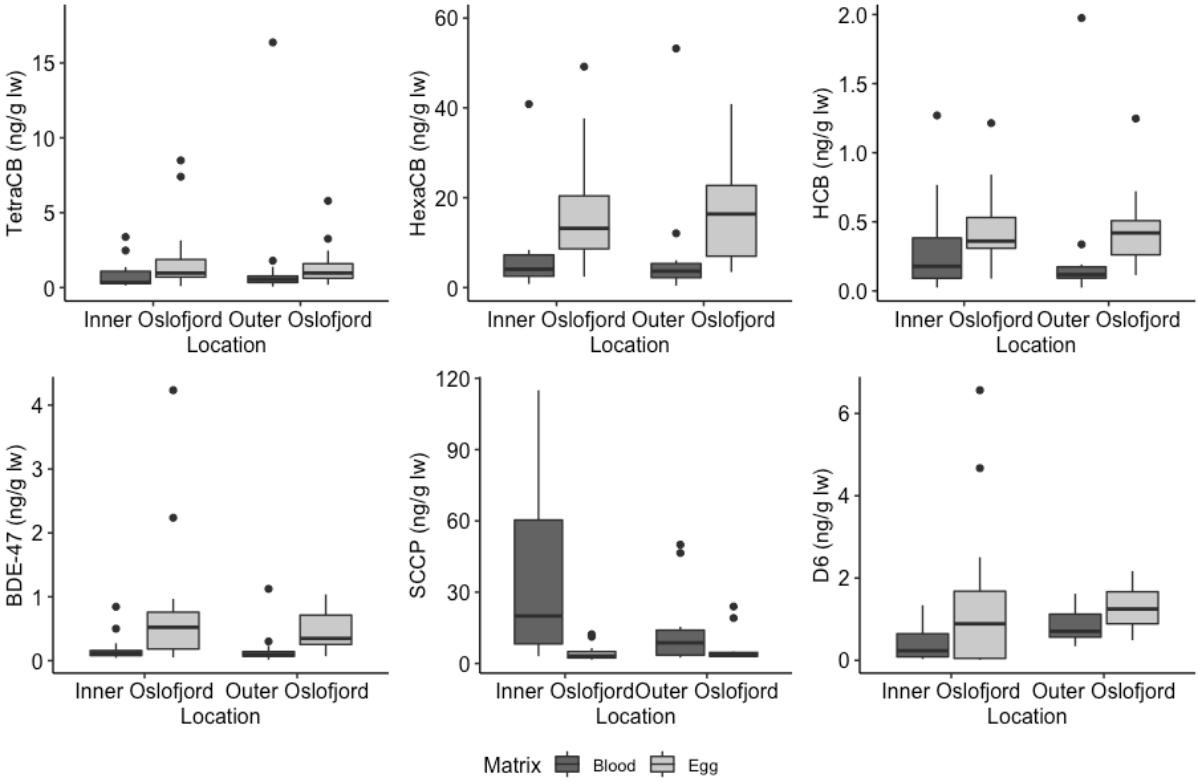

Figure 7. Boxplot showing the levels of TetraCB, HexaCB, HCB, BDE-47, SCCP and D6 (ng/g lw) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group.

Levels of D5 in females were higher in the inner fjord compared to the outer fjord (Wilcoxon rank sum tests, $p < 0.05$). The levels of D6 were slightly higher in the inner fjord females compared to the outer fjord females, albeit not significant (Wilcoxon rank sum tests, $p = 0.25$). SCCP had higher levels in females from the inner fjord compared to the outer fjord females (Wilcoxon rank sum tests, $p < 0.05$). No differences in MCCP levels were observed in the females between colonies (Wilcoxon rank sum tests, $p = 0.59$). All PFAS compounds

analysed showed higher levels in the outer fjord females compared to the inner fjord females (Wilcoxon signed rank test, $p < 0.05$).

3.3.3 Metal levels in females between colonies

Differences in the metal levels in females between colonies were investigated in ww. Iron (Fe), followed by zink (Zn) and copper (Cu), showed the highest levels in all four groups (Table 7). Boxplots showing the levels of Fe, Ni, Zn, Cu and Ag can be found in Appendix F.

No differences between the levels of Arsenic (As) was observed within or between eggs and females from the two colonies (Figure 8, Wilcoxon rank sum tests, $p > 0.05$). The cadmium (Cd) levels were higher in the outer fjord colony compared to the inner fjord colony (Wilcoxon rank sum test, $p = 0.02$). Lead (Pb) levels were higher in the inner fjord females compared to the outer fjord females (Welch's two-sample t-test, $p < 0.01$). No differences in mercury (Hg) levels were observed in females between the colonies (Wilcoxon rank sum test, $p > 0.05$). Of the essential metals, no differences in the levels of Fe, Ni, Cu, Zn or Ag in females was observed between colonies (Welch Two Sample t-tests, $p > 0.05$).

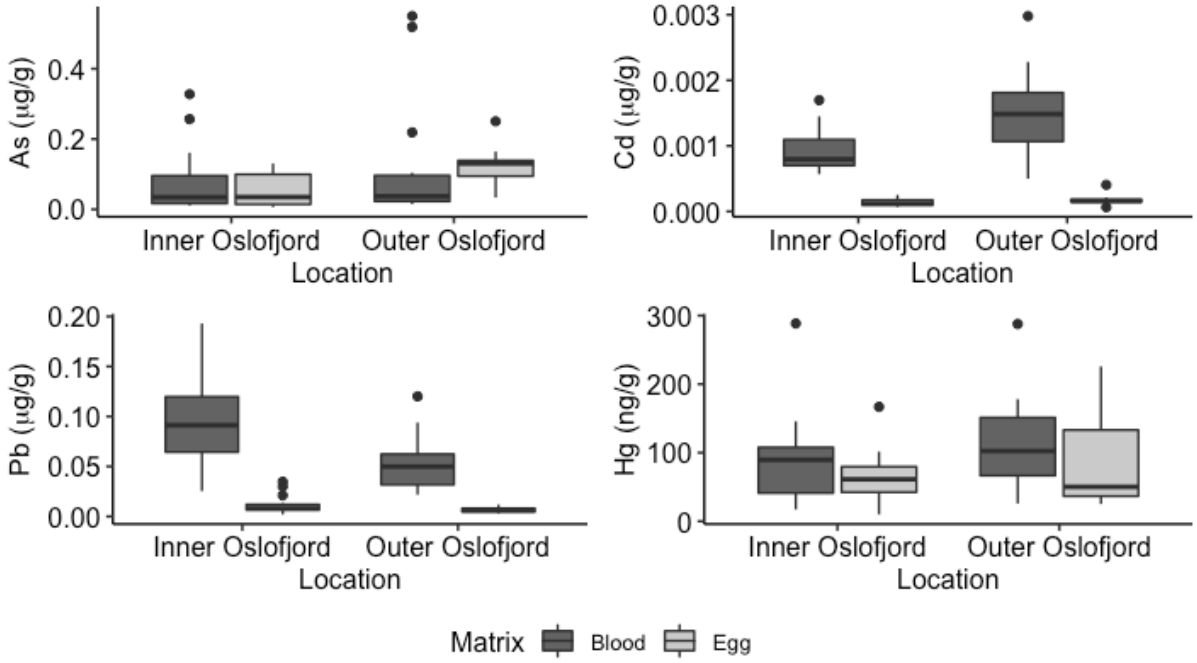

Figure 8. Boxplot showing different metal levels in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Levels of arsenic (As), cadmium (Cd), and lead (Pb) are presented in µg/g, while mercury (Hg) is reported in ng/g.

3.3.4 Organohalogen contaminants and biological variables in eggs

The two first principal components (PC1 and PC2) extracted from the PCA of the OHCs explained 58% of the total variation (Figure 9). Significant explanatory variables were $\delta^{15}N$ ($p = 0.03$) and $\delta^{13}C$ ($p = 0.04$) (RDA, permutation tests, $p < 0.05$). A biplot presenting the RDA ordination with the significant explanatory variables can be found in Appendix E. These explanatory variables cumulatively explained 13.7% of the constrained variation in the dataset. In the RDA, $\delta^{15}N$ and $\delta^{13}C$ each explained 8.9% and 8.3% of the variation, respectively. All OHCs showed increasing levels from left to right along PC1. By investigating the PCA visually, PFASs and chlorinated paraffins were positively correlated with each other and the explanatory variable $\delta^{15}N$ and egg stage. Furthermore, the PBDEs, PCBs, HCB and SCCP were positively correlated and were increasing with increasing $\delta^{13}C$.

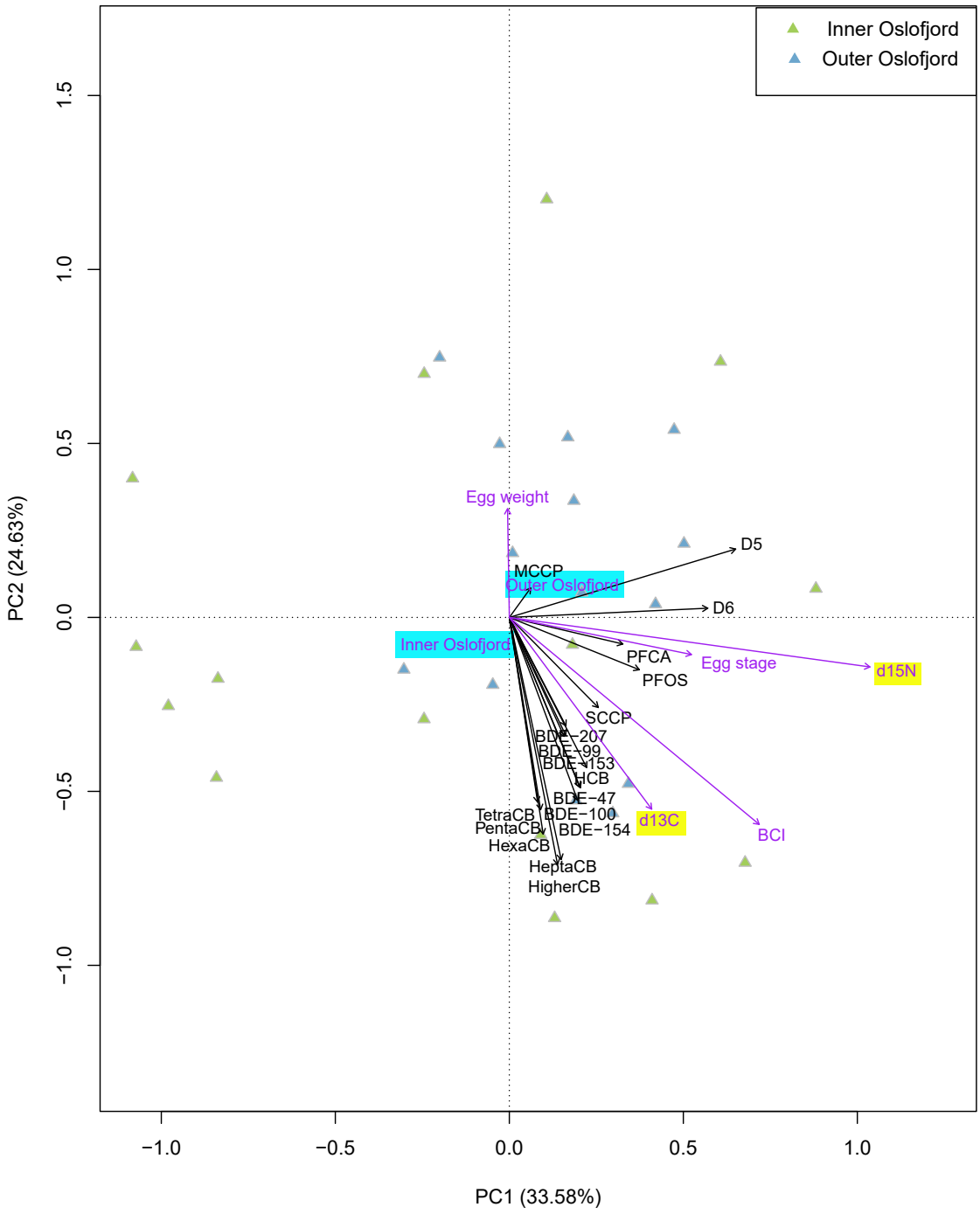

Figure 9. Principal component analysis biplot showing the organohalogen contaminant (OHC) levels (log-transformed contaminant concentrations ng/g ww) in herring gull (*Larus argentatus*) eggs (inner fjord n=15, outer fjord n=14) positioned according to their contaminant load. Lipid is used as a covariable. The herring gull individuals are represented as points in the plot, where the green points represent the gulls from the inner fjord and the blue points are the gulls from the outer fjord. Response loadings (contaminants) are represented as black arrows, and explanatory passive vectors as purple arrows and boxes (centroids). Significant explanatory variables are marked with a yellow box. Being passive, the explanatory variables did not influence the sample scores or the response variables. The direction and the length of the arrows indicate increasing concentration and amount of variation, respectively. The degree of correlation is indicated by the cosine of the angle between the arrows, where the smaller the cosine, the higher correlation there is between the variables. Arrows pointing in the same direction are positively correlated, arrows pointing in the opposite direction are negatively correlated, and arrows orthogonal to each other are uncorrelated. The amount of variance explained by PC1 and PC2 is shown on the x- and y-axis, respectively.

Based on the PCA biplot of OHC concentrations in herring gull eggs and how the different contaminants were grouped, linear regression analysis was performed to illustrate the interrelationship between the contaminants SCCP, PCB-153 and PFOS, to the explanatory variables ($\delta^{13}C$ and $\delta^{15}N$) within each colony (Figure 10). There was no correlation between the dietary markers and SCCP ($p > 0.05$). There was an intercorrelation between PCB-153 $\delta^{13}C$ ($p = 0.048$), showing an increase in PCB-153 with increasing $\delta^{13}C$. Similar trends were observed between PCB-153 and $\delta^{15}N$, but these were not significant ($p = 0.07$). PFOS and $\delta^{15}N$ showed an increase in contaminant levels with increasing $\delta^{15}N$ values ($p = 0.006$). No such trends were observed between PFOS levels and $\delta^{13}C$.

ANCOVA was performed using location as a covariable to investigate differences between the colonies in the increase or decrease of contaminant levels in the herring gull eggs. Location was not a significant explanatory variable but was included on the basis that it was an explanatory variable for the herring gull females. No differences in the increase or decrease in contaminant levels of SCCP, PCB-153 and PFOS in the eggs between the two colonies were observed.

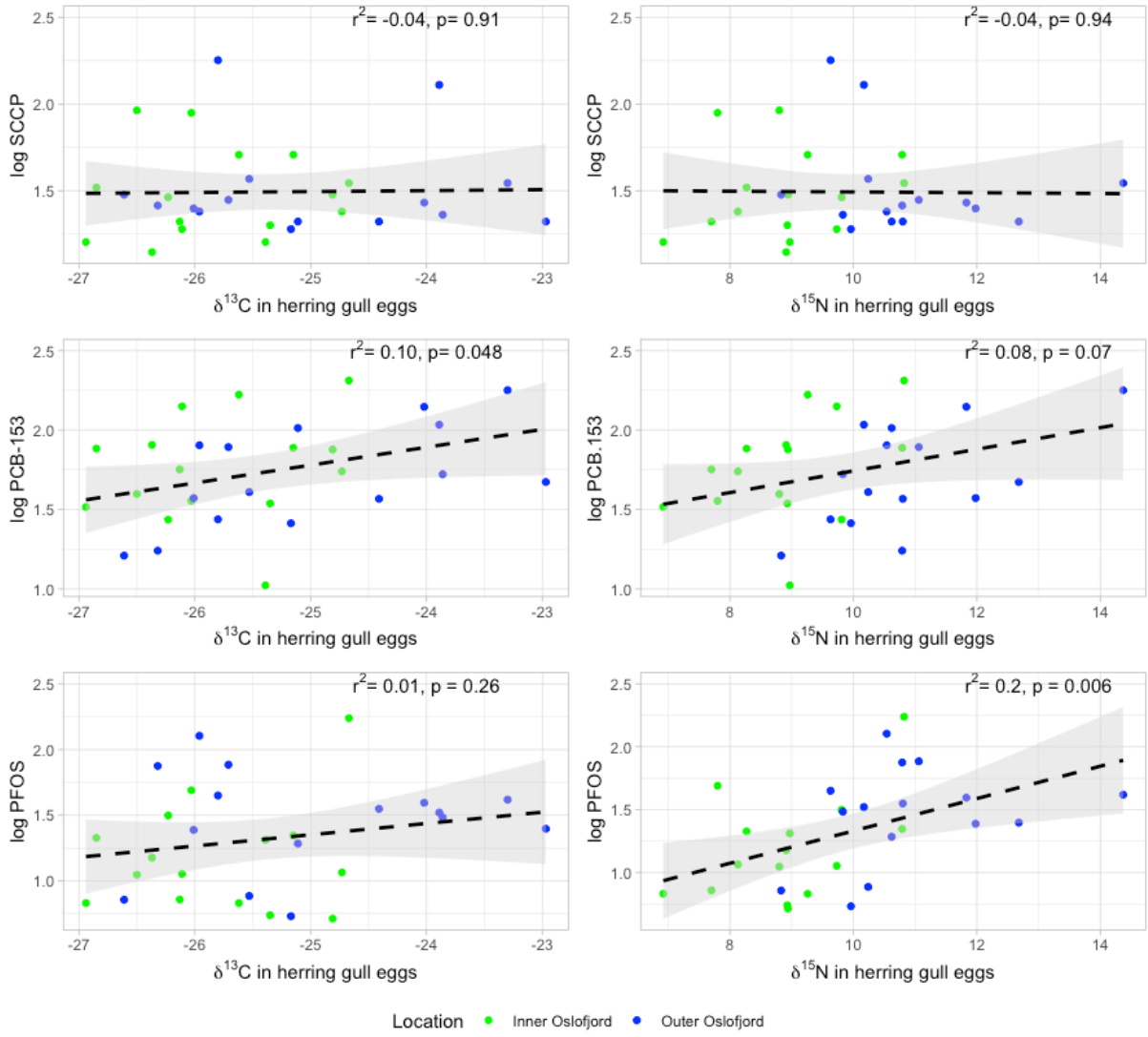

Figure 10. Linear regression scatter plots illustrating relationships between the individual contaminants SCCP, PCB-153 and PFOS (log transformed ng/g ww), and explanatory variables $\delta^{13}C$ and $\delta^{15}N$ in herring gull (*Larus argentatus*) eggs from the inner and outer Oslofjord. Significance level and regression coefficient is shown for each plot. 95% confidence is indicated by the grey area surrounding the dotted black lines.

Organohalogen contaminants levels in eggs between colonies

Differences in the levels of contaminants in eggs between the inner and outer fjord were investigated using t-tests. Differences in levels of the lipophilic contaminants were calculated in ng/g lw, while differences in PFAS levels were calculated in ng/g ww. Boxplots illustrating levels of PentaCB, HeptaCB, and HigherCB, as well as the individual contaminants not shown in Figure 7, can be found in Appendix F.

No differences in levels of PCBs, both when grouped based on their homologue groups (TetraCB, PentaCB, HexaCB, HeptaCB or HigherCB) or when investigated as individual congeners (n = 20) was observed in the eggs between the inner and outer fjord (Wilcoxon

rank sum tests, $p > 0.05$). The levels of HCB and the individual PBDEs were similar between the colonies (Wilcoxon rank sum tests, $p > 0.05$). The D5 levels were higher in eggs from the outer fjord compared to the inner fjord eggs (Wilcoxon rank sum tests, $p < 0.05$). An opposite trend was observed for the levels of D6, with higher levels in the inner compared to the outer fjord eggs (Figure 7, Wilcoxon rank sum tests, $p < 0.05$). No differences in SCCP and MCCP levels were observed in the eggs between the colonies (Wilcoxon rank sum tests, $p > 0.05$).

As observed in the female herring gulls, the PFAS levels were higher in the outer fjord eggs compared to the inner fjord eggs, with considerably higher levels of PFUdA. PFTrDA and PFOS were higher in the outer fjord eggs compared to the inner fjord eggs (Figure 11, Wilcoxon signed rank test, $p < 0.05$). The levels of PFDoA, and PFTeDA were also higher in the outer fjord gulls, while not to the same extent as the other three PFASs (Wilcoxon signed rank test, $p > 0.05$).

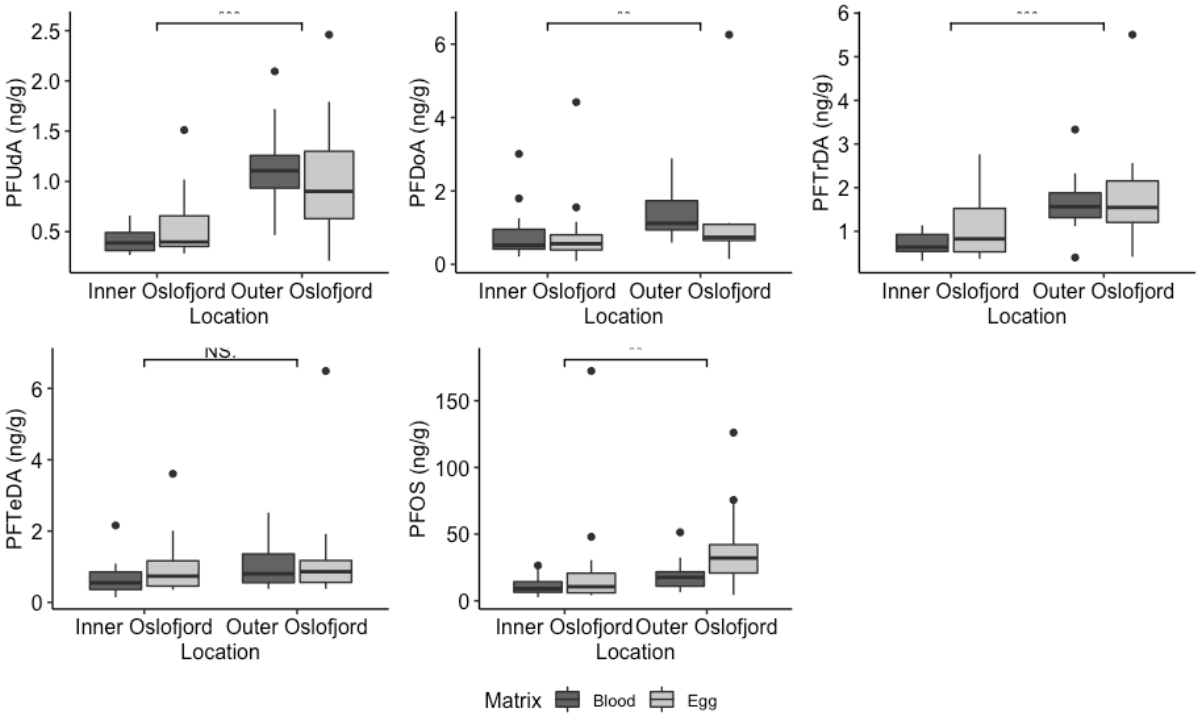

Figure 11. Boxplot showing different concentrations of PFAS compounds (ng/g ww) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. The lines at the top of the boxplot indicate if the differences between the two locations are significant or not, where significant results are shown as "*" signs. Note different scales.

3.4 Contaminant patterns

The contaminant pattern of the OHCs and the non-essential metals were investigated together (Figure 12). The contaminant levels in the inner fjord females were dominated by Pb (25.7%), followed by Hg (23.2%), As (20.9%) and Σ_2CP (20.6%). The most prevalent contaminants in the outer fjord females were As (27.7%), followed by Hg (25.7%), Σ_2CP (15.9%), and Pb (12.5%). The inner fjord eggs had a higher lipophilic contaminant load, with Σ_{20}PCB (45.3%) followed by Σ_2cVMS (12.0%) and Σ_2CP (11.4%) being the most prevalent. The outer fjord eggs showed similar patterns, where Σ_{20}PCB (29.0%) dominated, followed by Σ_2CP (15.9%), As (17.7%) and Σ_2cVMS (17.2%).

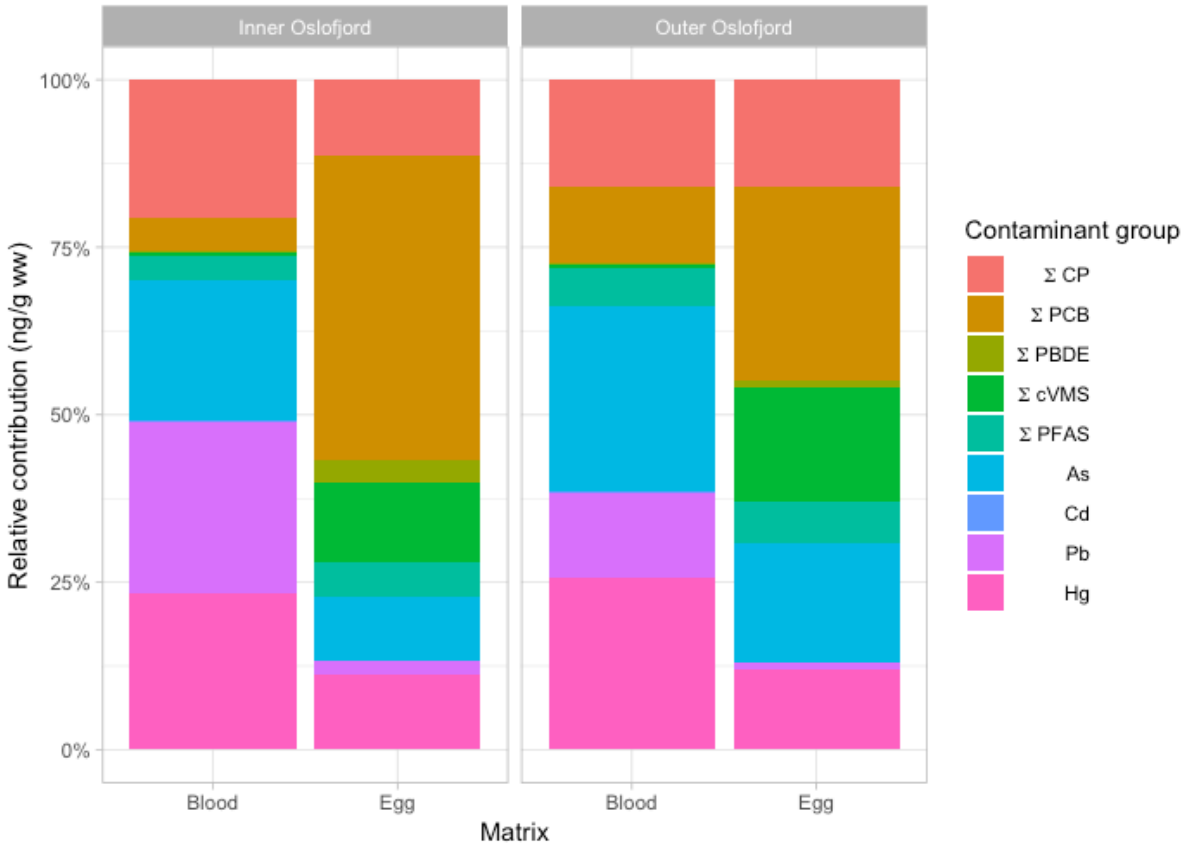

Figure 12. Relative contribution (%) of ∑CP, ∑PCB, ∑PBDE, ∑cVMS, ∑PFAS, As, Cd, Pb and Hg (ng/g ww) in herring gull (*Larus argentatus*) females (blood) and eggs from the inner and outer Oslofjord colonies (n = 15 in each of the four groups). ∑CP include the compounds SCCP and MCCP. ∑PCB include the PCB congeners -47, -66, -74, -99, -105, -118, -128, -138, -153, -156, -157, -167, -170, -180, -183, -187, -189, -194, -206, and -209. ∑PBDE include the BDE compounds -47, -99, -100, -153, -154, and -207. ∑cVMS include D5 and D6. ∑PFAS include the PFCA compounds PFDoA, PFTrDA, PFTeDA and PFUdA and the PFSA compound PFOS.

The pattern of PCB groups was similar in both blood and eggs in both colonies (Figure 13). HexaCB followed by PentaCB and HeptaCB dominated the contaminant pattern. The lowest levels of PCBs were the HigherCB. TetraCB were the second least prevalent group. Of $\sum_{20}$PCBs, PCB-153 was the dominating congener, followed by PCB-138 in both blood and eggs for both colonies.

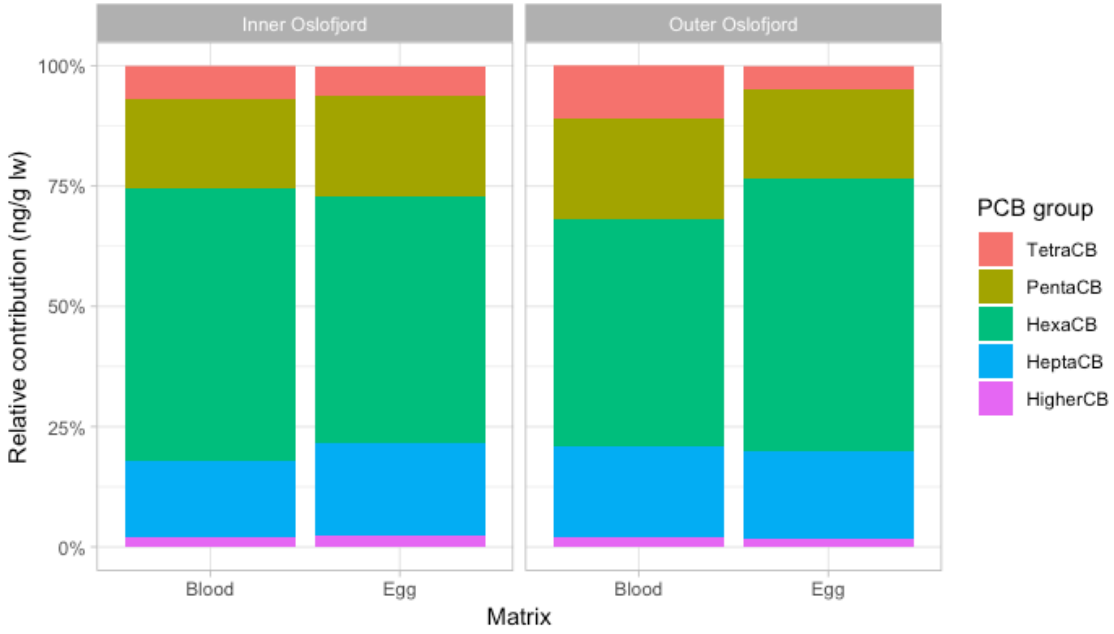

Figure 13. Relative contribution (%) of PCBs (based on ng/g lw) in herring gull (*Larus argentatus*) females (blood) and eggs from the inner and outer Oslofjord colonies (n = 15 in each of the four groups) from 2017. The PCBs are grouped based on number of chlorine substitutions on the PCB molecule. TetraCB include the PCB congeners -47, -99 and -74. PentaCB include the PCB congeners -99, -105, -118. HexaCB include the congeners -128, -138, -153, -156-, 157, -167. HeptaCB include PCB-170, -180, -183, -187, -189. HigherCB include the PCB congeners -194, 206, -209. Values are calculated and presented as the relative contribution of each contaminant group of the total contaminant load of the contaminant group (∑PCBs).

$\sum_{6}$PBDEs (Figure 14) was dominated by BDE-47 in all groups except in the eggs from the inner Oslofjord, where BDE-99 dominated. The second most abundant was BDE-99 from the outer fjord colony and in blood from the inner fjord. The second most abundant in the eggs was BDE-47. Overall, the PBDE pattern in the four groups was relatively similar. BDE-154 was the least prevalent PBDE compound in all four groups.

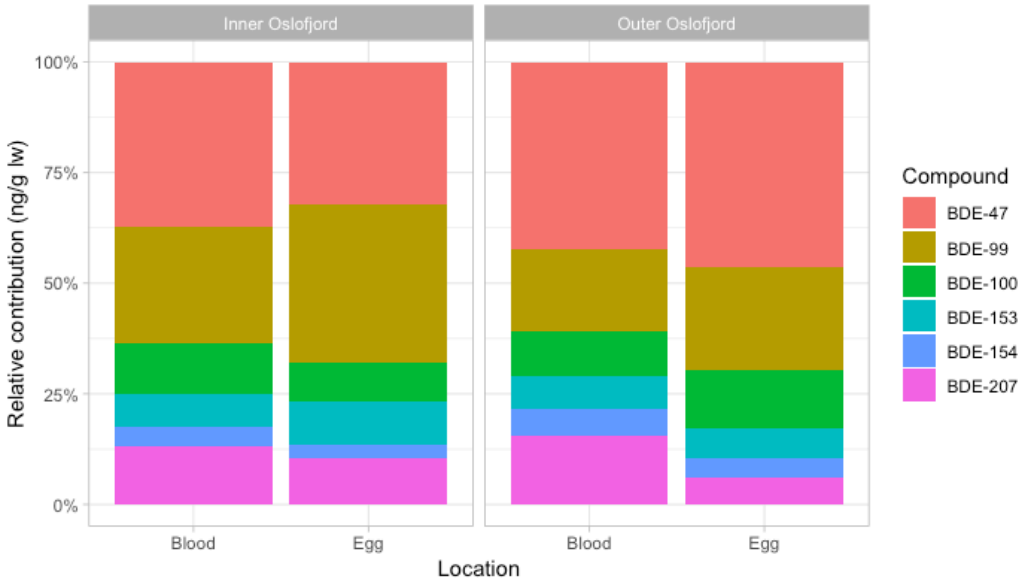

Figure 14. Relative contribution (%) of PBDEs (ng/g lw) in herring gull (*Larus argentatus*) females (blood) and eggs from the inner and outer Oslofjord (n = 15 in each of the four groups). Values are calculated and presented as the relative contribution of each contaminant of the total contaminant load of the contaminant group (∑PBDE).

PFOS was the most abundant $\sum_5$PFAS in all four groups (Figure 15). PFTrDA was the second most prevalent PFAS in the outer fjord colony and in eggs from the inner fjord. PFDoA was the second most prevalent PFAS compound in blood from the inner fjord. PFUdA was the least abundant PFAS in the inner fjord colony and in the outer fjord eggs, while PFTeDA was the least prevalent compound in blood from the outer fjord.

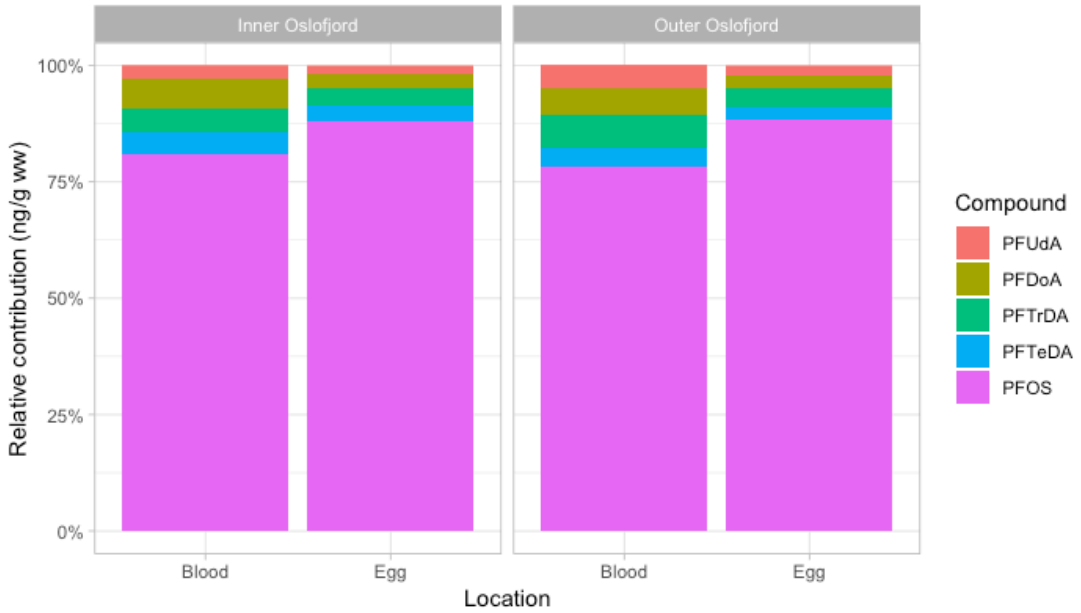

Figure 15. Relative contribution (%) of PFASs (ng/g ww) in herring gull (*Larus argentatus*) females (blood) and eggs from the inner and outer Oslofjord colonies (n = 15 in each of the four groups) from 2017. Values are calculated and presented as the relative contribution of each contaminant of the total contaminant load of the contaminant group (∑PFAS).

3.5 Maternal transfer of contaminants

3.5.1 Correlations between mother and egg

Correlation tests between mother and egg were conducted in lipid normalised levels for the lipophilic contaminants to account for the lipid differences between the matrices, while PFASs and metals were investigated in ww. Of the PCBs, only PCB-209 (ng/g lw) in blood and eggs from the inner Oslofjord correlated positively (Spearman's rank correlation test, $\rho = 0.61$, $p = 0.018$). No correlation was observed between mother and egg in the levels of CPs ($p > 0.05$). The levels of D5 were positively correlated between blood and eggs in the inner and outer fjord colonies (Spearman's rank correlation test, $\rho = 0.80$, $p < 0.01$, and $\rho = 0.78$, $p < 0.01$, respectively). Of the PBDEs, levels of BDE-154 and BDE-107 showed a positive correlation between blood and eggs in the inner fjord colony (Spearman's rank correlation test, $\rho = 0.61$, $p = 0.02$ and $\rho = 0.6$, $p = 0.01$, respectively). There was no correlation in the PFASs levels between blood and eggs (Spearman's rank correlation test, $p > 0.05$). Of the metals, Hg levels were positively correlated between blood and eggs in the inner and outer fjord (Spearman's rank correlation tests, $\rho = 0.87$, $p < 0.01$ and $\rho = 0.72$, $p < 0.01$, respectively).

3.5.2 Organohalogen contaminants between females and eggs

Differences in contaminant levels in lipophilic contaminants were investigated on a lw basis, while PFASs and metals were investigated on a ww basis. Log ratios of contaminant levels between eggs and whole-blood were used as a measure of transfer efficiency of the individual contaminants and metals, where 0 indicates a 1:1 relationship in the contaminant levels between mother and egg.

Polychlorinated biphenyls

The levels of TetraCB were higher in eggs compared to blood in the inner fjord colony (Pairwise Mann-Whitney U test, $p = 0.03$, Figure 7). No differences were observed between eggs and blood in the outer fjord colony (Pairwise Mann-Whitney U tests, $p = 0.73$). Within the individual congeners of TetraCB, PCB-47 had higher levels in eggs compared to blood (Pairwise Mann-Whitney U tests, $p < 0.05$) in the inner and the outer fjord colonies. PCB-74 did not show any difference between female and eggs within the colonies (Pairwise Mann-Whitney U tests, $p = 0.93$). The levels of PCB-66 were higher in eggs than in blood from the

inner fjord colony (Pairwise Mann-Whitney U tests, $p < 0.05$), but not in the Revlingen colony (Pairwise Mann-Whitney U test, $p = 0.12$).

Both the inner and outer fjord colonies had higher levels of PentaCB in eggs than in blood (Pairwise Mann-Whitney U tests, $p = 0.01$ and 0.02, respectively). Within the individual congeners of PentaCB, levels of PCB-99 and -118 were higher in eggs compared to blood (Pairwise Mann-Whitney U tests, $p < 0.05$) in both the inner and the outer fjord colony. Levels of PCB-105 were higher in eggs than in females from the inner fjord (Pairwise Mann-Whitney U test, $p < 0.05$), but not between blood and eggs in the outer fjord colony (Pairwise Mann-Whitney U test, $p > 0.05$).

Levels of HexaCB (Figure 7) were higher in eggs compared to blood in the inner and outer fjord colony (Pairwise Mann-Whitney U tests, $p = 0.02$ and 0.03, respectively). Levels of the individual congeners of HexaCB (PCB-128, -138, -153, -156, -157 and -167) were higher in eggs compared to blood (Pairwise Mann-Whitney U tests, $p < 0.05$) in both colonies.

Levels of the HeptaCBs were higher in the inner fjord and outer fjord eggs compared to blood samples from the same colonies (Pairwise Mann-Whitney U tests, $p < 0.01$ and 0.02, respectively). Similar to the HexaCB group, each individual congener of HeptaCB had higher levels in eggs compared to blood (Pairwise Mann-Whitney U tests, $p < 0.05$) in both colonies. The levels of HigherCB were higher in eggs than in blood, both in the inner fjord and in the outer fjord colony (Mann-Whitney U tests, $p < 0.01$ and 0.01, respectively). All three compounds within the group HigherCB (PCB-194, -206 and -209) were higher in eggs than in blood in each colony (Pairwise Mann-Whitney U tests, $p < 0.05$).

The egg:wholeblood ratio was similar between the different PCB congeners in each colony and was that of the most efficiently transferred lipophilic contaminants (Figure 16). Egg:wholeblood ratios of contaminants not displayed here can be found in Appendix G.

Polybrominated diphenyl ethers

The levels of BDE-47, -99, -100, -153, and -154 (ng/g lw) were higher in eggs compared to whole-blood in each of the two colonies (Pairwise Mann-Whitney U tests, $p < 0.05$). The inner fjord females showed higher levels of BDE-207 compared to their eggs (Pairwise Mann-Whitney U tests, $p < 0.05$), while no such differences were observed for the outer fjord

mothers compared to their eggs (Pairwise Mann-Whitney U tests, $p = 0.25$). As with the PCBs, the egg:wholeblood ratio was similar between the different PBDEs.

HCB, cyclic volatile methyl siloxanes, and chlorinated paraffins

HCB levels was higher in eggs compared to blood in herring gulls from the outer fjord (Pairwise Mann-Whitney U tests, $p < 0.05$). No difference was observed between blood and eggs in the inner fjord colony (Pairwise Mann-Whitney U tests, $p = 0.15$). D5 and D6 were detected in both blood and eggs from the two colonies, with significantly higher levels (Pairwise Mann-Whitney U tests, $p < 0.05$) in the eggs compared to the females in both colonies.

The transfer efficiency of short- and medium-chained chlorinated paraffins (SCCP and MCCP) was lower than the other lipophilic contaminants (Figure 16). Both CPs showed higher levels in blood compared to eggs in the inner fjord colony (Pairwise Mann-Whitney U tests, $p < 0.05$), but not for the outer fjord colony (Pairwise Mann-Whitney U tests, $p = 0.14$ and $p = 0.06$, respectively).

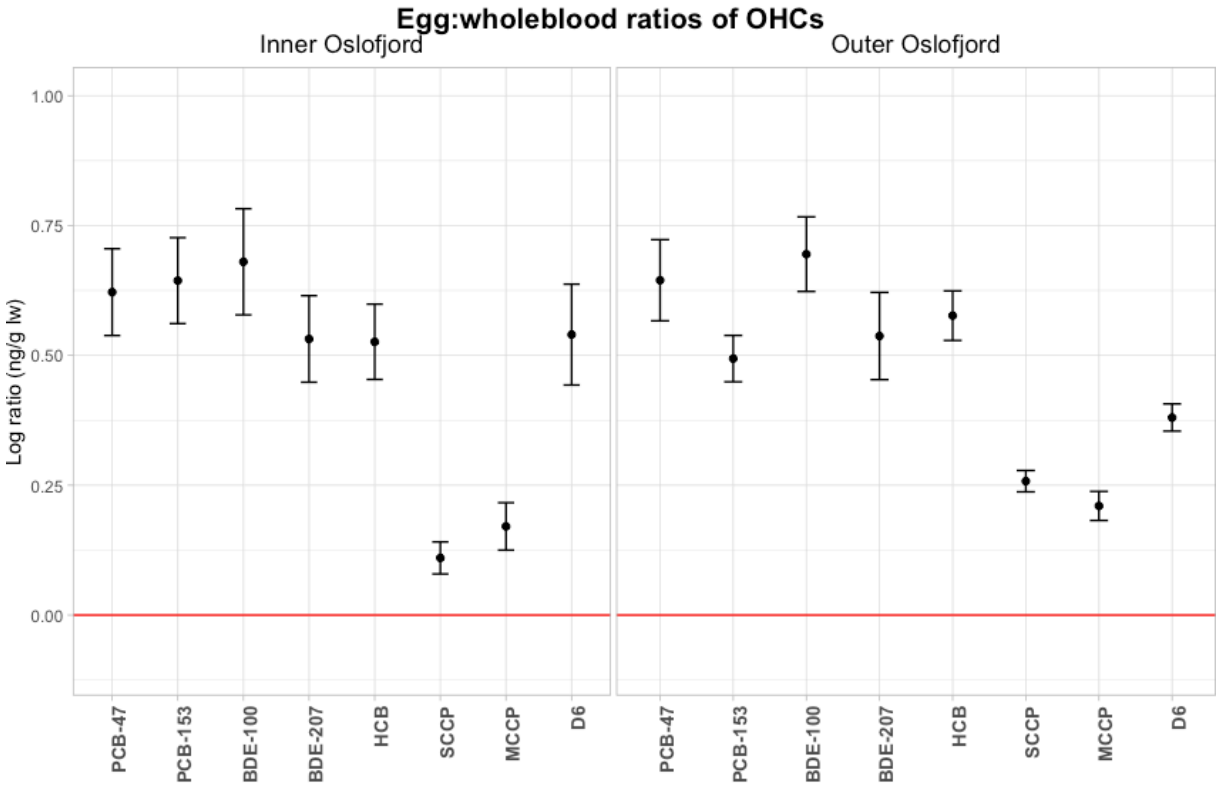

Figure 16. Log ratios of herring gull (*Larus argentatus*) females and eggs from the inner (n = 15) and outer (n = 15) Oslofjord colony for selected OHCs, presented as mean ratios (± SE). Ratios were based on mean concentrations (ng/g lw). The horizontal line (log ratio = 0) indicates a 1:1 relationship.

Per- and polyfluoroalkyl substances

Higher levels of PFDoA were observed in the outer fjord females compared to their eggs (Figure 10, Pairwise Mann-Whitney U test, $p < 0.05$). No difference was observed between the inner fjord females compared to their eggs for any of the PFAS compounds (Pairwise Mann-Whitney U test, $p > 0.05$). The egg:wholeblood ratios of the PFAS compounds differed between the two locations with overall higher ratios in the inner fjord colony compared to that of the outer fjord (Figure 17).

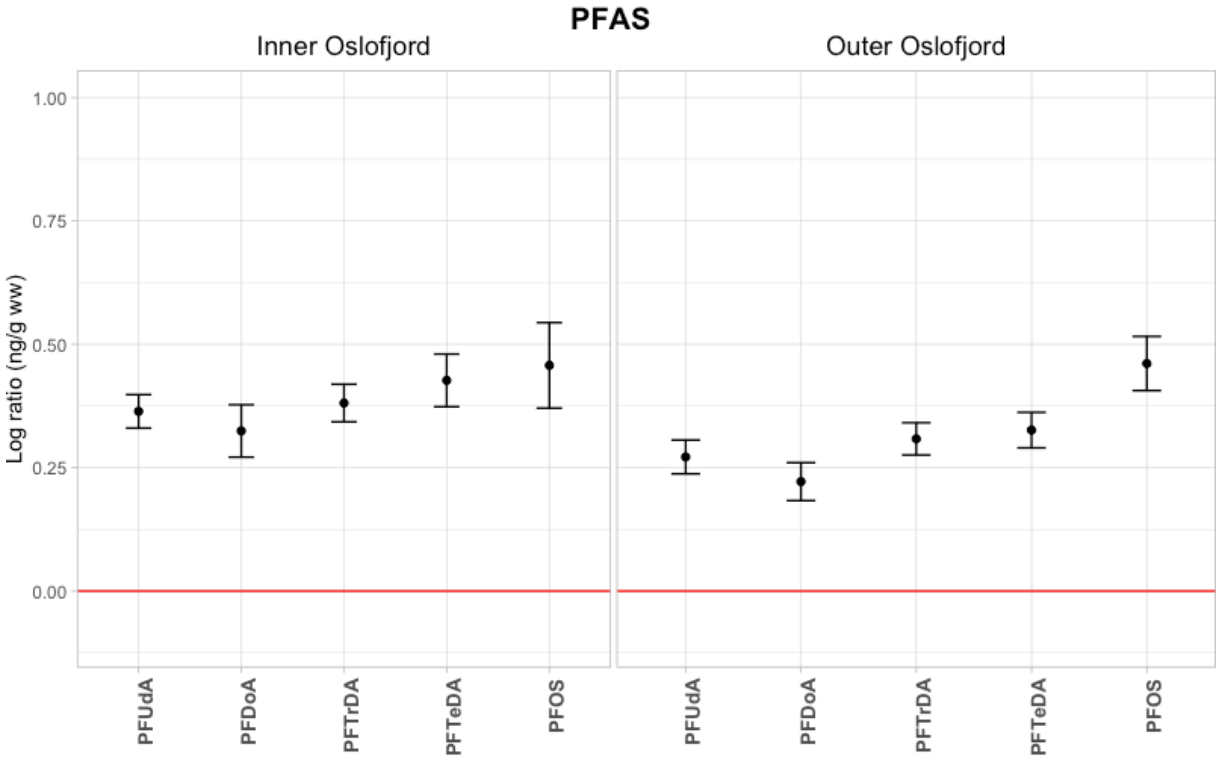

Figure 17. Log ratio of PFASs (ng/g ww) between herring gull (*Larus argentatus*) females and eggs in a) the inner Oslofjord (n = 15), and b) the outer Oslofjord (n = 15), presented as mean ratios (± SE). The PFCAs are ordered based on chain length (from shortest to longest), and the PFSA (PFOS) is placed to the far right. The horizontal line (log ratio = 0) indicates a 1:1 relationship.

3.5.3 Metal levels between mother and egg

As observed in the females, Fe followed by Zn and Cu were the three most prevalent metals in eggs from both colonies. The 4th and 5th most prevalent metals in eggs from the inner fjord were Hg and As, respectively. The opposite was observed in the outer fjord eggs, where the 4th most prevalent was As and the 5th Hg. The four least prevalent in eggs from both colonies were (in decreasing order) Ni, Pb, Ag and Cd.

No differences between the levels of As were observed between females and eggs from the two colonies (Pairwise Mann-Whitney U tests, $p > 0.05$). Cd levels were higher in females compared to eggs in both colonies (Pairwise Mann-Whitney U tests, $p < 0.01$), while no differences were observed in eggs between the two colonies (Wilcoxon rank sum test, $p = 0.19$). Levels of Pb were higher in females compared to eggs in the inner and outer fjord colonies (Pairwise Mann-Whitney U test and paired t-test, $p < 0.01$), while no differences between the Pb levels in eggs between the two colonies were observed (Wilcoxon rank sum test, $p = 0.12$). Hg levels were higher in the inner fjord females compared to their eggs, while no clear differences were observed between eggs and females in the outer fjord colony (Pairwise Mann-Whitney U tests, $p < 0.01$ and $p = 0.09$, respectively). No differences in Hg levels were observed in eggs between the colonies (Wilcoxon rank sum test, $p > 0.05$).

Of the essential metals, the levels of Fe were higher in females compared to blood from both colonies, while no differences were observed in eggs between colonies (Paired t-test and Welch Two Sample t-test, $p < 0.01$ and $p = 0.42$, respectively). The levels of Ni were higher in females compared to eggs from the inner fjord colony, while no differences were observed in between females and eggs in the outer fjord colony (Pairwise Mann-Whitney U tests, $p < 0.01$ and $p = 0.17$, respectively). The levels of Ni were higher in the outer fjord eggs compared to the inner fjord eggs (Wilcoxon rank sum test, $p = 0.03$). Cu levels was higher in eggs compared to females in both colonies (Paired t-tests, $p < 0.01$), while no differences were observed in Cu levels in eggs between colonies (Welch Two Sample t-test, $p = 0.47$). Similarly, the levels of Zn were higher in eggs compared to females in both colonies (Paired t-tests, $p < 0.01$) with no differences in eggs between colonies (Welch Two Sample t-test, $p = 0.71$). The levels of Ag were higher in the eggs from the outer fjord colony compared to the eggs from the inner fjord colony , while no differences in Ag levels was observed between mothers and offspring (Wilcoxon rank sum test and Pairwise Mann-Whitney U tests, $p < 0.01$ and $p > 0.05$).

The egg:wholeblood ratio in metals was not as high as for the lipophilic contaminants (Figure 18). For the essential metals, the ratio was higher for Cu and Zn compared to Fe and Ni. For the non-essential metals, As and Ag showed higher egg:wholeblood ratios than Cd, Pb and Hg.

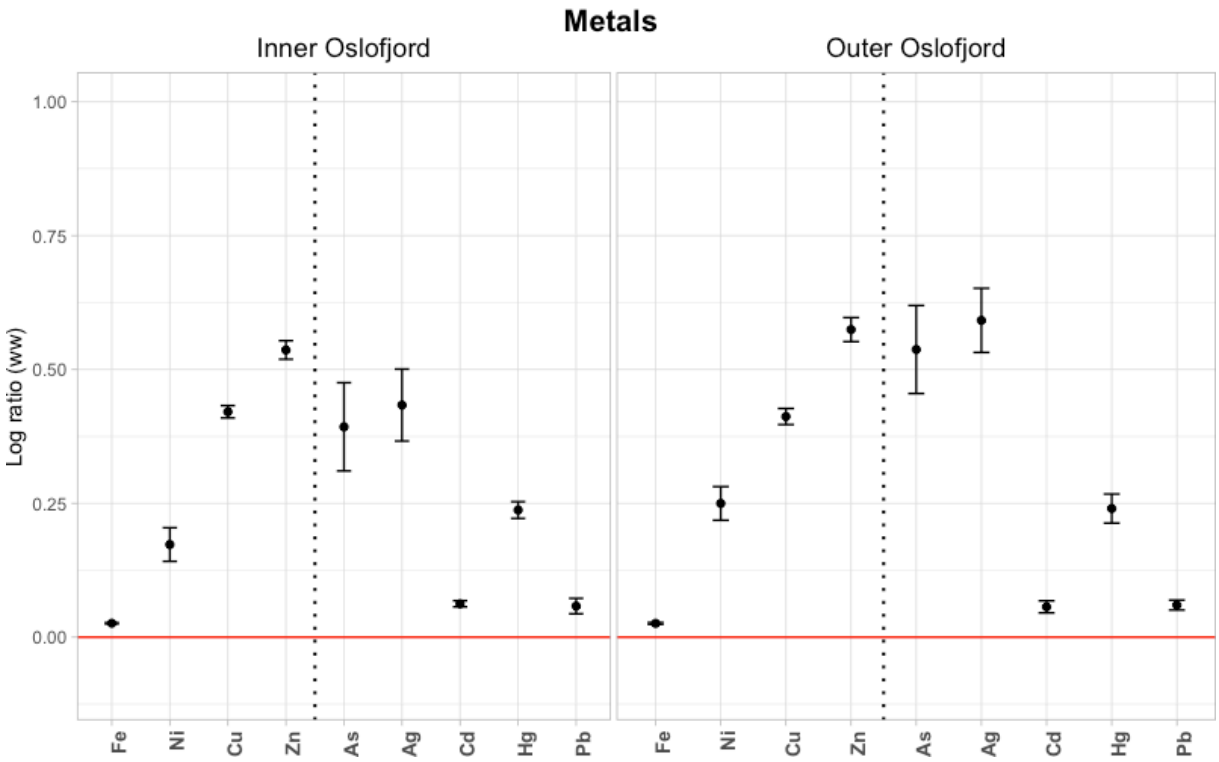

Figure 18. Log ratio between herring gull (*Larus argentatus*) females and eggs of the different metals. Iron (Fe), nickel (Ni), copper (Cu), zink (Zn), arsenic (As), silver (Ag), cadmium (Cd), lead (Pb) are in µg/g ww, while mercury (Hg) is in ng/g ww. The results are presented as mean ratios (± SE). The metals are positioned based on number of atoms, from lowest to highest. Ratios are shown for both a) the inner Oslofjord (n = 15) and b) the outer Oslofjord (n = 15) colonies. The black dotted vertical lines separate the essential (to the left) from the non-essential (to the right) metals. The horizontal line (log ratio = 0) indicates a 1:1 relationship.

4 Discussion

This study aimed to assess the influence of food source and trophic niche, as well as biological variables, on the contaminant status in herring gulls and the maternal transfer to their eggs. To answer this, the levels of organohalogen contaminants (OHCs) were quantified in herring gull females and their eggs in two colonies in the Oslofjord. In addition, dietary markers and biometric data were investigated.

4.1 Ecological niche

Ruus et al. (2017) found lower stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in herring gulls from the inner fjord compared to other marine species. In accordance with their findings, this present study discovered higher $\delta^{13}C$ and $\delta^{15}N$ in both females and eggs in the outer fjord compared to the inner fjord colony. As expected from H_1 and H_2, the results found in this study indicate that the inner fjord gulls have a more terrestrial diet and occupy a higher trophic niche compared to the outer fjord gulls.

Keilen (2017) revealed differences in the $\delta^{13}C$ values of female herring gulls within the Skjælholmen colony from the inner Oslofjord and divided these gulls into marine and terrestrial gulls. In the present study, the $\delta^{13}C$ variation between individuals in each colony was not as substantial, and the two colonies were investigated as two groups; inner and outer Oslofjord. The terrestrial and marine Oslofjord gulls from Keilen (2017) had mean $\delta^{13}C$ values of -24.2‰ and -21.8‰ and mean $\delta^{15}N$ values of 8.6‰ and 11.5‰ $\delta^{15}N$, respectively. These values are higher than those recorded in the inner Oslofjord females from the present study, with mean $\delta^{13}C$ values of -24.5‰ and $\delta^{15}N$ values of 8.4‰. These results suggest diet differences between the individual herring gulls within the same colony. Gull species have shown flexibility in their niche width and with dietary shifts in the absence of their main prey (González-Solís et al., 1997). Thus, these inter-annual differences between breeding seasons indicate a variation in each individual's diet specialisation or its availability of prey, as previously suggested by Leat et al. (2019).

$\delta^{13}C$ and $\delta^{15}N$ were positively correlated between mother and egg in each colony, suggesting that the isotopic ratio of the mother reflects that of the egg. No differences in correlation

concerning the stable isotopes were observed between the two colonies. The lack of correlation difference between colonies, together with the higher $\delta^{13}C$ and $\delta^{15}N$ in the outer fjord colony might suggest that the sequestering of the isotopes from mother to egg is similar, regardless of the diet source.

As well as a difference in $\delta^{13}C$ and $\delta^{15}N$ values between the inner and outer fjord colonies, lower $\delta^{13}C$ and higher $\delta^{15}N$ were found in eggs compared to females in both colonies. The higher $\delta^{13}C$ in the females indicates a more marine diet in the females compared to the eggs. Based on previous experience in capturing herring gulls in the Oslofjord combined with the egg development stages, the eggs were confirmed to be far along in their development. Thus, the difference in the stable isotopic values could be explained by differences in the diet of the female herring gulls from egg-laying compared to the time of blood sampling. The herring gull mothers might be feeding on more terrestrial food items before egg-laying and switching diets when they start nesting. A diet shift during egg-laying has previously been observed in the Eurasian dipper (*Cinclus cinclus*) and the American dipper (*Cinclus mexicanus*), where the females of the American dipper switched to feeding at a higher trophic level (TL) during egg-laying (Morrissey et al., 2010).

Another possible reason for the difference between the $\delta^{13}C$ values in the females compared to the eggs could be that the eggs arise from maternal tissues with other carbon isotope signatures than the ones observed in whole-blood. $\delta^{13}C$ and $\delta^{15}N$ differ between tissues in birds (Caut et al., 2009), which is further supported by the results found in the present study. Eggs are made up of nutrients derived from the diet of the adult females, so the values of $\delta^{13}C$ and $\delta^{15}N$ in the eggs should be related to those of the mother. Hobson (1995) found higher values of $\delta^{13}C$ and $\delta^{15}N$ in the eggshell yolk, albumen, and membranes of the eggs of the Japanese Quail (*Coturnix japonica*) relative to their diet and linked these findings to differences in biochemical and metabolic processes involved in tissue synthesis. Moreover, the differences in isotopic values in the two types of matrices could be explained by differences in lipid percentages. The C:N ratio in both whole blood and eggs was measured, revealing higher ratios for eggs than blood in both colonies. A C:N ratio over 3.5 suggests a presence of lipids. Lipids are around 6-7‰ depleted of ^{13}C relative to proteins, which might confound the interpretation (Sweeting et al., 2006). Whole-blood and eggs from both colonies in this study revealed mean C:N ratios over 3.5, suggesting the presence of lipids, which

might explain the $\delta^{13}C$ differences between blood and eggs, and also the differences observed between the females in each of the colonies.

4.2 Contaminant levels between colonies

The Urbanfjord programme has investigated the levels of OHCs in both herring gull females and their eggs from the inner Oslofjord for several years. The levels of PCBs, HCB, PBDEs, CPs and PFASs in the present study are similar to previous findings by the Urbanfjord programme from 2014, 2015 and 2016 (Ruus et al., 2015; Ruus et al., 2016; Ruus et al., 2017).

As mentioned, legacy persistent organic pollutants (POPs) bioaccumulate and biomagnify up the food web, leading to higher POP levels with increasing $\delta^{15}N$. Due to legislation (UNEP, 2009), and the following emission decline of POPs globally (EC and EPA, 2009), we expected (H_3 and H_4) the levels of the lipophilic legacy POPs (i.e., PCBs, HCB and PBDEs) and the more recently added PFOS, to be higher in the colony with the highest $\delta^{15}N$ values.

4.2.1 Lipophilic legacy persistent organic pollutants

Keilen (2017) found higher levels of lipophilic POPs in herring gulls from northern Norway and the marine Oslofjord gulls compared to the terrestrial inner Oslofjord gulls, linking these contaminant levels to diet. In contrast to the results found by Keilen (2017) and to our hypothesis (H_3), the PCBs, HCB and the majority of PBDEs did not correlate with the dietary markers ($\delta^{13}C$ and $\delta^{15}N$) in the females or differ between the two colonies. The lack of correlation between the dietary markers and the lipid soluble legacy POPs contradicts previous studies, e.g. Leat et al. (2019), linking increasing levels of POPs in seabirds to increasing levels of $\delta^{13}C$ and $\delta^{15}N$. Furthermore, the lack of correlation between these variables in the present study might be a result of differences in the individuals' prey specialisation or prey availability (Leat et al., 2019). It could also be explained by different turnover rates between contaminant levels and dietary markers. $\delta^{13}C$ and $\delta^{15}N$ in blood represent a short dietary accumulation of 3-4 weeks (Bearhop et al., 2002; Quillfeldt et al., 2010), which in this case was during the time of the breeding season. Blood contaminant levels reflect a more extended timeframe and could be influenced by the gulls' overwintering location (Baert et al., 2013; Borgå et al., 2005).

Despite the ban on PCBs, HCB and PBDEs (UNEP, 2001), considerable amounts of these contaminants may still discharge from urban sources such as waste landfills and building materials (Herzke et al., 2009). High levels of POPs have been linked to point sources from highly urbanised areas such as the inner Oslofjord (Ruus et al., 2017), possibly explaining the similarities in the contaminant levels between the inner and outer fjord colony, regardless of higher $\delta^{15}N$ in the outer fjord herring gulls. Also, several herring gulls have been observed on garbage dumps outside of Moss, close to the outer fjord colony (pers. comm. Morten Helberg), where they might be exposed to other levels of these contaminants than expected from the marine food web in the outer fjord. The marginal differences in biometric variables between colonies and between blood and eggs suggest that these will not influence the contaminant levels between colonies or matrices.

Polychlorinated biphenyls

The PCBs were grouped based on the number of chlorine substitutions in the molecule. Higher chlorinated PCB congeners are more bioaccumulative compared to the lower chlorinated PCBs, but they are also present at lower levels in the environment and adsorb to particles and soil to a higher degree (McFarland & Clarke, 1989). Moreover, these properties make the higher chlorinated PCBs less available to organisms. PCB congeners with lower chlorination tend to be more efficiently metabolised and eliminated from organisms (McFarland & Clarke, 1989). When the octanol-water partition coefficient (K_{OW}) reaches a certain point ($\log K_{OW} > 7$), the assimilation efficiency decreases, which was shown to be the case for hepta-, octa and nonaCBs (Fisk et al., 1998). These results are consistent with the ones found in this study, where the levels of hexaCBs are higher than hepta- and higherCB in females and eggs from both colonies. A maximum level of hexaCBs and lower levels of the higher chlorinated PCBs have been observed in glaucous gulls, black-legged kittiwakes (*Rissa tridactyla*) and alcids (Borgå et al., 2001; Borgå et al., 2005). Borlakoglu and Walker (1989) observed the same pattern in several piscivorous seabirds. Although the higher chlorinated PCBs are more bioaccumulative and the outer fjord colony had a higher $\delta^{15}N$, no differences in the levels of PCB-groups between colonies were discovered, which might be explained by the continued discharge of PCBs from urban sources (Herzke et al., 2009).

Hexachlorobenzene

HCB has been used for a variety of purposes in industry and agriculture, such as a porosity control agent, a fungicide and as a wool preserving agent (Becker et al., 2012). HCB has

higher volatility and a lower K_{OW} compared to other POPs (Barber et al., 2005), making it able to travel through air, water and sediment through multiple evaporation and deposition steps. This is often referred to as the 'grasshopper effect', where POPs evaporate from lower, warmer latitudes, and deposit at higher latitudes (Wania & Mackay, 1996). Keilen (2017) reported higher levels of HCB with increasing latitude and with increasing TL. Similarly, Kelly et al. (2007) found a strong positive relationship between increasing levels of HCB and TL in an Arctic food web. Although this present study found a higher value of $\delta^{15}N$ in the outer Oslofjord colony, no such relationships in the levels of HCB were discovered between the inner and outer Oslofjord colonies. Variability in the HCB levels was high for both females and eggs, possibly explaining the lack of a relationship between $\delta^{15}N$ and HCB between the two colonies.

Polybrominated diphenyl ethers

The levels of BDE-47 and BDE-99 in the inner fjord eggs in the present study were higher than the levels of the same compounds in herring gull eggs from more remote colonies in northern Norway (Sklinna and Røst; Huber et al., 2015), suggesting an urban influence of these legacy POPs. Urban PBDE exposure as well as bioaccumulation in the outer fjord colony due to higher $\delta^{15}N$ values might be the reason for the similar values observed in the herring gull females in the two colonies.

4.2.2 Cyclic volatile methyl siloxanes

The inner and outer Oslofjord is separated by a sill near Drøbak. Because of this sill, together with the semi-enclosed nature of the Oslofjord, substances from wastewater discharges below the pycnocline, i.e. the layer where the density gradient is greatest, will get trapped. Furthermore, higher levels of cyclic volatile methyl siloxanes (cVMSs) have been linked to proximity to human settlements, acting as point sources (Warner et al., 2010). It was therefore expected that the levels of cVMSs would be higher in the inner fjord close to highly urbanised areas compared to the outer fjord (H_3). Powell et al. (2018) found higher levels of the cVMSs D4, D5 and D6 in surface sediments from the inner Oslofjord compared to the outer Oslofjord, with exposure levels 2, 32 and 7 times higher for D4, D5 and D6, respectively. Furthermore, they found the highest cVMS lipid-normalised levels in biota (i.e. zooplankton, benthic macroinvertebrates, shellfish and finfish) from the inner Oslofjord. In the present study, the levels of D5 were, as expected (H_3), highest in the inner fjord female gulls, while

the levels of D6 did not show any clear difference in females between the colonies. D5 has been shown to bioaccumulate in the Lake Mjøsa food web from Norway with a trophic magnification factor (TMF) above 1 (Borgå et al., 2012), while the TMF for both D5 and D6 in the Oslofjord benthopelagic food web was below 1 (Powell et al., 2018). This might be explained by variability in TMFs in different food webs (Borgå et al., 2012). Thus, the higher levels of D5 in the inner fjord might be a result of exposure from urban areas.

Levels of D5 in female gulls from the Oslofjord have decreased during the last four years, with a mean of 7.10 ng/g ww in 2014, 4.53 ng/g ww in 2015 and 2.72 ng/g ww in 2016 (Ruus et al., 2015; Ruus et al., 2016; Ruus et al., 2017), suggesting that the reduction in production of this contaminant affects the levels in the gulls. However, mean levels of D5 in herring gull eggs in this study were 47 times higher than observed in herring gull eggs from more remote colonies in northern Norway (Sklinna and Røst; Huber et al., 2015), suggesting exposure from urban areas. D6 levels has shown a decrease in females, with blood levels in female herring gulls from inner fjord going from 2.55 ng/g ww in 2014 to 1.95 ng/g ww in 2015 and 0.19 ng/g ww in 2016. However, this study found higher levels of D6 in females than reported in 2016 (1.72 ng/g ww in the inner fjord and 0.55 ng/g ww in the outer fjord), indicating that the downward trend previously observed in females is not certain.

4.2.3 Chlorinated paraffins

Both of the chlorinated paraffins (CPs) SCCP and MCCP are on Norway's national priority list of contaminants that will be phased out, and there has been no registered use of SCCP in Norway since 2004. The compounds may still be found in the environment through emissions from older products still in use and their presence in imported products (Norwegian Environment Agency, 2018). Despite the ban, the levels of SCCP and MCCP in the Oslofjord females show no signs of decline during the last four years (Ruus et al., 2015; Ruus et al., 2016; Ruus et al., 2017). Considering that use of SCCP and MCCP is not restricted in the rest of the EU, the non-declining levels in the gulls might be explained by imported or old products containing these compounds. Furthermore, as expected (H_3), the levels of SCCP and MCCP were higher in the female gulls from the inner fjord compared to the outer fjord, with no correlation with the dietary markers. Thus, the higher levels in the inner fjord gulls might be explained by higher levels of CPs closer to urban areas.

4.2.4 Perfluoroalkyl substances

Despite the ban in using PFOS in fire-fighting foam in 2007 (UNEP, 2009), PFOS showed the highest levels of the perfluoroalkyl substances (PFAS) compounds in both blood and eggs from both colonies. These results are consistent with previous studies of PFAS, where PFOS is the predominant PFAS in the samples studied (Gebbink et al., 2011; Kallenborn & Berger, 2004; Verreault et al., 2005), owing to the highly bioaccumulative properties of PFOS (Kelly et al., 2009). The levels of the different PFAS compounds did not show any correlation between number of carbon atoms in the carbon chain in blood or eggs from the two locations. However, only four PFCAs and one PFSA were included in the statistical analysis, which might be too small a sample size to observe any trends in chain lengths.

The levels of the lipophilic legacy POPs did not differ in females or eggs between the inner and outer Oslofjord colony. However, contrary to expectations (H_4), the PFAS levels were higher in the outer fjord females compared to the inner fjord females. Rygge airport near the Revlingen colony has been used as a fire drill area for several decades, where the fire-fighting foams are an important point source of PFAS into the environment (Melnes & Mariussen, 2017). Fire-fighting foams containing PFAS have presumably been used at Rygge since the 1970s (Amundsen et al., 2016), but the exact PFAS mixture in these foams during the last 40-50 years is unknown. In 2007, fire-fighting foams containing PFOS were removed from the area and from 2015, all foams containing PFOS mixtures were replaced by Re-Healing Foams, which are PFAS-free (Amundsen et al., 2016). The spread of the PFAS compounds has resulted in levels two times higher than the environmental quality standard (EQS), i.e. the limit for environmental disturbances, for fish in the Vansjø Lake (Amundsen et al., 2016). The lake flows into the Mosselva river, before entering the Oslofjord and is therefore in close vicinity to the outer fjord colony. Thus, PFAS from fire-fighting foam is suggested to be the reason for the elevated levels of PFAS in the herring gulls from the outer fjord compared to the inner fjord females.

Higher levels of PFAS were observed in blood and eggs with higher $\delta^{13}C$ and $\delta^{15}N$. However, the increase in PFAS levels may not be correlated with $\delta^{15}N$ but could instead be explained by local PFAS exposure from the Rygge fire drill area. Higher levels due to local PFAS exposure are supported by other findings, such as elevated levels of PFAS in snow bunting (*Plectrophenax nivealis*) from two populated settlements, (Longyearbyen and Barentsburg),

compared to an abandoned Pyramiden area (Warner et al., 2019). Furthermore, higher PFAS levels have been found in soil and run-off water from fire-fighter training facilities in Longyearbyen, linking this to local source exposure (Skaar et al., 2019).

These results suggest that outer fjord colony presents a more local source of PFAS exposure than the Oslofjord ecosystem as such, and that when investigating the PFAS compounds in the Oslofjord system, herring gulls from the inner fjord do not provide a representative picture of the Oslofjord ecosystem.

4.2.5 Metals

Even though Arsenic (As) was the most prevalent contaminant in the outer fjord females, no differences in As levels in females between the inner and outer Oslofjord were observed. These findings are in agreement with Borgå et al. (2006), who found no potential for bioaccumulation of As in seabirds from the Barents Sea, and with Vizuete et al. (2019), stating that As is not of concern in terms of bioaccumulation in the food web. Cadmium (Cd) levels were higher in the outer fjord females compared to the inner fjord females, as expected (H_5), as Cd accumulates in aquatic organisms (Dubois & Hare, 2009).

Mercury (Hg) was the second most prevalent contaminant in the female herring gulls. Contrary to expectations (H_5), no differences in Hg levels were observed in females between the colonies. Hg, especially its methylated form (Sundberg et al., 1999), can bioaccumulate in both terrestrial, freshwater and marine food webs around the world (Campbell et al., 2005; Jarman et al., 1996), resulting in higher levels with higher $\delta^{15}N$. The contradictory results could be explained by the limited range in $\delta^{15}N$ values in females between the Oslofjord colonies. Similar results have been observed in the Central Barents Sea food web, where no correlation between Hg levels and TL was linked to a narrow trophic range in the food web (Borgå et al., 2006).

Pb occurs naturally in the environment from multiple sources such as volcanic activity and erosion. However, the majority of Pb is related to human activities (e.g. industry, gasoline, and agriculture) and is a common contaminant in industrial and urban areas. The most considerable rise in levels of Pb occurred between 1950-2000, attributable to the increased use of leaded gasoline (Abadin et al., 2007). The use of Pb in gasoline was eliminated under

the Clean Air Act by EPA in 1996 (EPA, 1996), leading to a reduction of this metal in the environment (Abadin et al., 2007). However, the present study showed that Pb is still present in urban areas. Pb dominated the contaminant load in the herring gull females from the inner fjord and the levels were higher than observed in the females from the outer fjord. Previous studies further support these results, finding higher levels of Pb in the house sparrow (*Passer domesticus*) and the common blackbird (*Turdus merula*) with increasing urbanisation (Bichet et al., 2013; Meillère et al., 2016).

It is important to consider where the majority of each metal can be found in the body, as whole-blood might not reflect the real body burden of each metal. For example, approximately 90% of the total Cd body burden is present in the liver and the kidney (Friberg et al., 1974). Dietz et al. (1996) investigated levels of Cd in ten seabird species and reported much higher levels of Cd in the kidney compared to liver, and lowest levels of Cd in muscle. These results might suggest that the levels of Cd detected in the herring gull blood in this thesis do not reflect the overall body burden of this contaminant.

4.3 Maternal transfer

Differences in contaminant levels could arise from differences in the egg-laying sequence between the chosen eggs. Becker and Sperveslage (1989) investigated organochlorine differences in the egg-laying sequence of herring gulls and found higher levels in the third eggs compared to the first ones (13-17%), linking this to increasing body fat conversion during egg production due to high energy demands. Furthermore, they found intra-clutch differences in the levels of Hg, but unlike the organochlorines, the highest levels were observed in the first egg laid compared to subsequent eggs (Becker et al., 1989; Becker, 1992). Becker and Sperveslage (1989) found larger inter-clutch differences compared to intra-clutch variation and concluded that each egg does reflect the mother. Furthermore, Verreault et al. (2006) investigated intra-clutch variability and found no differences between the first and the third laid egg in levels of ∑PCB, ∑OC and ∑PBDE. These results suggest that a randomised study, such as the one performed in the present study where eggs were collected at random from each nest, will even out the effect of the intra-clutch variability and not create any bias in contaminant levels between the eggs.

When investigating the correlations between mother and egg of the PCBs, only PCB-209 showed a positive correlation between mother and egg. The same trends were observed in PFASs and for several of the other contaminants, suggesting that higher level of contaminants in the mother does not indicate higher levels in the egg.

4.3.1 Lipophilic legacy persistent organic pollutants

The levels of PCBs in this study made up a more substantial proportion of the total contaminant load in eggs compared to the female gulls in both colonies, indicating that these lipophilic POPs are efficiently transferred to eggs. In addition, the majority of PBDEs were higher in eggs compared to females. These results were expected (H_6) and is supported from previous studies investigating maternal transfer of PCBs, showing how lipophilic contaminants get transferred from mother to egg during reproduction (Bjerk & Holt, 1971; Verreault et al., 2006). Verreault et al. (2006) investigated the maternal transfer of OHCs in glaucous gulls and found maternal transfer to favour contaminants with lower K_{OW} and/or less persistent compounds. The results of this thesis reveal contrasting results, with a higher maternal transfer efficiency of HeptaCB and HigherCB with a higher K_{OW} compared to the less chlorinated PCBs.

HCB levels were higher in eggs from the outer fjord colony, while no differences were found for HCB levels in the inner fjord colony, indicating that a larger quantity of lipophilic contaminants get transferred with a more marine diet. There was a positive correlation between the levels of PCBs, HCB and PBDEs and $\delta^{13}C$ in eggs, further indicating that more of these contaminants are transferred with a more marine diet, as expected (H_7). However, no differences were discovered between colonies, indicating that the dietary differences in the mothers do not affect the transfer efficiency of these legacy POPs.

4.3.2 Cyclic volatile methyl siloxanes

While levels cVMSs were higher in eggs compared to females in both colonies, the maternal transfer efficiency to eggs was higher for D5 than for D6 in both colonies. Similar results in eggs have been observed in glaucous-winged gulls (*Larus glaucescens*), California gulls (*Larus californicus*) and herring gulls from different colonies across Canada, where D5 was the most abundant cVMS, followed by D6 (Lu et al., 2017). Furthermore, levels of D5 followed by D6 dominated the cVMSs in bird eggs from northern Norway (Sklinna and Røst;

Huber et al., 2015). D5 has a log K_{OW} value of 5.2, compared to D6 with a value of 6.33, indicating that a higher log K_{OW} is not indicative of higher levels of cVMSs in gull eggs.

Another interesting discovery was the contrasting increase and decrease in the levels of cVMSs between mothers and eggs of the herring gulls from the inner fjord colony. The levels of D5 in the inner Oslofjord female gulls have decreased during the last four years, while the levels of D5 in the gull eggs from the same colonies show opposite trends, from 61.39 ng/g ww in 2014 over to 88.8 ng/g ww in 2015 to 205.43 ng/g ww in 2016 (Ruus et al., 2015; Ruus et al., 2016; Ruus et al., 2017). However, the D5 levels from the inner fjord eggs from this study were somewhat lower than observed in 2016, with a mean value of 56.12 ng/g ww. The conflicting results in D5 levels between mother and eggs could be explained by dietary differences in the mothers, as this study revealed both a more marine signal ($\delta^{13}C$) and a different trophic niche ($\delta^{15}N$) in the gulls from the outer fjord compared to the gulls from the inner fjord. Furthermore, the D5 levels were approximately four times higher in the outer fjord eggs compared to the inner fjord eggs, which suggests an increase in D5 with an increasing $\delta^{15}N$, as expected (H_7). These findings are further supported by the results in herring gull eggs from the same colony in 2016, with mean D5 levels of 205.43 ng/g ww and a $\delta^{15}N$ value of 10.0‰ (Ruus et al., 2017), both higher than observed in our colony. Thus, these results might suggest that D5, a lipophilic contaminant, is more efficiently transferred with a more lipid-rich and marine diet such as in the outer fjord gulls.

The same contrasting results in the two different matrixes can be seen for the levels of D6, with increasing levels in the herring gull females from 2014 to 2016 and decreasing levels in eggs during the same time period. However, the increase in D6 was not as substantial as observed for D5, with levels in eggs ranging from 10.29 ng/g ww in 2014, to 11.2 ng/g ww in 2015 and 13.62 ng/g ww in 2016 (Ruus et al., 2015; Ruus et al., 2016; Ruus et al., 2017). Moreover, the results of this current study revealed higher levels of D6 in the inner fjord eggs compared to the outer fjord eggs, which contradicts H_7 of increasing maternal transfer of lipophilic contaminants with a more marine signal (higher $\delta^{13}C$). However, the difference in levels of D6 between the inner and outer fjord eggs, being less substantial than the difference the levels of D5, might be explained by differences in an individual's prey specialisation (Leat et al., 2019).

4.3.3 Chlorinated paraffins

Even though the CPs are classified as persistent and bioaccumulative (REACH, 2019), the mother-to-egg transfer efficiency of SCCP and MCCP was lower than observed for the other lipophilic contaminants. The egg:wholeblood ratio for both CPs were higher in the outer fjord gulls, suggesting increasing levels of these lipophilic contaminants with increasing $\delta^{15}N$, and thus with a more marine and lipid-rich diet. However, there was no correlation with SCCP to $\delta^{13}C$ or $\delta^{15}N$ values in the eggs, indicating that an increase in $\delta^{15}N$ are not contributing to the higher levels of CPs transferred to the eggs in the outer fjord. However, as discussed above, differences in an individual's prey specialisation might contribute to these observed results.

4.3.4 Perfluoroalkyl substances

Except for the PFAS compounds PFUdA and PFDoA in the outer fjord colony, the levels of PFASs in both colonies were higher in the eggs compared to the females, suggesting that a considerable amount is transferred from mother to egg during reproduction. However, the levels were lower for the PFAS compounds compared to the majority of the lipophilic compounds, as expected (H_6). PFASs are amphipathic, binding to both serum albumin and lipoproteins (Armitage et al., 2013; Beesoon & Martin, 2015; Ng & Hungerbühler, 2014). Lipids constitute around 20-30% of the total egg mass (Astheimer & Grau, 1990). Protein is a limiting resource during egg production, possibly explaining this difference in maternal transfer ratios between the solely lipophilic and more protein-associated compounds (Bolton et al., 1993). The maternal transfer is likely to have a weaker effect on protein-associated compounds such as PFASs and Hg than lipophilic pollutants such as PCBs (Hitchcock et al., in press).

As expected from H_7, the increase in the protein-associated PFASs did not increase with a more marine diet (higher $\delta^{13}C$). However, in contrast to expectations (H_7), when using PFOS as an indication of the PFAS levels, higher PFAS levels were positively correlated with $\delta^{15}N$, suggesting that more PFAS gets transferred with a more marine diet. These results might be confounded by the higher PFAS levels in the outer fjord females as a result of local PFAS exposure, and not reflect a higher maternal transfer efficiency. Even though the PFAS levels were higher in the outer fjord colony, the egg:wholeblood ratios were higher in the inner fjord colony. As mentioned, proteins are a limiting resource in egg production (Bolton et al., 1993). Thus, if the herring gulls in the outer Oslofjord have less or equal the amount of proteins to

transfer to the eggs, as well as having higher levels of PFAS in their blood due to higher exposure, the result will be a lower egg:wholeblood ratio, as observed here.

Another possible explanation for this ratio discrepancy between colonies could be the influence of detection replacements when substituting the values under the LOD. Several values were under the LOD in both the inner fjord herring gull blood and eggs for the PFAS compounds PFUdA, PFDoA, PFTrDA and PFTeDA. The imputation method used when substituting these values uses the distribution of the data to replace the non-detects with new values, and even though this method is one of the most robust ones to use on these kinds of data sets, the exact values are not known. Substituting data could create a pattern different from the one observed if the real values were known.

4.3.5 Metals

The eggs had lower levels of Cd, Pb, and Hg than in blood of the female gulls, which is consistent with previous findings by Burger and Gochfeld (1996). Hg, especially its methylated form, binds to proteins (Sundberg et al., 1999), which are a limiting resource in egg reproduction (Bolton et al., 1993). The majority of Hg is sequestered in the albumen of eggs (Brasso et al., 2012; Heinz & Hoffman, 2004). Potential food limitations might affect the levels of Hg transferred from mother to egg, as some species acquire resources for albumen production exclusively from their local diet and not from endogenous body reserves (Hobson et al., 2015). Thus, the resources available in the mother at the time of albumen production might explain the mother-to-egg transfer ratio of Hg being below 1. These findings are consistent with results found by Hitchcock et al. (in press) where the authors found overall higher levels of PCBs and PFOS in male compared to female birds. The Hg levels between the sexes were similar, suggesting a less efficient maternal transfer of Hg compared to the lipophilic POPs (Hitchcock et al., in press). In the current study, levels of the essential metal Fe were higher in females than eggs. Fe is part of the haemoglobin molecule in red blood cells (Tymoczko et al., 2015), which is linked to this substantial difference. There were no differences between the levels of As in females and eggs, while the levels of Cu and Zn were higher in eggs than in females. Cu and Zn are essential metals needed for biological functions essential for the growth of future offspring (Davidson et al., 2007).

These results suggest that some of the contaminant burdens in the adult female gulls are offloaded to eggs during reproduction, especially the lipophilic contaminants. The eggs

appear to depollute the female's body, particularly the lipophilic contaminants for which the egg:wholeblood ratio was higher than observed for PFASs and metals. These results are supported by Hitchcock et al. (in press), who found lower levels of PCBs in females than in males during incubation.

5 Conclusions

The present study of environmental contaminants in herring gull found a more marine signal and a higher trophic niche in the outer Oslofjord colony compared to the inner Oslofjord colony. In addition, lower carbon and higher nitrogen isotopic values were found in eggs compared to blood, which might be explained by differences in the isotopic ratios in the mothers before and after egg-laying.

The levels of the lipophilic legacy POPs did not differ between the inner and outer colony and were uncorrelated with the dietary markers, which contradicts the expectations that the levels of these contaminants would increase with increasing $\delta^{15}N$. Even though these contaminants have been banned for several years, considerable amounts may still discharge from urban sources such as waste landfills and building materials, possibly explaining the similarities between the colonies. Higher levels of PFASs were observed in the outer fjord colony compared to the inner fjord colony, which was linked to local exposure from a previously used fire drill area near the outer fjord colony.

In both colonies, lipophilic contaminants were transferred from mother to egg to a higher degree than protein-associated contaminants and metals. PCBs dominated the contaminant load in the eggs from both colonies and their levels considerably higher than observed in the mothers. The legacy POPs (PCBs, HCB and PBDEs) in herring gull eggs were correlated with $\delta^{13}C$, suggesting that a higher contaminant load gets transferred with a more marine diet. However, no differences between colonies was observed for $\delta^{13}C$ and the levels of contaminants transferred, indicating that the transfer rate is similar between the females in the two colonies even though the diet is different.

6 Future perspectives

The contaminant load in herring gull females was investigated using whole-blood. This matrix may not be the best indicator of the total contaminant load in the gulls, as the contaminants have different properties (e.g. lipophilic, protein-associated). Investigating the contaminant load in feathers as well as whole-blood would provide a better picture of the contaminant burden, as gulls are known to sequester some contaminants in their feathers. In addition, protein values in both whole-blood and eggs should be measured to identify whether protein limitations could be the reason for the difference in protein-associated contaminants between the two colonies.

Differences in stable isotopes of carbon and nitrogen before and after egg-laying have been observed in other bird species. Thus, samples before and after egg-laying should be collected in the herring gull to be able to tell whether they switch to a more marine or terrestrial diet during the breeding period.

A C:N ratio higher than 3.5, as observed in the present study, suggests the presence of lipids, and lipid correction is recommended. To avoid a depletion of $\delta^{13}C$ in tissues in lipid-rich matrices, lipid extraction prior to stable isotope analysis should be performed.

References

Abadin, H., Ashizawa, A., Stevens, Y., Llados, F., Diamond, G., Sage, G., Citra, M., Quinones, A., Bosch, S., & Swarts, S. (2007). Public health statement. *Toxicological profile for lead*. Atlanta, GA: Agency for Toxic Substances and Disease Registry (ATSDR).

Abdullah, M. I., Ringstad, O., & Kveseth, N. J. (1982). Polychlorinated biphenyls in the sediments of the inner Oslofjord. *Water, Air, and Soil Pollution, 18*(4), 485-497.

Amos, H. M., Jacob, D. J., Streets, D. G., & Sunderland, E. M. (2013). Legacy impacts of all-time anthropogenic emissions on the global mercury cycle. *Global Biogeochemical Cycles, 27*(2), 410-421.

Amundsen, C. E., Joranger, T., & Sparrevik, M. (2016). *PFAS ved Rygge flystasjon. Tiltaksvurderinger 1.mars 2016 [In Norwegian]* (Report No. 867/2016). Forsvarsbygg, Oslo, Norway. Retrieved from https://www.forsvarsbygg.no/contentassets/392d6ac269554b578b9634e74641dc6a/tiltaksvurdering-pfas-rygge-flystasjon-1.mars-2016-endelig.pdf

Armitage, J. M., Arnot, J. A., Wania, F., & Mackay, D. (2013). Development and evaluation of a mechanistic bioconcentration model for ionogenic organic chemicals in fish. *Environmental Toxicology and Chemistry, 32*(1), 115-128.

Astheimer, L. B., & Grau, C. R. (1990). A comparison of yolk growth rates in seabird eggs. *Ibis, 132*(3), 380-394.

ATSDR. (2017). *The ATSDR 2017 substance priority list*. U.S. Department of Health and Human Services (HHS). Atlanta: Agency for Toxic Substances and Disease Registry Retrieved from https://www.atsdr.cdc.gov/spl/.

Azad, A. M., Frantzen, S., Bank, M. S., Johnsen, I. A., Tessier, E., Amouroux, D., Madsen, L., & Maage, A. (2019). Spatial distribution of mercury in seawater, sediment, and seafood from the Hardangerfjord ecosystem, Norway. *Science of The Total Environment, 667*, 622-637.

Baccarelli, A., Pfeiffer, R., Consonni, D., Pesatori, A. C., Bonzini, M., Patterson, D. G., Bertazzi, P. A., & Landi, M. T. (2005). Handling of dioxin measurement data in the presence of non-detectable values: Overview of available methods and their application in the Seveso chloracne study. *Chemosphere, 60*(7), 898-906.

Baert, J. M., Janssen, C. R., Borgå, K., & De Laender, F. (2013). Migration and opportunistic feeding increase PCB accumulation in Arctic seabirds. *Environmental Science & Technology, 47*(20), 11793-11801.

Barber, J. L., Sweetman, A. J., van Wijk, D., & Jones, K. C. (2005). Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. *Science of the Total Environment, 349*(1-3), 1-44.

Barceló, D., & Petrovic, M. (2008). Emerging contaminants in waste waters: Sources and occurence. In *Emerging contaminants from industrial and municipal waste. Occurence, analysis and effects* (Vol. 5, pp. 1-30). Berlin, Germany: Springer International Publishing.

Bearhop, S., Waldron, S., Votier, Stephen C., & Furness, Robert W. (2002). Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology, 75*(5), 451-458.

Becker, P. H., Conrad, B., & Sperveslage, H. (1989). Chlororganische verbindungen und schwermetalle in weiblichen silbermswen (*Larus argentatus*) und ihren eiern mit bekannter legefolge [In German]. *Vogelwarte, 35*, 1-35.

Becker, P. H., & Sperveslage, H. (1989). Organochlorines and heavy metals in herring gull (*Larus argentatus*) eggs and chicks from the same clutch. *Bulletin of Environmental Contamination and Toxicology, 42*(5), 721-727.

Becker, P. H. (1992). Egg mercury levels decline with the laying sequence in *charadriiformes*. *Bulletin of Environmental Contamination and Toxicology, 48*(5), 762-767.

Becker, S., Halsall, J. C., Tych, W., Kallenborn, R., Schlaback, M., & Manø, S. (2012). Changing sources and environmental factors reduce the rates of decline of organochlorine pesticides in the Arctic atmosphere. *Atmospheric Chemistry and Physics, 12*(9), 4033-4044.

Beesoon, S., & Martin, J. W. (2015). Isomer-specific binding affinity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) to serum proteins. *Environmental Science & Technology, 49*(9), 5722-5731.

Berge, J. A., Ranneklev, S., Selvik, J., & Steen, A. O. (2013). *Indre Oslofjord - Sammenstilling av data om miljøgifttilførsler og forekomst av miljøgifter i sediment [In Norwegian]* (Report No. 6565-2013). The Norwegian Institue of Water Research (NIVA), Oslo, Norway. Retrieved from https://niva.brage.unit.no/niva-xmlui/bitstream/handle/11250/216437/6565-2013_72dpi.pdf?sequence=1&isAllowed=y

Bichet, C., Scheifler, R., Cœurdassier, M., Julliard, R., Sorci, G., & Loiseau, C. (2013). Urbanization, trace metal pollution, and malaria prevalence in the house sparrow. *PloS one, 8*(1), e53866.

Bjerk, J. E., & Holt, G. (1971). Residues of DDE and PCB in eggs from herring gull (*Larus argentatus*) and common gull (*Larus canus*) in Norway. *Acta Veterinaria Scandinavica, 12*(3), 429-441.

Bolton, M., Monaghan, P., & Houston, D., C. . (1993). Proximate determination of clutch size in lesser black-backed gulls: The roles of food supply and body condition. *Canadian Journal of Zoology, 71*(2), 273-279.

Borgå, K., Gabrielsen, G. W., & Skaare, J. U. (2001). Biomagnification of organochlorines along a Barents Sea food chain. *Environmental Pollution, 113*(2), 187-198.

Borgå, K., Fisk, A. T., Hoekstra, P. F., & Muir, D. C. G. (2004). Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in Arctic marine food webs. *Environmental Toxicology and Chemistry, 23*(10), 2367-2385.

Borgå, K., Wolkers, H., Skaare, J. U., Hop, H., Muir, D. C. G., & Gabrielsen, G. W. (2005). Bioaccumulation of PCBs in Arctic seabirds: Influence of dietary exposure and congener biotransformation. *Environmental Pollution, 134*(3), 397-409.

Borgå, K., Campbell, L., Gabrielsen, G. W., Norstrom, R. J., Muir, D. C. G., & Fisk, A. T. (2006). Regional and species specific bioaccumulation of major and trace elements in Arctic seabirds. *Environmental Toxicology and Chemistry, 25*(11), 2927-2936.

Borgå, K., Fjeld, E., Kierkegaard, A., & McLachlan, M. S. (2012). Food web accumulation of cyclic siloxanes in Lake Mjøsa, Norway. *Environmental Science & Technology, 46*(11), 6347-6354.

Borlakoglu, J. T., & Walker, C. H. (1989). Comparative aspects of congener specific PCB metabolism. *European Journal of Drug Metabolism and Pharmacokinetics, 14*(2), 127-131.

Brasso, R. L., Abel, S., & Polito, M. J. (2012). Pattern of mercury allocation into egg components is independent of dietary exposure in gentoo penguins. *Archives of Environmental Contamination and Toxicology, 62*(3), 494-501.

Breivik, K., Bjerkeng, B., Wania, F., Helland, A., & Magnusson, J. (2004). Modeling the fate of polychlorinated biphenyls in the inner Oslofjord, Norway. *Environmental Toxicology and Chemistry, 23*(10), 2386-2395.

Bro, R., & Smilde, A. K. (2014). Principal component analysis. *Analytical Methods, 6*(9).

Buck, R. C., Franklin, J., Berger, U., Conder, J. M., Cousins, I. T., de Voogt, P., Jensen, A. A., Kannan, K., Mabury, S. A., & van Leeuwen, S. P. J. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment and Management, 7*(4), 513-541.

Burger, J., Fitch, M., Shugart, G., & Werther, W. (1979). Piracy in *Larus* gulls at a dump in New Jersey. *Proceedings of the Conference of the Colonial Waterbird Group, 3*, 87-98.

Burger, J., & Gochfeld, M. (1996). Heavy metal and selenium levels in Franklin's gull (*Larus pipixcan*) parents and their eggs. *Archives of Environmental Contamination and Toxicology, 30*(4), 487-491.

Campbell, L. M., Norstrom, R. J., Hobson, K. A., Muir, D. C. G., Backus, S., & Fisk, A. T. (2005). Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). *Science of The Total Environment, 351-352*, 247-263.

Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors (Δ15N and Δ13C): The effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology, 46*(2), 443-453.

Companioni-Damas, E. Y., Santos, F. J., & Galceran, M. T. (2012). Analysis of linear and cyclic methylsiloxanes in sewage sludges and urban soils by concurrent solvent recondensation – Large volume injection – Gas chromatography – Mass spectrometry. *Journal of Chromatography A, 1268*, 150-156.

Crawley, M. J. (2002). *Statistical computing: An introduction to data analysis using S-plus*. West Sussex, England: John Wiley & Sons.

Davidson, T., Ke, Q., & Costa, M. A. X. (2007). Chapter 5 - Selected molecular mechanisms of metal toxicity and carcinogenicity. In G. F. Nordberg, B. A. Fowler, M. Nordberg, & L. T. Friberg (Eds.), *Handbook on the toxicology of metals* (Third ed., pp. 79-100). Burlington: Academic Press.

de Wit, C. A. (2002). An overview of brominated flame retardants in the environment. *Chemosphere, 46*(5), 583-624.

de Wit, C. A., Alaee, M., & Muir, D. C. G. (2006). Levels and trends of brominated flame retardants in the Arctic. *Chemosphere, 64*(2), 209-233.

de Wit, C. A., Herzke, D., & Vorkamp, K. (2010). Brominated flame retardants in the Arctic environment — Trends and new candidates. *Science of The Total Environment, 408*(15), 2885-2918.

Demirtas, H., & Hedeker, D. (2008). Imputing continuous data under some non-Gaussian distributions. *Statistica Neerlandica, 62*(2), 193-205.

Denny, M. W., & Gaines, S. (2007). *Encyclopedia of tidepools & rocky shores* (1st ed.): University of California Press. Retrieved from https://books.google.no/books?id=uufQnE7MzMkC&pg=PA253&lpg=PA253&dq=#v=onepage&q&f=false

DeWitt, J. C. (2015). *Toxicological effects of perfuoroalkyl and polyfluoroalkyl substances*. New York, NY: Springer International Publishing.

Dietz, R., Riget, F., & Johansen, P. (1996). Lead, cadmium, mercury and selenium in Greenland marine animals. *Science of The Total Environment, 186*(1), 67-93.

Dietz, R., Sonne, C., Basu, N., Braune, B., O'Hara, T., Letcher, R. J., Scheuhammer, T., Andersen, M., Andreasen, C., Andriashek, D., Asmund, G., Aubail, A., Baagøe, H., Born, E. W., Chan, H. M., Derocher, A. E., Grandjean, P., Knott, K., Kirkegaard, M., Krey, A., Lunn, N., Messier, F., Obbard, M., Olsen, M. T., Ostertag, S., Peacock, E., Renzoni, A.,

Rigét, F. F., Skaare, J. U., Stern, G., Stirling, I., Taylor, M., Wiig, Ø., Wilson, S., & Aars, J. (2013). What are the toxicological effects of mercury in Arctic biota? *Science of The Total Environment, 443*, 775-790.

Dubois, M., & Hare, L. (2009). Subcellular distribution of cadmium in two aquatic invertebrates: Change over time and relationship to Cd assimilation and loss by a predatory insect. *Environmental Science & Technology, 43*(2), 356-361.

EC and EPA. (2009). *State of the Great Lakes 2009* (Report No. EPA 905-R-09-031). Environment Canada and the U.S. Environmental Protection Agency (EPA), Niagara Falls, Ontario. Retrieved from https://binational.net/wp-content/uploads/2014/11/En161-3-1-2009E.pdf

Eljarrat, E., & Barcelo, D. (2003). Priority lists for persistent organic pollutants and emerging contaminants based on their relative toxic potency in environmental samples. *TrAc-Trends in Analytical Chemistry, 22*(10), 655-665.

Elliott, K. H., Davis, M., & Elliott, J. E. (2014). Equations for lipid normalization of carbon stable isotope ratios in aquatic bird eggs. *PloS one, 9*(1), e83597-e83597.

EPA. (1996). *Federal register Vol. 61, No. 23. p. 3832*. U.S. Environmental Protection Agency (EPA). Retrieved from https://www.govinfo.gov/content/pkg/FR-1996-02-02/pdf/96-2231.pdf.

Ewins, P. J., Weseloh, D. V., Groom, J. H., Dobos, R. Z., & Mineau, P. (1994). The diet of herring gulls (*Larus argentatus*) during winter and early spring on the lower Great Lakes. *Hydrobiologica, 279*(1), 39-55.

Fisk, A. T., Norstrom, R. J., Cymbalisty, C. D., & Muir, D. C. G. (1998). Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environmental Toxicology and Chemistry, 17*(5), 951-961.

Fox, G. A., Gillman, A. P., Peakall, D. B., & Anderka, F. W. (1978). Behavioral abnormalities of nesting Lake Ontario herring gulls. *Journal of Wildlife Management, 42*(3), 477-483.

Fox, G. A., Jeffrey, D. A., Williams, K. S., Kennedy, S. W., & Grasman, K. A. (2007). Health of herring gulls (*Larus argentatus*) in relation to breeding location in the early 1990s. I. Biochemical measures. *Journal of Toxicology and Environmental Health, Part A, 70*(17), 1443-1470.

Friberg, L. T., Piscator, M., Nordberg, G. F., & Kjellstrom, T. (1974). *Cadmium in the environment II*. Cleveland, OH: CRC Press

Furness, R. W., & Camphuysen, K. (1997). Seabirds as monitors of the marine environment. *ICES Journal of Marine Science, 54*(4), 726-737.

Gebbink, W., Letcher, R., Hebert, C., & Weseloh, D. (2011). Twenty years of temporal change in perfluoroalkyl sulfonate and carboxylate contaminants in herring gull eggs from the Laurentian Great Lakes. *Journal of Environmental Monitoring, 13*(12), 3365-3372.

GESAMP. (1985). *Cadmium, lead and tin in the marine environment* (Report No. 56). GESAMP/IMO/FAO/UNESCO/WHO/IAEA/UN/UNEP, joint group of experts on the scientific aspects of marine pollution, Geneva, Switzerland. Retrieved from http://www.ais.unwater.org/ais/aiscm/getprojectdoc.php?docid=4060

Gilbertson, M., & Hale, R. (1974). Early embryonic mortality in a herring gull colony in Lake Ontario. *Canadian Field-Naturalist, 88*(3), 354-356.

Gilbertson, M., & Fox, G. A. (1977). Pollutant associated embryonic mortality of Great Lakes herring gulls. *Environmental Pollution, 12*, 211-216.

Gilman, A. P., Fox, G. A., Peakall, D. B., Teeple, S. M., Carroll, T. R., & Haymes, G. T. (1977). Reproductive parameters and egg contaminant levels of Great Lakes herring gulls. *Journal of Wildlife Management, 41*(3), 458-468.

González-Solís, J., Oro, D., Jover, L., Ruiz, X., & Pedrocchi, V. (1997). Trophic niche width and overlap of two sympatric gulls in the Southwestern Mediterranean. *Oecologia, 112*(1), 75-80.
Goyer, R. A. (1989). Mechanisms of lead and cadmium nephrotoxicity. *Toxicology Letters, 46*(1-3), 153-162.
Greenacre, M., & Primicerio, R. (2013). Ecological data and multivariate methods: The four corners of multivariate analysis. *Multivariate Analysis of Ecological Data* (pp. 25-32). Bilbao, Spain: Fundación BBVA.
Hartwig, A. (2013). Cadmium and cancer. In A. Sigel, H. Sigel, & R. K. O. Sigel (Eds.), *Cadmium: From toxicity to essentiality* (pp. 491-507). Dordrecht, Netherlands: Springer International Publishing.
Haukås, M., Berger, U., Hop, H., Gulliksen, B., & Gabrielsen, G. W. (2007). Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environmental Pollution, 148*(1), 360-371.
Hebert, C. E., Norstrom, R. J., & Weseloh, D. V. C. (2000). A quarter century of environmental surveillance: the Canadian wildlife service's Great Lakes herring gull monitoring programme. *Environmental Reviews, 7*(4), 147-166.
Hebert, C. E., Weseloh, D. V., Gauthier, L. T., Arts, M. T., & Letcher, R. J. (2009). Biochemical tracers reveal intra-specific differences in the food webs utilized by individual seabirds. *Oecologia, 160*(1), 15-23.
Heinz, G. H., & Hoffman, D. J. (2004). Mercury accumulation and loss in mallard eggs. *Environmental Toxicology and Chemistry, 23*(1), 222-224.
Helgason, L. B., Barrett, R., Lie, E., Polder, A., Skaare, J. U., & Gabrielsen, G. W. (2008). Levels and temporal trends (1983-2003) of persistent organic pollutants (POPs) and mercury (Hg) in seabird eggs from northern Norway. *Environmental Pollution, 155*(1), 190-198.
Helsel, D. R. (2005). More than obvious: Better methods for interpreting nondetect data. *Environmental Science & Technology, 39*(20), 419A-423A.
Helsel, D. R. (2006). Fabricating data: How substituting values for nondetects can ruin results, and what can be done about it. *Chemosphere, 65*(11), 2434-2439.
Helsel, D. R. (2010). Much ado about next to nothing: Incorporating nondetects in science. *The Annals of Occupational Hygiene, 54*(3), 257-262.
Herzke, D., Nygård, T., Berger, U., Huber, S., & Røv, N. (2009). Perfluorinated and other persistent halogenated organic compounds in European shag (*Phalacrocorax aristotelis*) and common eider (*Somateria mollissima*) from Norway: A suburban to remote pollutant gradient. *Science of The Total Environment, 408*(2), 340-348.
Hitchcock, D. J., Andersen, T., Varpe, Ø., & Borgå, K. Effect of reproductive investment on sex-specific pollutant accumulation in seabirds: A meta-analysis. Manuscript submitted for publication.
Hobson, K. A., & Clark, R. G. (1992). Assessing avian diets using stable isotopes I: Turnover of 13C in tissues. *The Condor: Ornithological Applications, 94*(1), 181-188.
Hobson, K. A. (1995). Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: Patterns of isotopic fractionation and turnover. *The Condor: Ornithological Applications, 97*(3), 752-762.
Hobson, K. A., Jaatinen, K., & Öst, M. (2015). Differential contributions of endogenous and exogenous nutrients to egg components in wild Baltic common eiders (*Somateria mollissima*): a test of alternative stable isotope approaches. *The Auk, 132*(3), 624-633.
Huber, S., Warner, N. A., Nygard, T., Remberger, M., Harju, M., Uggerud, H. T., Kaj, L., & Hanssen, L. (2015). A broad cocktail of environmental pollutants found in eggs of three

seabird species from remote colonies in Norway. *Environmental Toxicology and Chemistry, 34*(6), 1296-1308.
ITRC. (2018). Naming conventions and physical and chemical properties of per- and polyfluoroalkyl substances (PFAS). *PFAS fact sheets.* Retrieved from https://www.itrcweb.org. Accessed April 10 2019.
Jakob, E. M., Marshall, S. D., & Uetz, G. W. (1996). Estimating fitness: A comparison of body condition indices. *Oikos, 77*(1), 61-67.
Jarman, W. M., Hobson, K. A., Sydeman, W. J., Bacon, C. E., & McLaren, E. B. (1996). Influence of trophic position and feeding location on contaminant levels in the Gulf of the Farallones food web revealed by stable isotope analysis. *Environmental Science & Technology, 30*(2), 654-660.
Johansen, P., Hansen, M. M., Asmund, G., & Nielsen, P. B. (1991). Marine organisms as indicators of heavy metal pollution - Experience from 16 years of monitoring at a lead zinc mine in Greenland. *Chemistry and Ecology, 5*(1-2), 35-55.
Jones, K. C., & de Voogt, P. (1999). Persistent organic pollutants (POPs): State of the science. *Environmental Pollution, 100*(1-3), 209-221.
Jones, P. D., Hu, W., De Coen, W., Newsted, J. L., & Giesy, J. P. (2003). Binding of perfluorinated fatty acids to serum proteins. *Environmental Toxicology and Chemistry, 22*(11), 2639-2649.
Järup, L., Berglund, M., G., E. C., Nordberg, G. F., & M, V. (1998). Health effects of cadmium exposure - A review of the literature and a risk estimate. *Scandinavian Journal of Work, Environment and Health, 24*, 1-51.
Kallenborn, R., & Berger, U. (2004). *Perfluorinated alkylated substances (PFAS) in the nordic environment*. Copenhagen, Danmark: Nordic Council of Ministers
Keilen, E. K. (2017). *Levels and effects of environmental contaminants in herring gull (Larus argentatus) from an urban and a rural colony in Norway.* (Master's thesis), University of Oslo, Oslo, Norway.
Keith, J. A. (1966). Reproduction in a population of herring gulls (*Larus argentatus*) contaminated by DDT. *Journal of Applied Ecology, 3*.
Kelly, B. C., Ikonomou, M. G., Blair, J. D., Morin, A. E., & Gobas, F. A. P. C. (2007). Food web–specific biomagnification of persistent organic pollutants. *Science, 317*(5835), 236-239.
Kelly, B. C., Ikonomou, M. G., Blair, J. D., Surridge, B., Hoover, D., Grace, R., & Gobas, F. A. P. C. (2009). Perfluoroalkyl contaminants in an Arctic marine food web: Trophic magnification and wildlife exposure. *Environmental Science & Technology, 43*(11), 4037-4043.
KEMI. (2015). *Occurence and use of highly fluorinated substances and alternatives.* (Report No. 7/15). Swedish Chemical Agency (KEMI). Retrieved from https://www.kemi.se/en/global/rapporter/2015/report-7-15-occurrence-and-use-of-highly-fluorinated-substances-and-alternatives.pdf
Landolph, J. R. (1994). Molecular mechanisms of transformation of C3H/10T1/2 C1 8 mouse embryo cells and diploid human fibroblasts by carcinogenic metal compounds. *Environmental Health Perspectives, 102*(3), 119-125.
Lapworth, D. J., Baran, N., Stuart, M. E., & Ward, R. S. (2012). Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environmental Pollution, 163*, 287-303.
Leat, E. H. K., Bourgeon, S., Hanssen, S. A., Petersen, A., Strøm, H., Bjørn, T. H., Gabrielsen, G. W., Bustnes, J. O., Furness, R. W., Haarr, A., & Borgå, K. (2019). The effect of long-range transport, trophic position and diet specialization on legacy

contaminant occurrence in great skuas, *Stercorarius skua*, breeding across the Northeast Atlantic. *Environmental Pollution, 244*, 55-65.
Lehnherr, I. (2014). Methylmercury biogeochemistry: A review with special reference to Arctic aquatic ecosystems. *Environmental Reviews, 22*(3), 229-243
Letcher, R. J., Bustnes, J. O., Dietz, R., Jenssen, B. M., Jørgensen, E. H., Sonne, C., Verreault, J., Vijayan, M. M., & Gabrielsen, G. W. (2010). Exposure and effects assessment of persistent organohalogen contaminants in Arctic wildlife and fish. *Science of The Total Environment, 408*(15), 2995-3043.
Lipnick, R. L., Hermens, J. L. M., Jones, K. C., & Muir, D. C. G. (2001). History of persistent, bioaccumulative, and toxic chemicals. In *Persistent, bioaccumulative and toxic chemicals I. Fate and exposure* (Vol. 772, pp. 1-12). Washington, DC: American Chemical Society.
Lu, Z., Martin, P. A., Burgess, N. M., Champoux, L., Elliott, J. E., Baressi, E., De Silva, A. O., de Solla, S. R., & Letcher, R. J. (2017). Volatile methylsiloxanes and organophosphate esters in the eggs of european starlings (*Sturnus vulgaris*) and congeneric gull species from locations across Canada. *Environmental Science & Technology, 51*(17), 9836-9845.
Lubran, M. M. (1980). Lead toxicity and heme biosynthesis. *Annals of Clinical and Laboratory Science, 10*(5), 402-413.
McFarland, V. A., & Clarke, J. U. (1989). Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: Considerations for a congener-specific analysis. *Environmental Health Perspectives, 81*, 225-239.
Meillère, A., Brischoux, F., Bustamante, P., Michaud, B., Parenteau, C., Marciau, C., & Angelier, F. (2016). Corticosterone levels in relation to trace element contamination along an urbanization gradient in the common blackbird (*Turdus merula*). *Science of The Total Environment, 566-567*, 93-101.
Melnes, M., & Mariussen, E. (2017). *Perfluorerte alkylerte stoffer (PFAS) - En litteraturstudie om PFAS i akvatisk miljø, effekter og kunnskapshull [In Norwegian]* (Report No. 17/00531). Forsvarets forskningsinstitutt (FFI), Kjeller, Norway. Retrieved from https://www.ffi.no/no/Rapporter/17-00531.pdf
Mizutani, H., Kabaya, Y., & Wada, E. (1991). Nitrogen and carbon isotope compositions relate linearly in cormorant tissues and its diet. *Isotopenpraxis Isotopes in Environmental and Health Studies, 27*(4), 166-168.
Morrissey, C. A., Elliott, J. E., & Ormerod, S. J. (2010). Diet shifts during egg laying: Implications for measuring contaminants in bird eggs. *Environmental Pollution, 158*(2), 447-454.
Ng, C. A., & Hungerbühler, K. (2014). Bioaccumulation of perfluorinated alkyl acids: Observations and models. *Environmental Science & Technology, 48*(9), 4637-4648.
Norwegian Environment Agency. (2017, 09 June). Siloksaner [In Norwegian]. Retrieved from https://www.miljostatus.no/siloksaner. Accessed May 15 2019.
Norwegian Environment Agency. (2018, 15 Jan). Klorerte parafiner (SCCP and MCCP) [In Norwegian]. Retrieved from https://www.miljostatus.no/klorerte-parafiner. Accessed May 15 2019.
OECD. (2018). *Toward a new comprehensuve global database og per- and polyluoroalkyl substances (PFASs): Summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances (PFASs)* (Report No.39). Organisation for Economic Co-operation and Development (OECD), Paris, France. Retrieved from https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-JM-MONO(2018)7&doclanguage=en
Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Simpson, G. L., Solymos, P., Stevens, M. H., Eduard, S., & Wagner, H. (2019). Vegan: Community ecology package.

Palmer, M. W., McGlinn, D. J., Westerberg, L., & Milberg, P. (2008). Indices for detecting differences in species composition: Some simplifications of RDA and CCA. *Ecology, 89*(6), 1769-1771.

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics, 18*, 293-320.

Pitt, J. J. (2009). Principles and applications of liquid chromatography - Mass spectrometry in clinical biochemistry. *The Clinical Biochemist Reviews, 30*(1), 19-34.

Powell, D. E., Schøyen, M., Øxnevad, S., Gerhards, R., Böhmer, T., Koerner, M., Durham, J., & Huff, D. W. (2018). Bioaccumulation and trophic transfer of cyclic volatile methylsiloxanes (cVMS) in the aquatic marine food webs of the Oslofjord, Norway. *Science of The Total Environment, 622-623*, 127-139.

Quillfeldt, P., Masello, J. F., McGill, R. A., Adams, M., & Furness, R. W. (2010). Moving polewards in winter: A recent change in the migratory strategy of a pelagic seabird? *Frontiers in Zoology, 7*(15), 1-11.

REACH. (2019). Candidate list of substances of very high concern for authorisation. Retrieved from https://echa.europa.eu/web/guest/candidate-list-table. Accessed April 26 2019.

Riget, F., Bignert, A., Braune, B., Stow, J., & Wilson, S. (2010). Temporal trends of legacy POPs in Arctic biota, an update. *Science of the Total Environment, 408*(15), 2874-2884.

Ringmerking.no. (2019). Colour ringing [In Norwegian]. Retrieved from https://www.ringmerking.no/cr/. Accessed April 12 2019.

Russell, R. W., Gobas, F. A. P. C., & Haffner, G. D. (1999). Maternal transfer and in ovo exposure of organochlorines in oviparous organisms: A model and field verification. *Environmental Science & Technology, 33*(3), 416-420.

Ruus, A., Ugland, K. I., & Skaare, J. U. (2002). Influence of trophic position on organochlorine concentrations and compositional patterns in a marine food web. *Environmental Toxicology and Chemistry, 21*(11), 2356-2364.

Ruus, A., Allan, I., Beylich, B., Bæk, K., Schlabach, M., & Helberg, M. (2015). *Environmental Contaminants in an Urban Fjord, 2014* (Report No. 6684-2015). The Norwegian Institute of Water Research (NIVA), Oslo, Norway. Retrieved from https://www.miljodirektoratet.no/globalassets/publikasjoner/m375/m375-komplett-liten.pdf

Ruus, A., Bæk, K., Petersen, K., Allan, I., Beylich, B., Schlabach, M., Warner, N., & Helberg, M. (2016). *Environmental Contaminants in an Urban Fjord, 2015* (Report No. 7073-2016). The Norwegian Institute of Water Research (NIVA), Oslo, Norway. Retrieved from https://www.miljodirektoratet.no/globalassets/publikasjoner/m601/m601.pdf

Ruus, A., Bæk, K., Petersen, K., Allan, I., Beylich, B., Schlabach, M., Warner, N., Borgå, K., & Helberg, M. (2017). *Environmental Contaminants in an Urban Fjord, 2016* (Report No. 7199-2017). The Norwegian Institute of Water Research (NIVA), Oslo, Norway. Retrieved from https://www.miljodirektoratet.no/globalassets/publikasjoner/m812/m812.pdf

Ruus, A., Bæk, K., Petersen, K., Allan, I., Beylich, B., Schlabach, M., Warner, N., Borgå, K., & Helberg, M. (2019). *Environmental Contaminants in an Urban Fjord, 2017* (Report No. 7368-2018). The Norwegian Institute of Water Research (NIVA), Oslo, Norway. Retrieved from https://www.miljodirektoratet.no/globalassets/publikasjoner/m1131/m1131.pdf

Skaar, J. S., Ræder, E. M., Lyche, J. L., Ahrens, L., & Kallenborn, R. (2019). Elucidation of contamination sources for poly- and perfluoroalkyl substances (PFASs) on Svalbard (Norwegian Arctic). *Environmental Science and Pollution Research, 26*(8), 7356-7363.

Sparks, T. H., Scott, W. A., & Clarke, R. T. (1999). Traditional multivariate techniques: Potential for use in ecotoxicology. *Environmental Toxicology and Chemistry, 18*(2), 128-137.

Sundberg, J., Ersson, B., Lönnerdal, B., & Oskarsson, A. (1999). Protein binding of mercury in milk and plasma from mice and man — A comparison between methylmercury and inorganic mercury. *Toxicology, 137*(3), 169-184.

Sweeting, C. J., Polunin, N. V. C., & Jennings, S. (2006). Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry, 20*(4), 595-601.

Sørmo, E. G., Lie, E., Ruus, A., Gaustad, H., Skaare, J. U., & Jenssen, B. M. (2011). Trophic level determines levels of brominated flame-retardants in coastal herring gulls. *Ecotoxicology and Environmental Safety, 74*(7), 2091-2098.

Thorstensen, H. S. (2019). *Comparing contaminant occurence and maternal transfer in herring gull and common eider in the urban inner Oslofjord, Norway.* (Master's thesis), University of Oslo (UiO), Oslo, Norway.

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. *Oecologia, 57*(1), 32-37.

Tymoczko, J. L., Berg, J. M., & Stryer, L. (2015). Chapter 9 - Hemoglobin, an allosteric protein. *Biochemistry: A short course. Third edition* (pp. 149-159). New York, NY: W. H. Freeeman & Company.

UNEP. (2001). *Final act of the conference of plenipotentiaries in the stockholm convention on persistent organic pollutants*. Geneva, Switzerland: United Nations Environment Programme (UNEP). Retrieved from http://chm.pops.int/Portals/0/Repository/convention_text/UNEP-POPS-COP-CONVTEXT-FULL.English.PDF

UNEP. (2009). *Stockholm Convention on Persistent Organic Pollutants (POPs)*. Geneva, Switzerland: United Nations Environment Programme (UNEP) Retrieved from https://www.wipo.int/edocs/lexdocs/treaties/en/unep-pop/trt_unep_pop_2.pdf

Van Wijngaarden, R. P. A., Van Den Brink, P. J., Oude Voshaar, J. H., & Leeuwangh, P. (1995). Ordination techniques for analysing response of biological communities to toxic stress in experimental ecosystems. *Ecotoxicology, 4*(1), 61-77.

Verreault, J., Houde, M., Gabrielsen, G. W., Berger, U., Haukås, M., Letcher, R. J., & Muir, D. C. G. (2005). Perfluorinated alkyl substances in plasma, liver, brain, and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. *Environmental Science & Technology, 39*(19), 7439-7445.

Verreault, J., Villa, R. A., Gabrielsen, G. W., Skaare, J. U., & Letcher, R. J. (2006). Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. *Environmental Pollution, 144*(3), 1053-1060.

Vizuete, J., Pérez-López, M., Míguez-Santiyán, M. P., & Hernández-Moreno, D. (2019). Mercury (Hg), lead (Pb), cadmium (Cd), selenium (Se), and arsenic (As) in liver, kidney, and feathers of gulls: A review. In P. de Voogt (Ed.), *Reviews of environmental contamination and toxicology volume 247* (pp. 85-146). Amsterdam, The Netherlands: Springer International Publishing.

Vogel, J. C., & van der Merwe, N. J. (1977). Isotopic evidence for early maize cultivation in New York state. *American Antiquity, 42*(2), 238-242.

Walker, C. H., Sibly, R. M., Hopkin, S. P., & Peakall, D. B. (2012). Major classes of pollutants. In *Principles of ecotoxicology, fourth edition* (pp. 1-386). Boca Raton, FL: CRC Press.

Wang, D.-G., Norwood, W., Alaee, M., D Byer, J., & Brimble, S. (2013). Review of recent advances in research on the toxicity, detection, occurrence and fate of cyclic volatile methyl siloxanes in the environment. *Chemosphere, 93*(5), 711-725.

Wania, F., & Mackay, D. (1996). Tracking the distribution of persistent organic pollutants. *Environmental Science & Technology, 30*(9), 390A-396A.
Warner, N. A., Evenset, A., Christensen, G., Gabrielsen, G. W., Borgå, K., & Leknes, H. (2010). Volatile siloxanes in the European Arctic: Assessment of sources and spatial distribution. *Environmental Science & Technology, 44*(19), 7705-7710.
Warner, N. A., Sagerup, K., Kristoffersen, S., Herzke, D., Gabrielsen, G. W., & Jenssen, B. M. (2019). Snow buntings (*Plectrophenax nivealis*) as bio-indicators for exposure differences to legacy and emerging persistent organic pollutants from the Arctic terrestrial environment on Svalbard. *Science of The Total Environment, 667*, 638-647.
Wellington Laboratories. (2012). Reference and handling guide: Perfluoroalkyl compounds. Retrieved from https://well-labs.com/docs/pfc_reference_handling_guide.pdf. Accessed April 10 2019.
Whitlock, M., & Schluter, D. (2015). *The analysis of biological data*. Greenwood Village, CO: Roberts and Company Publishers, Inc.
World Population Review. (May 12 2019). Oslo population. Retrieved from http://worldpopulationreview.com/world-cities/oslo-population/. Accessed April 15 2019.

Appendices

Appendix A: Optimized parameters for the Q-ToF-HRMS instrument during PFAS analysis.

Table A1: Optimized parameters used for the The Xevo G2-S Q-ToF-HRMS instrument (Waters).

Parameters	**Settings**
Capillary voltage	0.7 kV
Desolvation temperature	500°C
Source temperature	120°C
Nitrogen desolvation gas flow	800 L/h

Appendix B: Internal standards for lipophilic contaminant analysis.

Table B1: Amount of internal standard added to each glass column during the extraction phase.

Name	Volume (µl)
PCBI	20
PBDEI	20
DBDPE	20
CPI	20
PESTI	50

Appendix C: Number of detected values for each contaminant analysed.

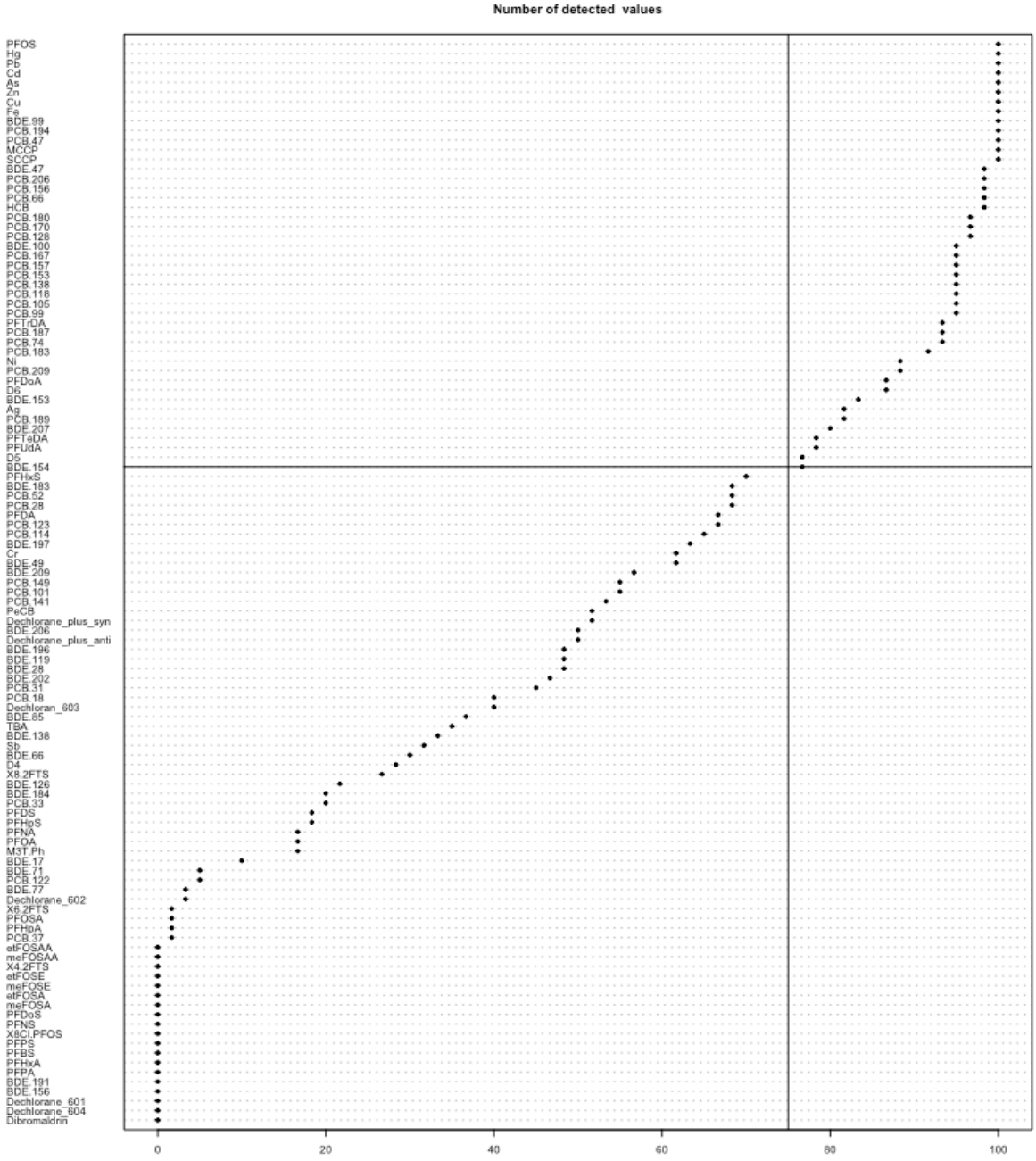

Figure C1. Dot chart representing the number of detected values for all the different contaminants analysed. The cut-off value was set to "the peak", shown by the vertical and horizontal lines at 75%. All compounds with detected values under 75% were removed and not used for further analysis.

Table C1. Number of detected values over LOD from highest to lowest in all the 60 samples taken from herring gull (*Larus argentatus*) females and eggs from the inner and outer Oslofjord. Values under 75% LOD (from 45 detected values and below, i.e. PFHxS) were not used in the statistical analyses.

Contaminant	**Number of detected values over LOD**
PFOS	60
Hg	60
Pb	60
Cd	60
As	60
Zn	60
Cu	60
Fe	60
BDE-99	60
PCB-194	60
PCB-47	60
MCCP	60
SCCP	60
BDE-47	59
PCB-206	59
PCB-156	59
PCB-66	59
HCB	59
PCB-180	58
PCB-170	58
PCB-128	58
BDE-100	57
PCB-167	57
PCB-157	57
PCB-153	57
PCB-138	57
PCB-118	57
PCB-105	57
PCB-99	57
PFTrDA	56
PCB-187	56

Table C1 continued. Number of detected values over LOD from highest to lowest in all the 60 samples taken from herring gull (*Larus argentatus*) females and eggs from the inner and outer Oslofjord. Values under 75% LOD (from 45 detected values and below, i.e. PFHxS) were not used in the statistical analyses.

Contaminant	**Number of detected values over LOD**
PCB-74	56
PCB-183	55
Ni	53
PCB-209	53
PFDoA	52
D6	52
BDE-153	50
Ag	49
PCB-189	49
BDE-207	48
PFUdA	47
PFTeDA	47
D5	46
BDE-154	46
PFHxS	42
PBDE-183	41
PCB-52	41
PCB-28	41
PFDA	40
PCB-123	40
PCB-114	39
BDE-197	38
Cr	37
BDE-49	37
BDE-209	34
PCB-149	33
PCB-101	33
PCB-141	32
PeCB	31
Dechlorane plus syn	31

Table C1 continued. Number of detected values over LOD from highest to lowest in all the 60 samples taken from herring gull (*Larus argentatus*) females and eggs from the inner and outer Oslofjord. Values under 75% LOD (from 45 detected values and below, i.e. PFHxS) were not used in the statistical analyses.

Contaminant	**Number of detected values over LOD**
BDE-206	30
Dechlorane plus anti	30
BDE-196	29
BDE-119	29
BDE-28	29
BDE-202	28
PCB-31	27
PCB-18	24
Dechlorane-603	24
BDE-85	22
TBA	21
BDE-138	20
Sb	19
BDE-66	18
D4	17
X8-2FTS	16
BDE-126	13
BDE-184	12
PCB-33	12
PFDS	11
PFHpS	11
PFNA	10
PFOA	10
M3T-Ph	10
OC	8
BDE-17	6
BDE-71	3
PCB-122	3
BDE-77	2
Dechlorane-602	2

Table C1 continued. Number of detected values over LOD from highest to lowest in all the 60 samples taken from herring gull (*Larus argentatus*) females and eggs from the inner and outer Oslofjord. Values under 75% LOD (from 45 detected values and below, i.e. PFHxS) were not used in the statistical analyses.

Contaminant	**Number of detected values over LOD**
X6-2FTS	1
PFOSA	1
PFHpA	1
PCB-37	1
EHMC	0
BP3	0
etFOSAA	0
meFOSAA	0
X4-2FTS	0
eFOSE	0
meFOSE	0
etFOSA	0
meFOSA	0
PFDoS	0
PFNS	0
X8Cl-PFOS	0
PFPS	0
PFBS	0
PFHxA	0
PFPA	0
BDE-191	0
BDE-156	0
Dechlorane-601	0
Dechlorane-604	0
Dibromaldrin	0

Appendix D: Table of biological data

Table D1. Biometric data for each herring gull (*Larus argentatus*) female from the inner Oslofjord (Skjælholmen) (n=15) and outer Oslofjord (Revlingen) Oslofjord (n=15).

Location	Colour ring	Species	Sex	Wing length (mm)	Weight (g)	Head length (mm)	Bill height (mm)	BCI
Søndre Skjælholmen	J5549	Herring gull	F	432	930	117.4	16.8	33.71
Søndre Skjælholmen	JCL23	Herring gull	F	418	870	120.5	17.8	-60.61
Søndre Skjælholmen	JCL59	Herring gull	F	427	770	110.9	16.3	-42.74
Søndre Skjælholmen	JCL67	Herring gull	F	427	870	115.7	17.2	-3.19
Søndre Skjælholmen	JCL68	Herring gull	F	437	890	120.9	18.9	-52.06
Søndre Skjælholmen	JCL72	Herring gull	F	430	990	117	18.3	99.42
Søndre Skjælholmen	JCP52	Herring gull	F	422	930	117.8	18.4	32.05
Søndre Skjælholmen	JJP01	Herring gull	F	426	885	118.8	16.85	-26.90
Søndre Skjælholmen	JJP03	Herring gull	F	434	910	118.4	17.6	0.44
Søndre Skjælholmen	JJP05	Herring gull	F	436	965	120.8	16.7	24.54
Søndre Skjælholmen	JJP06	Herring gull	F	437	860	117.2	16.7	-35.46
Søndre Skjælholmen	JJP07	Herring gull	F	438	950	120.4	17.7	13.90
Søndre Skjælholmen	JJP18	Herring gull	F	429	830	113.6	NA	-17.42
Søndre Skjælholmen	JJP19	Herring gull	F	429	900	117.4	17.7	4.72
Søndre Skjælholmen	JJP21	Herring gull	F	415	900	115.8	17.1	29.60
Store Revlingen	J884A	Herring gull	F	430	1000	119.7	18.1	91.81
Store Revlingen	JJP33	Herring gull	F	440	NA	NA	NA	NA
Store Revlingen	JJP24	Herring gull	F	440	935	116.5	16.1	-24.61
Store Revlingen	JJP25	Herring gull	F	447	1050	118.2	18.2	58.61
Store Revlingen	JJP27	Herring gull	F	428	900	117.7	17.3	-0.46
Store Revlingen	JJP28	Herring gull	F	429	830	119.2	NA	-73.84
Store Revlingen	JJP32	Herring gull	F	429	925	118.2	17.5	20.19
Store Revlingen	JJP34	Herring gull	F	439	935	119	17.35	-17.36
Store Revlingen	JJP35	Herring gull	F	414	875	113.7	17.7	38.31
Store Revlingen	JJP36	Herring gull	F	440	960	117.8	17.6	1.65
Store Revlingen	JJP39	Herring gull	F	415	820	118.1	17.65	-17.25

Table D1 continued. Biometric data for each herring gull (*Larus argentatus*) female from the inner (Skjælholmen) (n=15) and outer (Revlingen) Oslofjord (n=15).

Location	**Colour ring**	**Species**	**Sex**	**Wing length (mm)**	**Weight (g)**	**Head length (mm)**	**Bill height (mm)**	**BCI**
Store Revlingen	JJP41	Herring gull	F	443	955	115.8	17.5	-19.79
Store Revlingen	JJP42	Herring gull	F	426	845	116.5	18.3	-46.96
Store Revlingen	JJP46	Herring gull	F	438	930	120.7	19.8	-15.88
Store Revlingen	JJP47	Herring gull	F	412	830	116.8	18	5.98

Table D2: Biological measurements showing values for lipid %, egg weight (g), and egg stage in individual eggs from Skjælholmen (n=15) and Revlingen (n=15).

Location	Colour ring	Lipid %	Egg weight (g)	Egg stage
Søndre Skjælholmen	J5549	9.82	88.2	1
Søndre Skjælholmen	JCL23	7.9	62.43	4
Søndre Skjælholmen	JCL59	8.96	79.97	3
Søndre Skjælholmen	JCL67	8.12	84.52	2
Søndre Skjælholmen	JCL68	9.5	86.66	2
Søndre Skjælholmen	JCL72	7.7	85	4
Søndre Skjælholmen	JCP52	7.15	68.39	4
Søndre Skjælholmen	JJP01	8.1	74.21	3
Søndre Skjælholmen	JJP03	6.85	80.75	4
Søndre Skjælholmen	JJP05	6.77	72.86	4
Søndre Skjælholmen	JJP06	8.8	91.48	3
Søndre Skjælholmen	JJP07	9.97	69.47	4
Søndre Skjælholmen	JJP18	7.82	74.52	4
Søndre Skjælholmen	JJP19	6.82	97.78	2
Søndre Skjælholmen	JJP21	5.92	61.8	5
Store Revlingen	J884A	6.25	87.44	4
Store Revlingen	JJP23	7.4	87.82	4
Store Revlingen	JJP24	6.45	80.83	4
Store Revlingen	JJP25	3.98	90.37	5
Store Revlingen	JJP27	7.9	86.94	1
Store Revlingen	JJP28	4.6	88.3	5
Store Revlingen	JJP32	8.25	81.36	3
Store Revlingen	JJP34	5.34	90.25	4
Store Revlingen	JJP35	5.81	75.81	5
Store Revlingen	JJP36	8.6	84.08	3
Store Revlingen	JJP39	7.96	67	3
Store Revlingen	JJP41	9.15	89.15	2
Store Revlingen	JJP42	9.3	80.28	2
Store Revlingen	JJP46	7.1	71.93	5
Store Revlingen	JJP47	8.2	73.54	4

Table D3. Stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and C:N ratios in herring gull (*Larus argentatus*) females and their eggs from the inner (Skjælholmen, n=30) and outer (Revlingen, n=30) Oslofjord.

Fargering	Matrix	Location	$\delta^{13}C$	$\delta^{15}N$	C:N ratio
J5549	Blood	Søndre Skjælholmen	-24.36	10.17	5.02
JCL23	Blood	Søndre Skjælholmen	-24.71	7.74	3.41
JCL59	Blood	Søndre Skjælholmen	-25.18	7.39	4.49
JCL67	Blood	Søndre Skjælholmen	-24.05	8.41	3.49
JCL68	Blood	Søndre Skjælholmen	-24.87	9.09	5.30
JCL72	Blood	Søndre Skjælholmen	-24.27	7.73	3.58
JCP52	Blood	Søndre Skjælholmen	-24.11	8.47	4.00
JJP01	Blood	Søndre Skjælholmen	-24.16	9.06	3.66
JJP03	Blood	Søndre Skjælholmen	-24.68	7.67	3.89
JJP05	Blood	Søndre Skjælholmen	-24.24	8.08	3.93
JJP06	Blood	Søndre Skjælholmen	-24.79	9.19	4.93
JJP07	Blood	Søndre Skjælholmen	-24.38	8.48	4.43
JJP18	Blood	Søndre Skjælholmen	-24.19	8.36	3.71
JJP19	Blood	Søndre Skjælholmen	-24.02	8.70	3.67
JJP21	Blood	Søndre Skjælholmen	-24.74	8.10	3.97
J5549	Egg	Søndre Skjælholmen	-25.15	10.79	8.35
JCL23	Egg	Søndre Skjælholmen	-26.13	7.70	7.94
JCL59	Egg	Søndre Skjælholmen	-26.94	6.92	9.10
JCL67	Egg	Søndre Skjælholmen	-26.37	8.91	8.86
JCL68	Egg	Søndre Skjælholmen	-26.11	9.73	9.00
JCL72	Egg	Søndre Skjælholmen	-25.62	9.26	6.53
JCP52	Egg	Søndre Skjælholmen	-26.03	7.80	7.94
JJP01	Egg	Søndre Skjælholmen	-26.50	8.80	8.17
JJP03	Egg	Søndre Skjælholmen	-25.39	8.97	5.93
JJP05	Egg	Søndre Skjælholmen	-24.73	8.13	6.32
JJP06	Egg	Søndre Skjælholmen	-26.23	9.81	6.88
JJP07	Egg	Søndre Skjælholmen	-26.85	8.27	8.85
JJP18	Egg	Søndre Skjælholmen	-24.67	10.82	6.57
JJP19	Egg	Søndre Skjælholmen	-25.35	8.93	6.23

Table D3 continued. Stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and C:N ratios in herring gull (*Larus argentatus*) females and their eggs from the inner (Skjælholmen, n=30) and outer (Revlingen, n=30) Oslofjord.

Fargering	**Matrix**	**Location**	**$\delta^{13}C$**	**$\delta^{15}N$**	**C:N ratio**
JJP21	Egg	Søndre Skjælholmen	-24.81	8.94	4.85
J884A	Blood	Store Revlingen	-21.85	12.27	3.49
JJP23	Blood	Store Revlingen	-24.58	8.81	3.38
JJP24	Blood	Store Revlingen	-23.32	9.63	3.25
JJP25	Blood	Store Revlingen	-22.26	10.58	3.40
JJP27	Blood	Store Revlingen	-24.76	9.66	3.46
JJP28	Blood	Store Revlingen	-23.72	9.63	3.45
JJP32	Blood	Store Revlingen	-24.09	9.99	3.48
JJP34	Blood	Store Revlingen	-23.77	10.45	3.43
JJP35	Blood	Store Revlingen	-24.96	9.34	3.34
JJP36	Blood	Store Revlingen	-24.21	9.18	3.37
JJP39	Blood	Store Revlingen	-24.14	9.56	3.36
JJP41	Blood	Store Revlingen	-22.08	12.69	3.26
JJP42	Blood	Store Revlingen	-24.78	9.64	3.52
JJP46	Blood	Store Revlingen	-23.58	10.20	3.41
JJP47	Blood	Store Revlingen	-24.70	8.55	3.43
J884A	Egg	Store Revlingen	-24.02	11.83	6.60
JJP23	Egg	Store Revlingen	-26.61	8.83	7.85
JJP24	Egg	Store Revlingen	-25.17	9.96	5.67
JJP25	Egg	Store Revlingen	-22.97	12.68	5.70
JJP27	Egg	Store Revlingen	-25.96	10.54	6.86
JJP28	Egg	Store Revlingen	-23.86	9.83	5.20
JJP32	Egg	Store Revlingen	-25.71	11.06	7.77
JJP34	Egg	Store Revlingen	-23.89	10.17	4.95

Table D3 continued. Stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and C:N ratios in herring gull (*Larus argentatus*) females and their eggs from inner (Skjælholmen, n=30) and outer (Revlingen, n=30) Oslofjord.

Fargering	**Matrix**	**Location**	**$\delta^{13}C$**	**$\delta^{15}N$**	**C:N ratio**
JJP35	Egg	Store Revlingen	-24.41	10.80	4.81
JJP36	Egg	Store Revlingen	-26.01	11.98	8.16
JJP39	Egg	Store Revlingen	-25.53	10.24	6.63
JJP41	Egg	Store Revlingen	-23.30	14.37	7.36
JJP42	Egg	Store Revlingen	-25.80	9.63	7.02
JJP46	Egg	Store Revlingen	-25.11	10.62	6.22
JJP47	Egg	Store Revlingen	-26.32	10.79	8.33

Appendix E: Principal component analysis and redundancy analysis

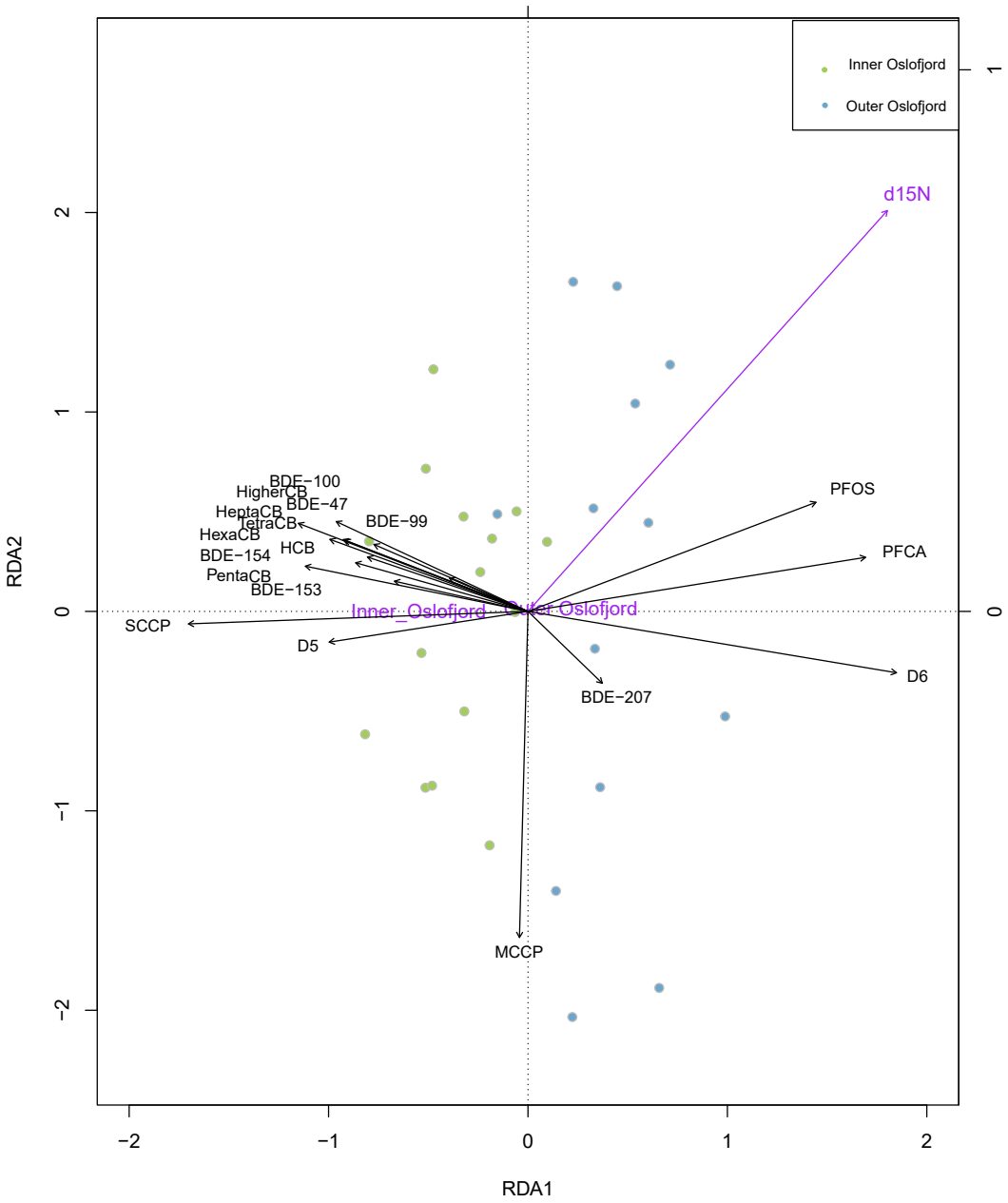

Figure E1. RDA showing the explanatory environmental variables location, $\delta^{13}C$ and $\delta^{15}N$. The points in the plot are the individual adult herring gulls (*Larus argentatus*). The black arrows are the contaminants, while the purple arrows are the explanatory variables.

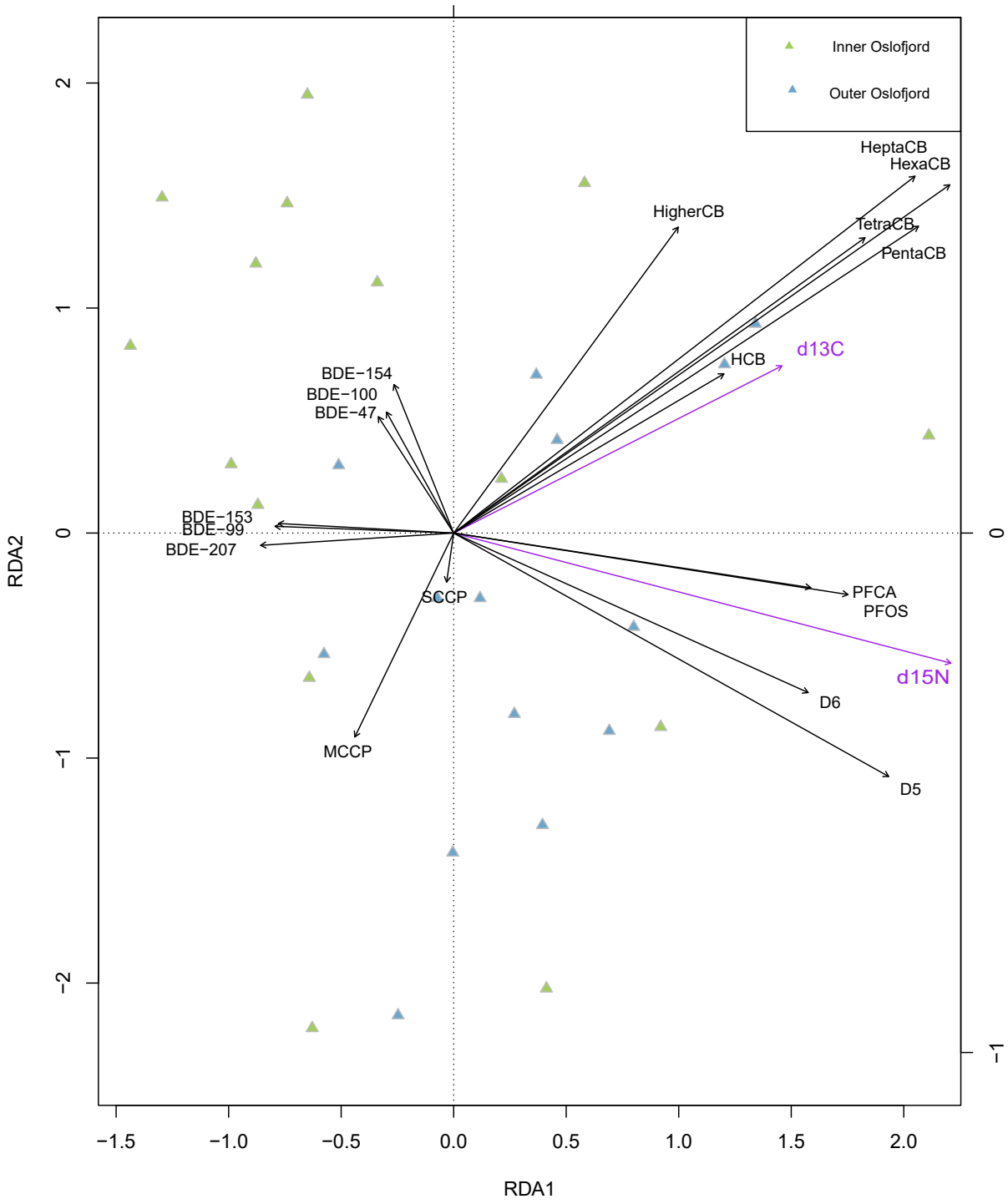

Figure E2. RDA showing the explanatory environmental variables δ13C and δ15N. The points in the plot are the individual adult herring gulls (*Larus argentatus*). The black arrows are the contaminants, while the purple arrows are the explanatory variables.

a) Concentration of metals in adult herring gulls

b) Concentration of metals in herring gull eggs

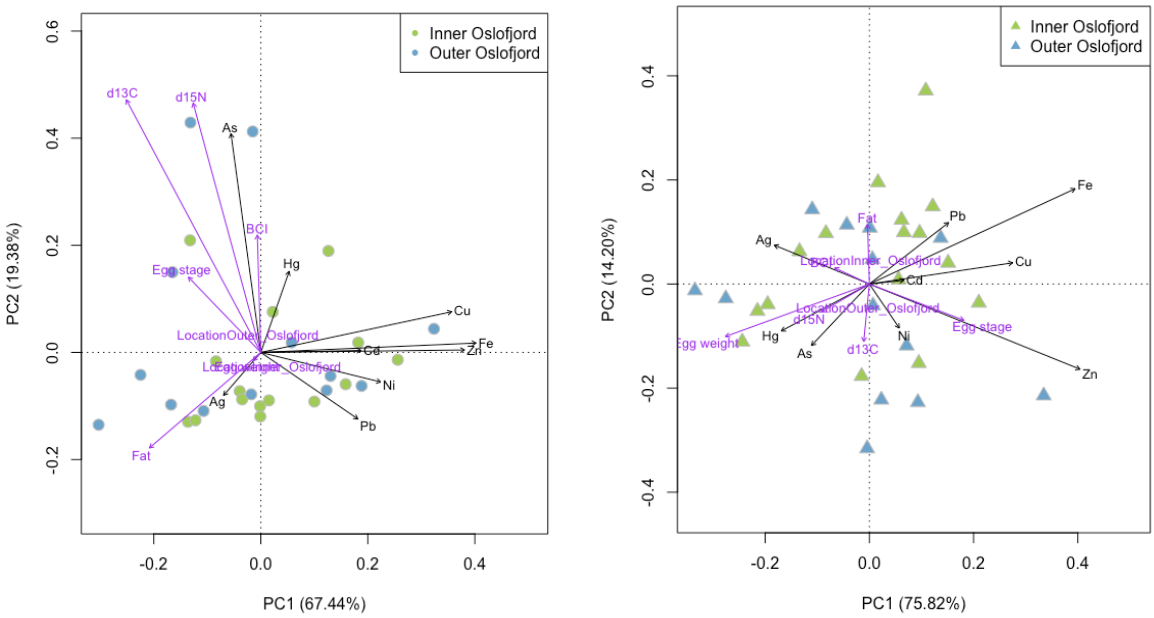

Figure E3. Principal component analysis showing the concentrations of the metals As, Hg, Cu, Cd, Fe, Zn, Ni, Ag, and Pb (log10-transformed concentrations, µg/g ww) in a) adult female herring gulls (*Larus argentatus*) (n = 30) and b) herring gull eggs (n = 30). The herring gull individuals are represented as points in the plot, where the green points represent the gulls from the inner fjord and the blue points are the gulls from the outer fjord. Response loadings (contaminants) are represented as black arrows, and explanatory passive vectors as purple arrows. Being passive, the explanatory variables did not influence the sample scores or the response variables. The direction and the length of the arrows indicate increasing concentration and amount of variation, respectively. The degree of correlation is indicated by the cosine of the angle between the arrows, where the smaller the cosine, the higher the correlation between the variables. Arrows pointing in the same direction are positively correlated, arrows pointing in the opposite direction are negatively correlated, and arrows orthogonal to each other are uncorrelated.

Appendix F: Contaminant levels of individual contaminants

Table F1. Mean ± standard deviation (SD) and range (Min – Max) organic contaminant concentrations (ng/g ww) in herring gull (*Larus argentatus*) females and their eggs from the inner and outer Oslofjord.

	Inner Oslofjord		**Outer Oslofjord**	
Mean ± SD **Min – Max**	**Females n=15**	**Egg n=15**	**Females n=15**	**Egg n=15**
PCB-47	0.25 ± 0.18 0.05 – 0.60	3.59 ± 3.78 0.18 – 14.70	0.85 ± 2.56 0.04 – 10.10	2.70 ± 2.53 0.43 – 8.89
PCB-66	0.83 ± 0.97 0.07 – 3.63	7.98 ± 10.65 0.27 – 37.10	3.47 ± 11.11 0.11 – 43.60	4.55 ± 4.70 0.60 – 17.20
PCB-74	0.46 ± 0.52 0.09 – 2.00	4.26 ± 5.48 0.16 – 19.30	1.45 ± 4.23 0.04 – 16.70	2.75 ± 2.83 0.35 – 10.10
PCB-99	1.24 ± 0.93 0.29 – 3.40	17.74 ± 17.44 1.39 – 70.00	4.20 ± 12.96 0.19 – 51.00	13.76 ± 11.62 2.49 – 40.30
PCB-105	0.62 ± 0.51 0.02 – 1.55	9.30 ± 10.45 0.47 – 40.40	2.03 ± 6.42 0.08 – 25.20	6.08 ± 5.62 1.06 – 19.40
PCB-118	1.84 ± 1.46 0.35 – 5.26	26.00 ± 26.12 1.74 – 103.00	5.00 ± 15.15 0.31 – 59.70	17.92 ± 15.34 3.49 – 53.60
PCB-128	0.48 ± 0.40 0.12 – 1.73	6.69 ± 5.28 0.65 – 21.70	1.42 ± 4.04 0.07 – 16.00	5.81 ± 4.76 1.08 – 17.30
PCB-138	3.70 ± 3.36 0.76 – 14.50	46.56 ± 36.24 5.69 – 142.00	8.40 ± 22.09 0.60 – 88.00	39.61 ± 30.61 8.40 – 111.00
PCB-153	5.75 ± 5.37 1.71 – 23.00	73.31 ± 55.51 9.53 – 204.00	11.35 ± 28.24 1.00 – 113.00	64.93 ± 48.02 15.20 – 177.00
PCB-156	0.23 ± 0.19 0.06 – 0.80	3.16 ± 2.36 0.41 – 9.55	0.58 ± 1.64 0.04 – 6.50	2.44 ± 1.91 0.55 – 6.77
PCB-157	0.05 ± 0.05 0.02 – 0.20	0.69 ± 0.54 0.07 – 2.11	0.13 ± 0.38 0.01 – 1.52	0.55 ± 0.43 0.11 – 1.56
PCB-167	0.14 ± 0.15 0.03 – 0.62	1.74 ± 1.45 0.20 – 5.23	0.33 ± 0.97 0.02 – 3.82	1.82 ± 0.99 0.33 – 3.54
PCB-170	0.45 ± 0.38 0.13 – 1.63	4.20 ± 2.56 0.53 – 10.10	1.20 ± 3.62 0.06 – 14.30	4.39 ± 3.57 0.89 – 13.50
PCB-180	1.37 ± 1.01 0.50 – 4.43	19.84 ± 11.78 3.31 – 43.90	4.68 ± 14.48 0.22 – 57.00	15.26 ± 10.70 4.13 – 40.00
PCB-183	0.26 ± 0.15 0.09 – 0.59	6.28 ± 4.32 0.90 – 17.90	1.02 ± 2.80 0.07 – 11.10	4.33 ± 3.16 1.12 – 12.20

Table F1 continued. Mean ± standard deviation (SD) and range (Min – Max) organic contaminant concentrations (ng/g ww) in herring gull (*Larus argentatus*) females and their eggs from Skjælholmen and Revlingen.

	Inner Oslofjord		**Outer Oslofjord**	
Mean ± SD **Min – Max**	**Females n=15**	**Egg n=15**	**Females n=15**	**Egg n=15**
PCB-187	0.81 ± 0.56 0.19 – 2.48	18.27 ± 12.60 4.12 – 50.20	2.84 ± 7.95 0.20 – 31.50	12.71 ± 8.22 3.19 – 28.80
PCB-189	0.03 ± 0.03 0.01 – 0.14	0.39 ± 0.26 0.08 – 0.88	0.08 ± 0.21 0.01 – 0.82	0.28 ± 0.20 0.08 – 0.77
PCB-194	0.21 ± 0.16 0.07 – 0.65	4.46 ± 3.34 0.91 – 12.80	0.59 ± 1.77 0.03 – 6.98	2.31 ± 1.15 0.68 – 4.89
PCB-206	0.08 ± 0.08 0.03 – 0.32	0.93 ± 0.73 0.19 – 2.91	0.26 ± 0.80 0.01 – 3.16	0.61 ± 0.49 0.14 – 1.91
PCB-209	0.07 ± 0.09 0.01 – 0.32	0.68 ± 0.73 0.09 – 2.35	0.15 ± 0.46 0.01 – 1.80	0.54 ± 0.43 0.13 – 1.45
BDE-47	0.33 ± 0.29 0.09 – 1.00	6.14 ± 8.68 0.36 – 34.30	0.51 ± 1.20 0.06 – 4.83	3.13 ± 2.08 0.51 – 7.86
BDE-99	0.26 ± 0.46 0.04 – 1.81	6.75 ± 15.06 0.33 – 53.90	0.18 ± 0.27 0.04 – 1.14	1.56 ± 1.03 0.46 – 3.66
BDE-100	0.10 ± 0.10 0.02 – 0.31	1.71 ± 2.43 0.12 – 9.80	0.11 ± 0.23 0.01 – 0.93	0.91 ± 0.61 0.15 – 2.35
BDE-153	0.07 ± 0.09 0.01 – 0.35	1.84 ± 3.68 0.10 – 14.00	0.10 ± 0.29 0.01 – 1.13	0.46 ± 0.26 0.17 – 1.15
BDE-154	0.04 ± 0.04 0.01 – 0.13	0.62 ± 0.75 0.06 – 2.82	0.09 ± 0.30 0.01 – 1.17	0.31 ± 0.23 0.07 – 0.91
BDE-207	0.10 ± 0.14 0.01 – 0.57	1.95 ± 3.97 0.07 – 14.00	0.10 ± 0.18 0.02 – 0.64	0.41 ± 0.37 0.03 – 1.49
PFUdA	0.41 ± 0.11 0.27 – 0.66	0.60 ± 0.33 0.28 – 1.51	1.15 ± 0.41 0.47 – 2.10	1.00 ± 0.60 0.21 – 2.46
PFDoA	0.85 ± 0.73 0.21 – 3.01	0.87 ± 1.05 0.10 – 2.42	1.41 ± 0.73 0.59 – 2.89	1.09 ± 1.47 0.15 – 6.26
PFTrDA	0.72 ± 0.24 0.32 – 1.14	1.06 ± 0.69 0.37 – 2.77	1.64 ± 0.65 0.40 – 3.33	1.76 ± 1.24 0.42 - 5.51
PFTeDA	0.66 ± 0.50 0.15 – 2.16	1.00 ± 0.86 0.36 – 3.61	1.02 ± 0.63 0.38 – 2.52	1.26 ± 1.51 0.38 – 6.49

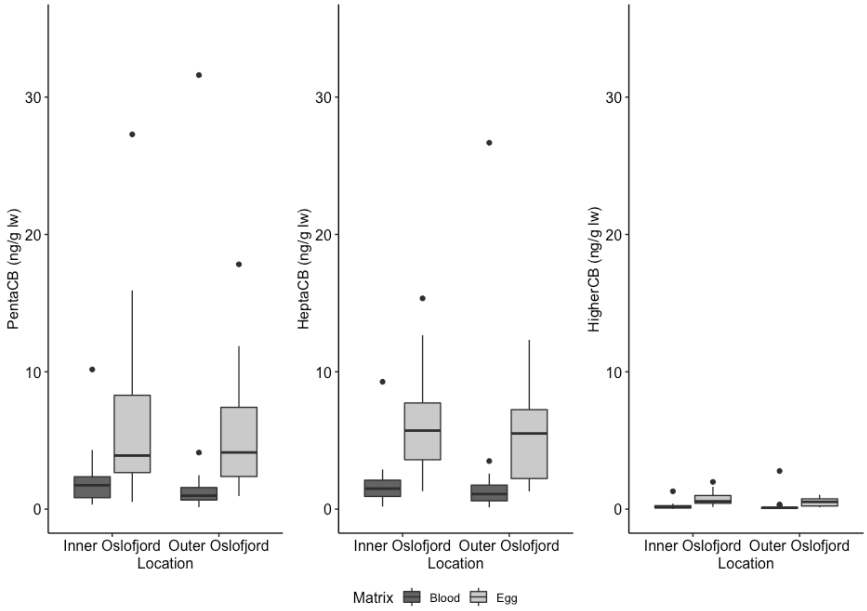

Figure F1. Concentrations of three of the PCB groups: PentaCB, HeptaCB, and HigherCB (ng/g lw) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. Note different scales.

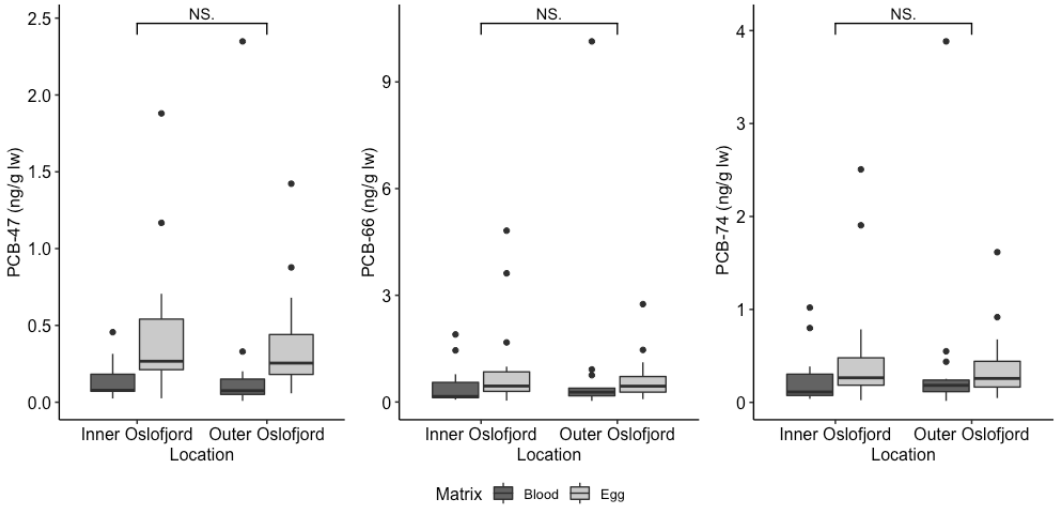

Figure F2. Concentrations of the different PCB congeners (ng/g lw) in TetraCB (PCB-47, -66, -74) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. Note different scales. The bar overlying the boxplots in each plot shows whether there is a significant difference between the locations or not, where "NS" means "not significant".

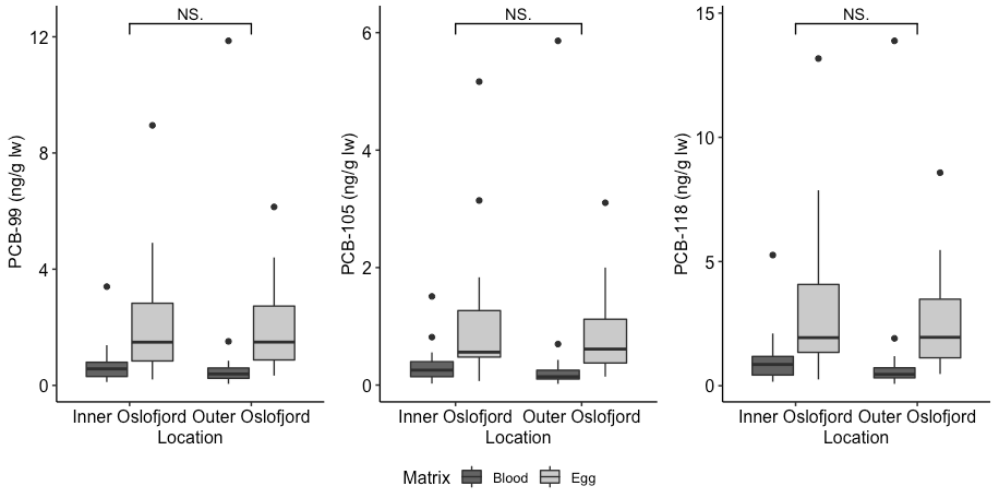

Figure F3. Concentrations of the different PCB congeners (ng/g lw) in PentaCB (PCB-99, -105, and -118) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. Note different scales. The bar overlying the boxplots in each plot shows whether there is a significant difference between the locations or not, where "NS" means "not significant".

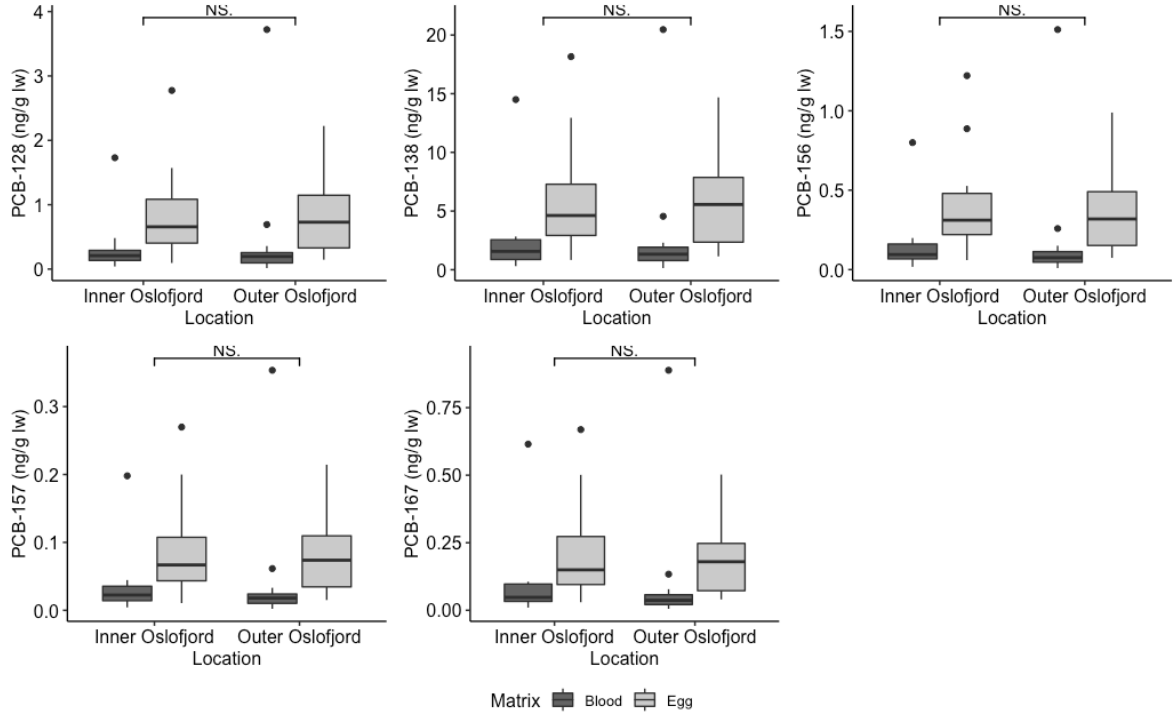

Figure F4. Concentrations of the different PCB congeners (ng/g lw) in HexaCB (PCB-128, -138, -156, -157, and -167) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values in each group. Note different scales on the axes. The bar overlying the boxplots in each plot shows whether there is a significant difference between the colonies (locations) or not, where "NS" means "not significant".

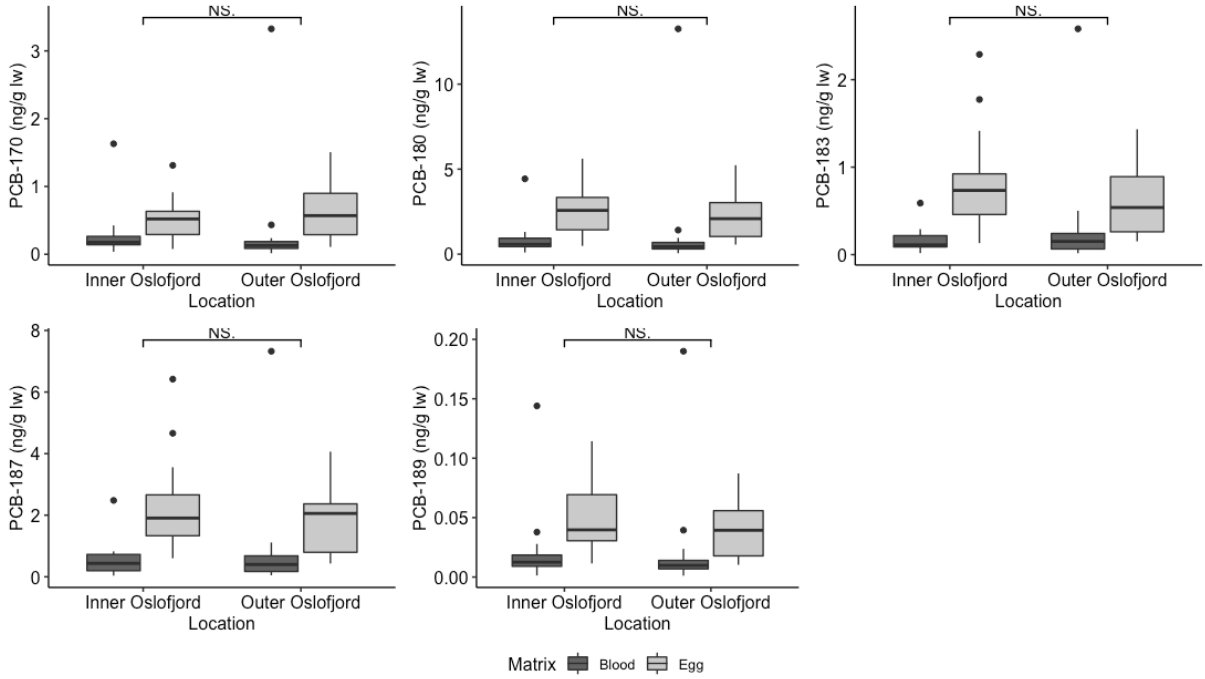

Figure F5. Concentrations of the different PCB congeners (ng/g lw) in HeptaCB (PCB-170, -180, -183, -187, and -189) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. Note different scales. The bar overlying the boxplots in each plot shows whether there is a significant difference between the locations or not, where "NS" means "not significant".

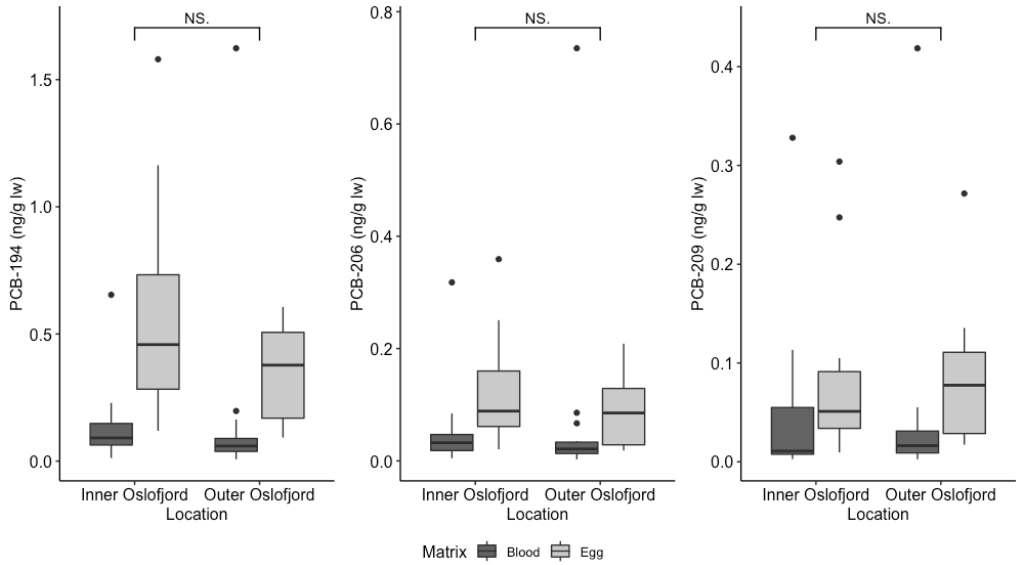

Figure F6. Concentrations of the different PCB congeners (ng/g lw) in HigherCB (PCB-194, -206, and -209) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. Note different scales. The bar overlying the boxplots in each plot shows whether there is a significant difference between the locations or not, where "NS" means "not significant".

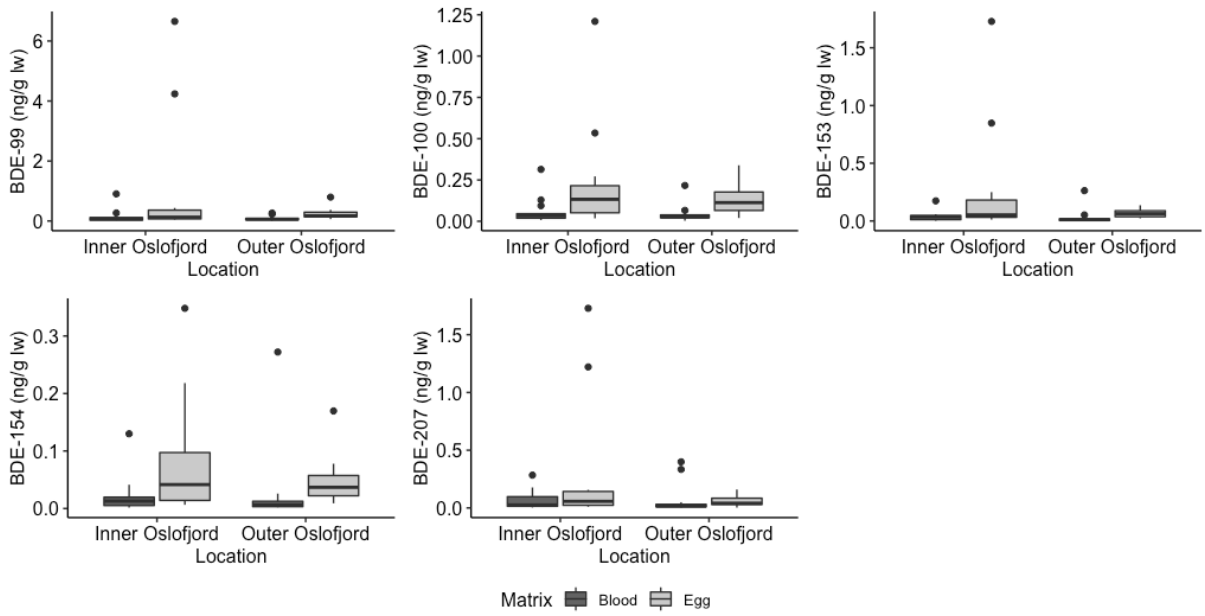

Figure F7. Concentrations of the PBDEs BDE- 99, -100, -153, -154, and -207 (ng/g lw) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. Note different scales.

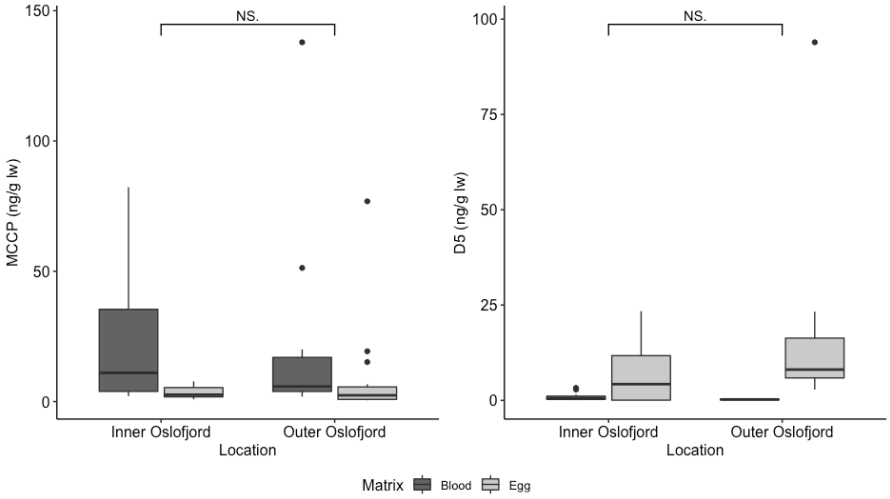

Figure F8. Concentrations of MCCP and D5 (ng/g lw) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. Note different scales. The bar overlying the boxplots in each plot shows whether there is a significant difference between the locations or not, where "NS" means "not significant".

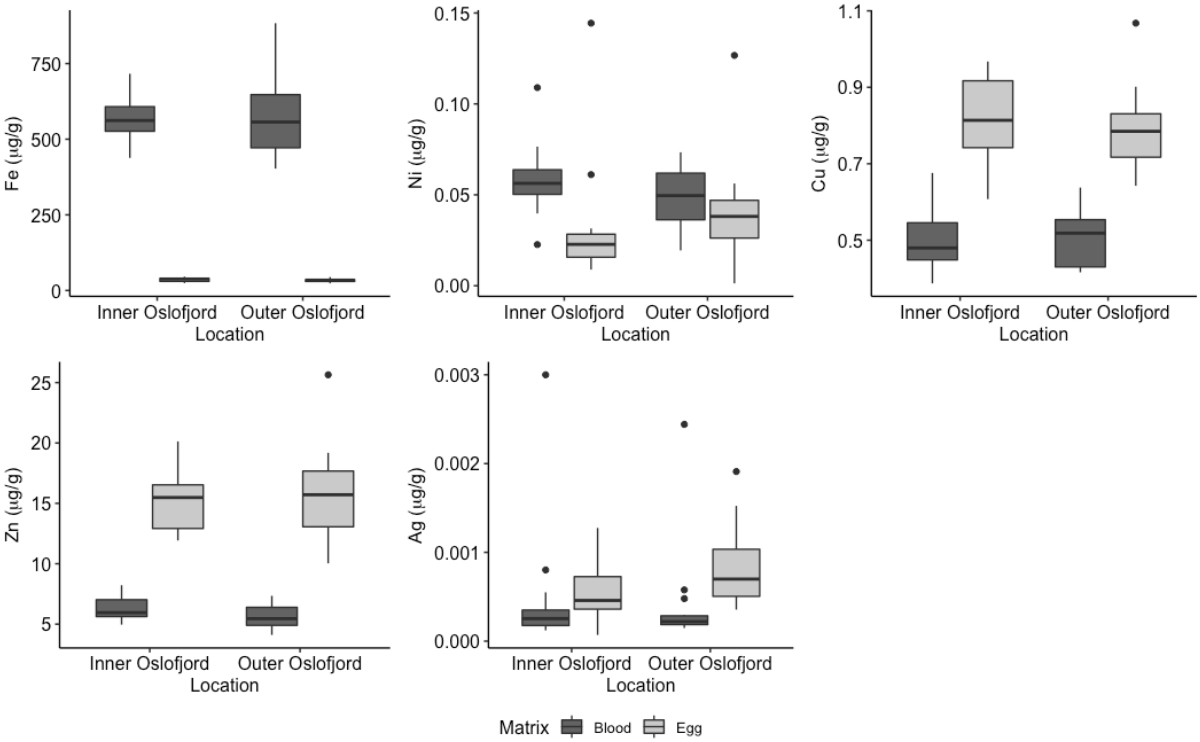

Figure F9. Concentrations of the different metals. Fe, Ni, Cu, Zn, and Ag (µg/g) in herring gull (*Larus argentatus*) females (n = 15) and eggs (n = 15) from the inner Oslofjord and females (n = 15) and eggs (n = 15) from the outer Oslofjord. Each of the boxes stretch from first to third quartile, with a black horizontal line representing the median. The whiskers represent the standard deviation (SD). The points are the extreme values (outliers) in each group. Note different scales.

Appendix G: Maternal transfer

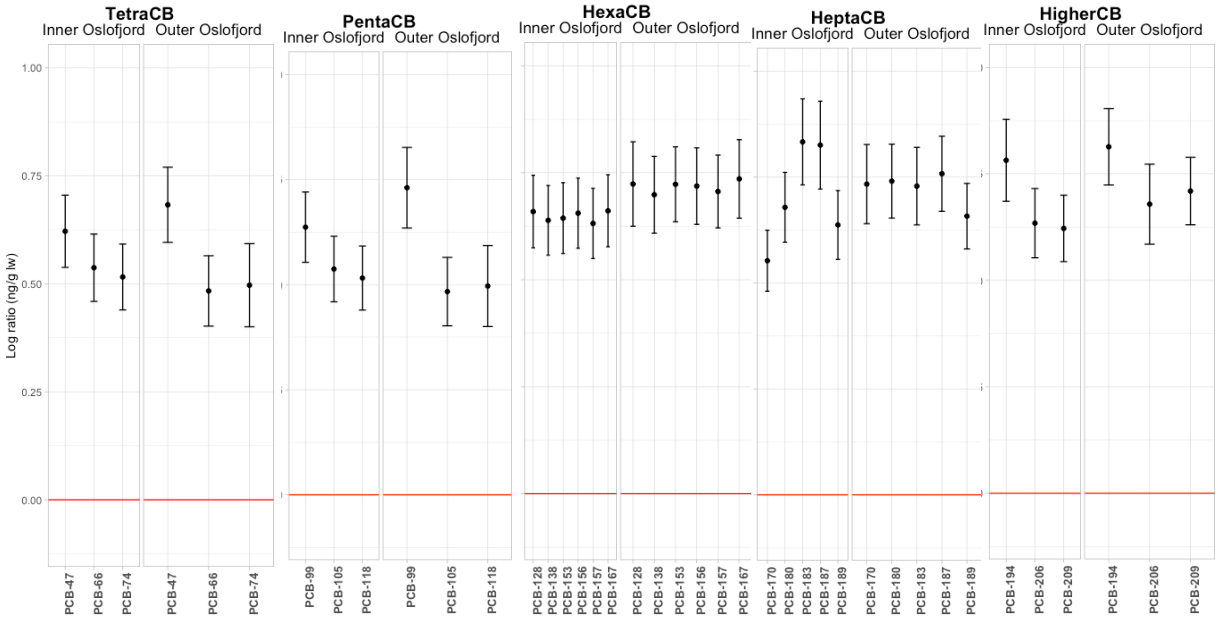

Figure G1: Log ratios of egg:wholeblood in herring gulls (*Larus argentatus*) from the inner and outer Oslofjord colony for the PCB groups TetraCB, PentaCB, HexaCB, HeptaCB and HigherCB, presented as mean ratios (± SE). Ratios were based on mean concentrations (ng/g lw). The horizontal line indicates a 1:1 relationship.

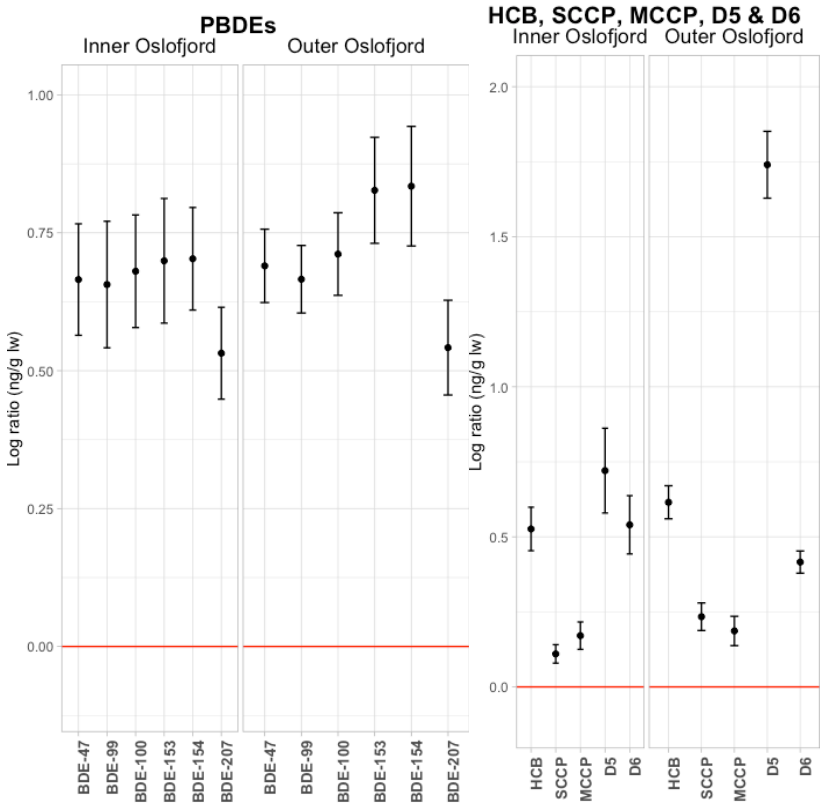

Figure G2. Ratios of egg:wholeblood in herring gulls (*Larus argentatus*) for the PBDEs, HCB, SCCP, MCCP, D5 and D6 presented as mean ratios (± SE). Ratios were based on mean concentrations (ng/g lw). The red horizontal indicates a 1:1 relationship.

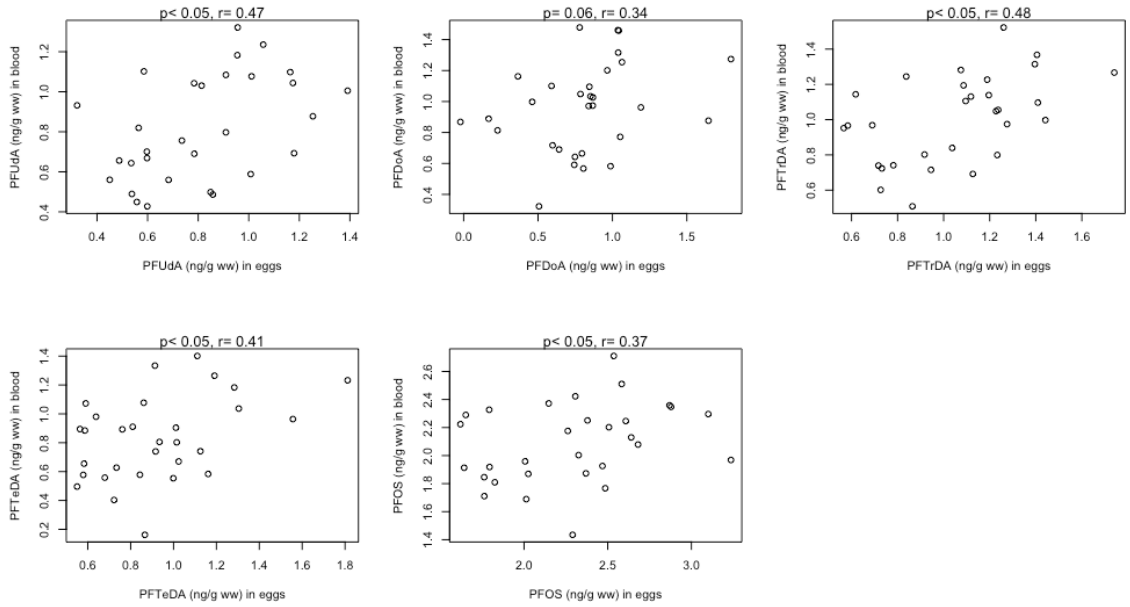

Figure G3. Correlation between blood and eggs in herring gulls (*Larus argentatus*) of different PFAS homologs (ng/g ww). Significance value and correlation coefficient are listed above each plot.

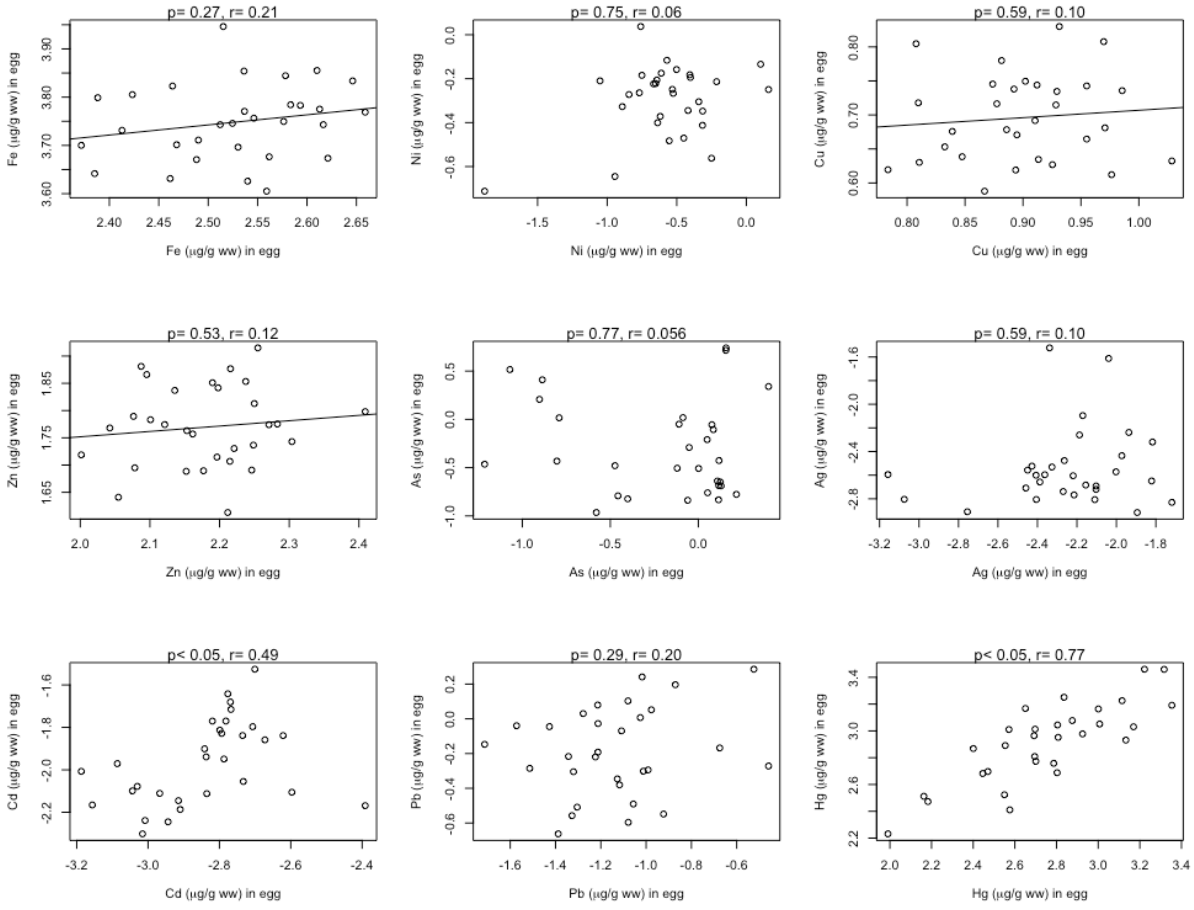

Figure G5. Correlation between herring gull (*Larus argentatus*) blood and eggs of different metals, where Fe, Ni, Cu, Zn, As, Ag, Cd and Pb are reported in µg/g ww and Hg in ng/g ww. Significance value (p) and correlation coefficient (r) are listed above each plot. Correlation lines are listed when the values are normally distributed.