Comparing contaminant occurrence and maternal transfer in Herring Gull and Common Eider in the urban inner Oslofjord, Norway

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Master thesis in Toxicology

Institute of Biosciences
Faculty of Mathematics and Natural Sciences

UNIVERSITY OF OSLO

60 credits

1.06.2019

2019

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http://www.duo.uio.no

Print: Reprosentralen, Universitetet i Oslo

This project was carried out in collaboration with the Norwegian Institute for Water Research (NIVA), the Norwegian Institute for Air Research (NILU) and UiO, under contract from the Norwegian Environment Agency (Miljødirektoratet).

Acknowledgements

First of all I want to thank my supervisors for letting me conduct this project, and for feedback along the way. Thank you to Anders for giving me this thesis and letting me join the Urban fjord project, and for always finding time for a talk. Thank you to Katrine for letting me be a part of your group, and for teaching me so much. And thank you to Morten for patience in the field, and for teaching me about.

To all the people that have helped me: To everyone at the MILKEM lab at NILU for making me feel so welcome during my stay in the lab, and especially to Inger Cristin Steen for teaching me so much about lab work, and also for having so much faith in me. To Kine Bæk at NIVA for letting me join the homogenisation, and for making it such a good time. To Christian Alexander Schöpke at IFE for teaching me about stable isotopes and showing me your lab, and Jan Thomas Rundberget at NIVA for teaching me about PFASs.

Thank you to everyone in the tox group for invaluable feedback, I have learned so much from all of you. A special big thank you to Danny for always being so helpful.

Also thank you to everyone at room 4611 for all the good times. Most of all to Nina for being my partner on the Urban fjord project. Thank you for all your help, proofreading, fun in the field, and all your good stories. I don't think I could have done it alone. Also a special thanks to Sindre and Eirik for proofreadings, discussions, and most of all for always solving my problems, and always having time to take a break. And thanks to Emelie for making sure I didn't forget things.

Finally, the biggest thank you to Hilde, Ingvild and Ylva for being with me every day, and for always supporting me.

Abstract

Environmental monitoring is performed across the world for determination of contamination status of ecosystems. For coastal marine ecosystems, seabirds are commonly used as indicators of contaminant levels high in the food web. A species that is widely used for this purpose is the Herring Gull *(Larus argentatus)*. The Herring Gull is an opportunistic species, foraging from both marine and anthropogenic sources. This poses the question of how well it is suited for its role as indicator of the status of marine food webs in urban environments. This will be addressed by comparing contaminant occurrence in relation to ecological niche of Herring Gull and the marine benthic-feeding Common Eider *(Somateria molissima)*.

Blood and eggs of herring gull and eider duck were collected from the inner Oslofjord during the breeding season in May 2017. A total number of 60 samples were collected; 15 blood samples and 15 eggs from each species. The samples were analysed for a range of legacy and emerging environmental contaminants in the laboratories at the Norwegian Institute for Water Research (NIVA), the Norwegian Institute for Air Research (NILU) Kjeller and NILU Tromsø, and $\delta^{15}N$ and $\delta^{13}C$ stable isotopes at Institute for Energy Technology (IFE). In addition, determination of lipid content was performed at NILU.

The stable isotope analysis indicated that the Herring Gull do not belong to the marine food web, which the Common Eider belonged to. Concentrations of lipophilic contaminants were higher in blood of Common Eider than in blood of Herring Gull, and this could be related to ecological niche, but also to breeding ecology. In addition, the results were discussed in the light of matrix composition and implications for environmental monitoring.

Abbreviations

ANCOVA	Analysis of covariance
BCI	Body condition index
C:N	Carbon:nitrogen ratio
CW	Carbon weight
df	Degrees of freedom
EU	European Union
GC	Gas chromatography
HCB	Hexachlorobenzene
Hg	Mercury
IFE	Institute for Energy Technology
K_{ow}	Octanol-water partition coefficient
Lw	Lipid weight
LOD	Limit of detection
Log	Logarithm
LOQ	Limit of quantification
MeHg	Methyl mercury
MS	Mass spectrometer
N_2	Nitrogen gas
NA	Not analysed
Ni	Nickel
NILU	Norwegian Institute for Water Research
NIVA	Norwegian Institute for Air Research
NW	Nitrogen weight
OB	Organobromine
OC	Organochlorine
OF	Organofluorine
PBDE	Polybrominated diphenyl ether
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PFAS	Perfluorinated alkyl substance

PFDA	Perfluorodecanoic acid
PFDoA	Perfluorododecanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFTrDA	Perfluorotridecanoic acid
PFuDA	Perfluoroundecanoic acid
POP	Persistent organic pollutant
RDA	Redundancy analyses
SD	Standard deviation
SE	Standard error
UiO	University of Oslo
UNEP	United Nations Environment Programme
Ww	Wet weight
$\delta^{13}C$	Carbon isotope
$\delta^{13}N$	Nitrogen isotope

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1 Introduction

1.1 Contaminated ecosystems

Human activities have introduced contaminants to ecosystems all over the world (Huber et al., 2015). Areas close to emissions and high densities of people, such as urban areas, have been particularly exposed to different forms of pollution (Herzke et al., 2009; Ruus et al., 2019).

1.1.1 Fate in the ecosystem and trophic transfer

When contaminants are released to the aquatic environment, their fate in, and influence on, the environment depend on physical, chemical and biological factors of the compound and ecosystem (K. C. Jones & de Voogt, 1999; Wania & Mackay, 1996). Important properties of a compound determining its fate include persistence in the environment, recalcitrance against degradation, and bioavailability to be accumulated in biota, e.g. through lipophilicity or affinity to proteins (Borgå et al., 2004). Compounds not eliminated after entering an organism may be transferred to organisms of higher trophic position in the food web, leading to biomagnification (Borgå et al., 2001). Biomagnification occurs when the concentration of a compound in the body exceeds the concentration in the diet due to dietary accumulation (Borgå et al., 2004; Gobas & Morrison, 2000). Slow elimination causes concentration in an organism to not reach equilibrium with that in food or water, and biomagnification will thus occur (Borgå et al., 2004). Chemicals with fast elimination rates are less likely to biomagnify. Lipophilic compounds are stored in the fatty tissues of organisms, leading to low elimination rates and thus high probability of biomagnification (K. C. Jones & de Voogt, 1999). This is the case for the group of chemicals known as persistent organic pollutants (POPs) (K. C. Jones & de Voogt, 1999).

In addition to POPs being organic and having potential for accumulation in food chains, these compounds are not easily degraded in the environment, and are found in high concentrations (K. C. Jones & de Voogt, 1999). This means that they have the potential of remaining in nature for long periods of time after release is stopped, and of being transported to remote areas, posing further threat to the environment (Beyer et al., 2000; UNECE, 1979). Some POPs are known and suspected to have a range of effects on biota, as reviewed by Letcher et

al. (2010). As a result of high concentrations due to bioaccumulation, harmful effects of POPs might be of special concern for top predators. This include humans as well as predators such as birds and marine mammals (K. C. Jones & de Voogt, 1999). Effects linked to mixtures of POPs in the environment include reduced reproductive success, deformities, behavioural changes, and impaired immune function in seabirds (Bosveld & Berg, 1994; J. O. Bustnes et al., 2004; Giesy et al., 1994; Helberg et al., 2005; Prestt et al., 1970).

1.2 Environmental monitoring

Environmental monitoring is performed to assess the health of ecosystems. Chemicals in the environment can impact ecosystems and subsequently also human health. For this reason, screening of contamination status of ecosystems is a vital part of environmental monitoring programs, and is required under the EU Water Framework Directive (European Commission, 2000). In addition, information retrieved during environmental monitoring is important as effectiveness measurements under the Stockholm convention (Harner et al., 2015).

1.2.1 Seabirds in monitoring

Monitoring of contaminant status of an ecosystem requires measurements of contaminant concentrations in biota, water and sediments. Species selected for this role should represent the state of the ecosystem, and rapidly mirror any ecosystem change. In coastal marine ecosystems, seabirds are commonly used as indicators of contaminant concentrations high in the food web (Furness & Greenwood, 2013). Seabirds have been identified as effective biomonitors of coastal ecosystem health (reviewed by Burger & Gochfeld, 2004). Contaminant concentrations in seabirds have been shown to differ between species (Borgå et al., 2005; J. Elliott et al., 2015; Haukås et al., 2007; Savinov et al., 2003). The interspecies differences can be related to exposure, such as differences in diet and thus trophic position as well as physiology and life history traits such as biotransformation capacity and elimination through maternal transfer in egg production (Borgå et al., 2004; Hitchcock et al., in review; Hop et al., 2002). Different species will reflect the contaminant status of the ecosystem differently, which is consequently important for the choice of indicator species in monitoring.

The Great Lakes Herring Gull monitoring program is an example of how monitoring projects can not only assess the ecologic status of local ecosystems and effects of impacts on these, but

also contribute long-term studies for the understanding of ecosystem dynamics (Hebert et al., 2011). What began as monitoring in response to population declines suspected to be caused by pollution (Gilbertson & Fox, 1977), has contributed to knowledge used to construct contaminant models (Comba et al., 1993; Hebert et al., 2011).

The European Herring Gull (*Larus argentatus)* has an important function as a study species in a wide range of research on accumulation of contaminants in ecosystems e.g. (Weseloh et al., 1979). The choice of herring gull as monitoring species is justified for several reasons, including trophic position and ecology (Hebert et al., 2011). Herring Gull is a widely distributed species, found in both coastal and inland areas of north eastern North America, western Europe and central Asia (Morris et al., 2003; K. M. Olsen, 2010). This makes it well suited as a study species for comparing studies from different areas.

Herring Gull is a generalist, capable of feeding from both marine, terrestrial, and anthropogenic sources (Burger et al., 1980). Their diet include fish, insects, small mammals and birds, as well as human garbage and animal carcasses (Morris et al., 2003). The species is capable of adapting to urban environments (Coulson, 2015), and is often known to individually specialise on specific diets (Morris et al., 2003).

The Norwegian Oslofjord is an urban, polluted area. Decades of emissions from industry and other human impacts such as runoff from agriculture, traffic and sewage, have resulted in a marine ecosystem affected by high concentrations of both organic and inorganic contaminants (Grung et al., 2011). The Oslofjord ecosystem has since 2013 been monitored through the program Environmental Contaminants in an Urban Fjord (Urban fjord) (Ruus et al., 2014; Ruus, Allan, et al., 2015; Ruus et al., 2017, 2019; Ruus et al., 2016b). Every year, water and sediments, and selected biota representing the marine food web, are sampled from the inner Oslofjord and analysed for a range of environmental contaminants. Furthermore, analysis of stable isotopes of both carbon and nitrogen are used to describe the carbon source and trophic status of the biota. At the initiation of the Urban Fjord monitoring programme, Herring Gull was selected as the seabird indicator of the Oslofjord marine food web. The other organisms representing the marine food web are Blue Mussel *(Mytilus Edulis)*, Polychaetes, Krill (Euphausiacea), Shrimp *(Pandalus borealis)* and Cod *(Gadus morhua)*

In the first years of the Urban fjord programme, it was noted, based on the results of stable isotope analysis, that a significant part of the Herring Gull diet was not represented by the

selected food web (Ruus et al., 2014). Since 2015, Herring Gull has been included in the programme as a representative of an urban fjord inhabitant, but not as a representative of the marine food web (Ruus et al., 2016a). This motivates the inclusion of another species that could be more suited to represent the Oslofjord food web.

In 2017, the Common eider (*Somateria molissima)* was included to test its applicability in the monitoring programme (Ruus et al., 2019). Common Eider is a marine benthic-feeding species (Cramp, 1977). The diet is mainly Blue Mussels and other marine invertebrates such as crustaceans and polychaetes (Huber et al., 2015; Larsen & Guillemette, 2000), and Common Eider is therefore considered a midtrophic predator (Huber et al., 2015). Because of the marine diet, Common Eider is expected to serve as a good indicator of contaminant exposure in the marine food web.

Because contaminants have different affinities to different tissue types depending on their chemical properties, the choice of matrix is important when designing monitoring programmes. Blood sampling is a relatively non-invasive method, and has been shown to reflect contaminants levels of an organism (Henriksen et al., 1998; Marsili et al., 1996). It is therefore preferred over other destructive sampling methods such as that of liver or brain tissue (Friend et al., 1979). Eggs of seabirds have been widely used for contaminant monitoring (Focardi et al., 1988; P Mineau et al., 1984), and are recognised as a good monitoring matrix because sampling is reasonably uninvasive (Furness & Greenwood, 2013). Egg production is a known route of elimination of contaminants for female birds (Drouillard & Norstrom, 2001; Fernie et al., 2000; Verboven et al., 2009), and eggs can thus reflect female contaminant levels (Lewis & Furness, 1993)

1.3 Stable isotopes

Stable isotopes are used as dietary markers in food web studies (Wada et al., 1991). In natural systems fractionation processes results in different ratios of heavy and light isotopes of elements in different scenarios (Briscoe & Robinson, 1925). Ratios of the heavy and light isotopes are used to indicate degree of fractionation in a given system, and thereby properties of the system. In order to compare these relative ratios across studies, values are reported as the deviation of the sample ratio to the ratio of an international standard for that specific element (McKinney et al., 1950). These values are denoted δ.

Carbon source of the diet can be assessed through analysis of ratios of stable isotopes ^{13}C and ^{12}C. Organic material of terrestrial origin will have a more depleted $\delta^{13}C$ than material of marine origin because of differences in the photosynthetic carbon fixation of terrestrial and marine primary producers (Peterson & Fry, 1987). Trophic status can be assessed using the isotopic ratio of ^{14}N and ^{15}N, based on retention of the heavy isotope in the body compared to the lighter, which is excreted and metabolised to a greater degree (Peterson & Fry, 1987). This leads to higher $\delta^{15}N$ values in predators relative to their prey (Fry 1988, Hobson 1992 (Mizutani et al., 1991).

1.4 Contaminants measured in Urban Fjord

To reach the monitoring aims of the Urban fjord programme, a broad range of contaminants of different origins and properties are analysed (Ruus et al., 2019). The compound groups analysed in Herring Gull in the Urban fjord 2017 programme were chlorinated compounds, brominated compounds, siloxanes, phenolic compounds, metals and fluorinated compounds (Ruus et al., 2019). Because of limited funding, only a few of the contaminant groups analysed in Herring Gull were analysed in Common Eider. Here, only contaminants analysed in both species will be included. The contaminant groups analysed in both species were polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), polybrominated biphenyl ethers (PBDEs), per- and polyfluoroalkyl substances (PFASs) and mercury (Hg). These POPs and the element Hg represent groups with different status regarding use, regulation and fate in ecosystems.

Organohalogens are a group of POPs containing organic chemicals where carbon is covalently bound to halogens; chlorine, bromine, fluorine or iodine (Letcher et al., 2010). In this study, organochlorines (OC), organobromines (OB) and organofluorines (OF) are represented. The chlorinated compounds include PCBs and HCB, the brominated include PBDEs, and the fluorinated compounds include PFASs.

PCBs are industrial chemicals with a varying number of chlorine substitutions (1-10) (Ballschmiter & Zell, 1980). The number and positions of the chlorine substitutions are important for the properties of the congener. Commercial production of PCB started in 1929 (de Vogt and Brinkman 1989). Applications include hydraulic fluids, cooling liquids, plasticisers, lubricants, inks and paints. In many industrialised countries, including Norway,

use and production of PCBs have been restricted since the 1970s (K. C. Jones & de Voogt, 1999). As a result of increasing knowledge and concern about the effects of environmental contaminants on health and environment, the Stockholm Convention was signed in 2001, and has been effective since 2004 (UNEP, 2001). The first 12 contaminants addressed in the Stockholm Convention are known as the dirty dozen, and PCBs were among these. (UNEP, 2001). Regardless of the ban, PCBs are still found in the environment. This is both due to their persistent properties, and due to products containing PCB still being in use or not properly handled at end of life of the products.

HCB is a compound consisting of a benzene ring with six chlorine substitutions. It was previously used as a pesticide. HCB is formed in combustion processes and is produced as by-products in production of other chlorinated compounds and in a range of industry processes. Like the PCBs, HCB is also one of the Stockholm Convention's dirty dozen, and a worldwide ban has been effective since 2004. In Norway, it has been on the priority list of chemicals to be reduced since 1997.

PBDEs are used as flame retardants. They are structurally similar to the PCBs. While PCBs are biphenyls with a number of chlorine substitutions, PBDEs are biphenyl ethers with bromine substitutions. The difference between a biphenyl and a biphenyl ether is that in the biphenyl ether, the phenyls are connected by an oxygen atom. In Norway, BDEs have been on the government's priority list since 1997. Today, many PBDEs are also regulated under the Stockholm convention.

PFASs are a group of perfluorinated hydrocarbons which are widely used in industry and consumer products, for example as water repellents or in fire-fighting foams. PFASs are aliphatic hydrocarbons, meaning that they are chain-structures, as opposed to the aromatic PCBs and PBDEs. In the case of PFASs, all the carbon atoms in the chain have fluorine substitutions.

The initially produced long-chained PFASs were first included on the Stockholm convention in 2009. Other PFAS are still produced and used in numerous applications. Today, additional PFASs, including Perfluorooctanoic acid (PFOA) and Perfluorohexanesulfonic acid (PFHxS) are under evaluation for listing. Environmental properties of the new short-chained PFASs, which are emerging as the long-chained PFASs are banned, are not yet well understood, and the use of these chemicals is increasing (Brendel et al., 2018). In Norway, the first PFAS,

Perfluorooctane sulfonate (PFOS), was added to the priority list in 2002. Since then more PFASs have been added to the list as well. Fire-fighting foams containing PFASs have been banned in Norway since 2007, but PFASs can still be detected in the environment in significantly higher concentrations in areas where foams have been used extensively, e.g. close to airports.

Mercury is a naturally occurring element, but it has also been widely used for many industrial purposes. Application is now restricted to scientific usage due to its toxicity. Emissions of mercury is controlled worldwide by the 2013 Minamata convention. Norway has had a ban on manufacturing and importing mercury since 2008. In its inorganic form, the toxicity of mercury is limited because of its insolubility. However, like other elements, mercury has the ability to form covalent bonds. Through covalently binding to organic groups, metals can form compounds with characteristics differing from the original, relatively unreactive element. The most toxic of these species is methylated mercury. MeHg has higher bioavailability than inorganic mercury, and is known to accumulate and magnify in food chains (Amlund et al., 2007; J. E. Elliott, 2005; Savinov et al., 2003).

1.5 Aims and hypotheses

The aim of this study was to examine the suitability of Herring Gull and Common Eider as indicators of the contamination status of marine food webs in urban environments. This was addressed by comparing contaminant concentrations and patterns in relation to carbon source and trophic status of Herring Gull and Common Eider, by evaluating the use of whole blood or eggs, as well as by comparing maternal transfers to eggs in the two species, with the following objectives and hypotheses:

Objective I: Assess the suitability of Herring Gull and Common Eider as representatives of the marine food web of the inner Oslofjord using stable isotope analysis

> **H 1.1:** Based on knowledge about the feeding ecology of the two species, Herring Gull does not feed exclusively from the marine food web, whereas Common Eider does
>
> **H 1.2:** Based on knowledge about feeding ecology, Herring Gull has a low trophic status relative to Common Eider and does not have a food web baseline in the marine food web

Objective II: Compare concentrations and patterns of contaminants in Herring Gull and Common Eider, and evaluate the importance of species differences in ecological niche, metabolism and matrix differences, for environmental monitoring

> **H 2.1:** Based on knowledge about accumulation of contaminants in marine food webs, concentrations will be higher in the marine feeding Common Eider than in the terrestrially influenced Herring Gull. Species differences can be described by the dietary descriptors δ15N and δ13C
>
> **H 2.2:** Contaminant patterns will be influenced by the metabolic abilities of the species, with patterns dominated by recalcitrant contaminants in the more effective metaboliser, Herring Gull. This will be tested by comparing relative contribution of contaminant groups to the different species and matrices.
>
> **H 2.3:** Differences in matrix will impact the differences in concentrations and patterns between the species with differences in lipid content affecting the concentrations of lipophilic contaminants. This will be tested by assessing the impacts of lipid standardisation on the interpretation of the results

Objective III: Evaluate the influence of diet and breeding strategy on maternal transfer of contaminants, and their implications for monitoring

H 3.1: Higher contaminant concentrations in the mother following a more marine diet will lead to higher concentrations transferred to eggs. Breeding strategy will also influence the maternal transfer, with higher investment in reproduction leading to a higher degree of maternal transfer, and lipid dynamics during breeding impacting the results.

2 MATERIALS AND METHODS

The data used in this study was collected as part of the Urban fjord 2017 programme. I participated in the sampling and chemical analysis during summer 2018. Therefore, the data in this study was not sampled by me, but both the field and lab work was done in the same way the two years. I participated in sampling of Herring Gull at Søndre Skjælholmen and of Common Eider at Husbergøya in May 2018. In June 2018 I homogenised and distributed samples at NIVA, and performed lipid determination and sample preparation for POP analysis of Herring Gull eggs at NILU.

2.1 Study area and field procedures

Herring Gulls and Common Eiders were collected during the breeding season in May 2017.

Herring Gull samples were collected on Søndre Skjælholmen, a small island located in the inner Oslofjord, with close proximity to Oslo city centre. The exact position is at 59° 51'N, 10° 43'E in Nesodden municipality, Akershus county (Figure 1). Herring Gull is the dominating breeding species here (Bergan & Andersen, 2017), but several other seabirds, including Lesser Black-backed Gulls (*L. fuscus*), Barnacle Goose (*Branta leucopsis*) and common eider are also present on the island (Bergan & Andersen, 2017). The southern part of the island and the nearby sea area has been protected as a nature reserve since 2008, restricting the use of motorised vehicles in and near the breeding colonies during the breeding season (15. April – 15. July).

Common Eider samples were collected from three different sites in the Inner Oslofjord; Søndre Skjælholmen (5 females), Husbergøya (6 females) and Raudskjæra (4 females). Husbergøya is located north of Søndre Skjælholmen in Nesodden municipality (Figure 1), at 59°51'N, 10°42'. The island has had status as nature reserve since 2008, and is a breeding area for several bird species (Bergan & Andersen, 2017). Raudskjæra is located in Asker municipality, Akershus county (Figure 1) at 59°50'N, 10°32'E, and is not a nature reserve.

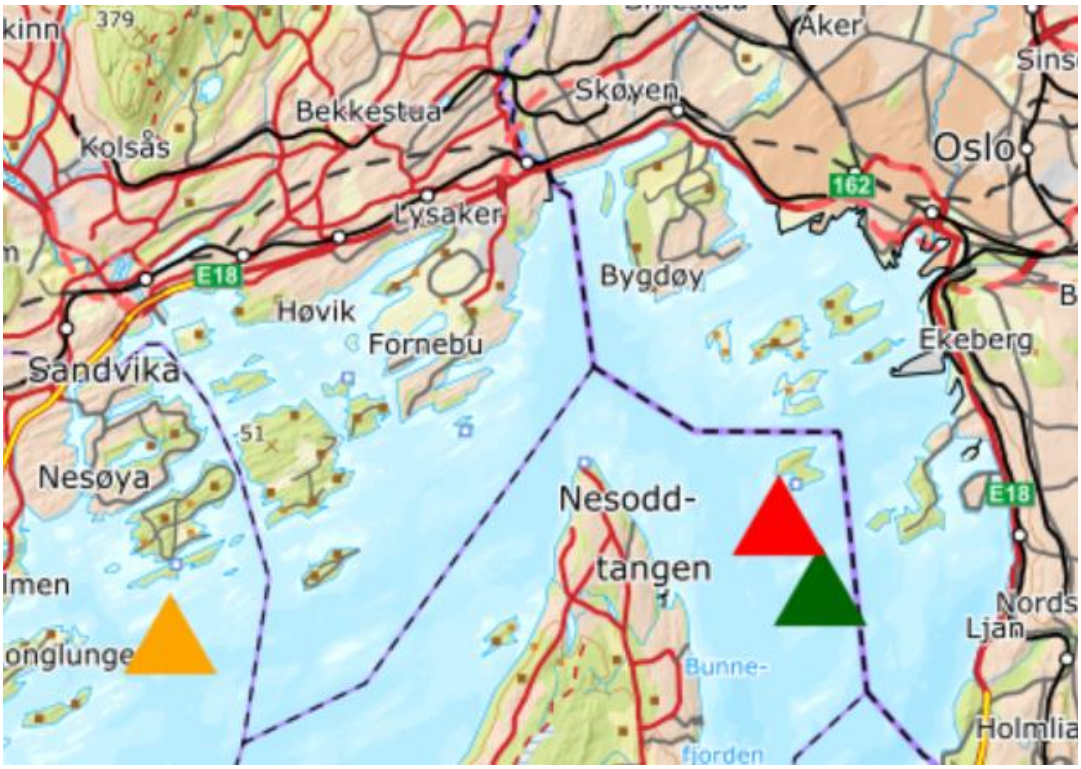

Figure 1: Map of the Inner Oslofjord with the study sites marked: Søndre Skjælholmen (green), Husbergøya (red) and Raudskjæra (orange)

From a total of 15 Herring Gull nests, a blood sample (5 mL) from a Herring Gull female and one of her egg were collected May 14th, 2017. The numbers of eggs in the nest were registered. Nests with three eggs were preferred, and nests with only one egg were avoided in order to make the sampling as non-destructive for the birds as possible. The Herring Gulls were caught using walk-in traps placed over the nests. As the gull entered the trap, the open door closed, leaving the bird trapped on its nest. This ensured that the sampled egg belonged to the sampled female. Both male and female Herring Gulls incubate eggs, but blood samples were only collected from females. Head length measured from the tip of the bill to the back of the head were used for determination of sex. Because the measurement among sexes varies geographically, a head-length criteria based on the local gulls in the Oslofjord was used. Adult, breeding individuals with head length less than 121mm were considered female, while individuals with head length greater than 123mm were considered males. To ensure only sampling of females, only birds with head length less than 120 mm were sampled. The trapped bird was taken away from the site of capture in a dark bag to prevent disturbance of the colony. After removal from the colony, a blood sample was drawn from a vein on the

underside of the wing. In addition to the blood sample, biometric measurements, head length, wing length, bill height and body weight were taken of each bird. Finally, the birds were also tagged and registered in a population monitoring program.

Common Eiders were sampled from 15 nests on May 7th and May 26th (Husbergøya), May 9th (Raudskjæra) and May 19th (Søndre Skjælholmen), 2017. From each nest, a blood sample (5 mL) from a breeding female and one egg from the same nest was sampled. Also here, the sampling was designed to disturb the birds as little as possible; numbers of eggs in the nests were registered, nests with more than three eggs were preferred, and nests with only one egg were avoided. The females were sampled using hand nets on the nests. Only females incubate the eggs, and because of sexual dimorphism, visual sex determination is sufficient for Common Eiders. In all other aspects, the setup was the same as for Herring Gull. The trapped bird was taken a short way away from the nest, and a blood sample was taken from the vein beneath the wing. Measurements of head length, wing length and body weight were made, and the birds were tagged and registered for population monitoring.

2.2 Chemical analyses

All contaminant and stable isotope analyses were performed on whole blood and homogenate of whole eggs.

2.2.1 Stable isotopes

Stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ in blood and eggs was performed by staff at the Stable Isotope Laboratory at the Institute for Energy Technology (IFE), Kjeller.

Samples were dried at 80°C, homogenised with a mortar and combusted in a Eurovector EA3080 elemental analyser at 1700°C in the presence of oxygen (O_2) and chromium (III) oxide (Cr_2O_3). NOx was reduced to N2 at 650°C in the presence of Copper (Cu). After combustion and H_2O removal, N_2 and CO_2 were separated on a 2m Poraplot Q gas chromatograph (GC) column. When separated, N_2 and CO_2 were transferred directly to a Horizon Isotope Ratio Mass Spectrometer (IRMS) from Nu-instruments for determination of isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$. The stable isotope ratios were expressed as δ values

relative to the internationally accepted standards, PeeDee Belemnite (PDB) marine fossil limestone formation (Vienna) for $\delta^{13}C$, and atmospheric nitrogen (N_2) for $\delta^{15}N$, and calculated as shown in Equation 1. In addition, carbon weight (CW), nitrogen weight (NW) and carbon:nitrogen ratio (C:N) were quantified the by comparison of the chromatographic peak areas to known standards.

$$\delta 13C \text{ or } \delta 15N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000, \text{ with } R = \frac{^{13}C}{^{12}C} \text{ or } R = \frac{^{15}N}{^{14}N} \qquad \text{(Equation 1)}$$

2.2.2 Contaminants

Frozen eggs were thawed and homogenised at the Norwegian Institute of Water Research (NIVA), Oslo. Homogenised samples were distributed in appropriate quantities for analyses. Prior to homogenisation, the eggs were opened and visually classified according to their level of development. There were 5 levels, making up this classification scale: 1: without signs of fetus. 2: no clear fetus, but signs of development, such as presence of blood. 3: clear fetus, no or little sign of feathers. 4: fetus with feathers – still with vitellus (yolk sac). 5: near hatching. After classification, eggs were homogenised using an Ultraturrax blender.

2.2.3 PCB and PBDE

Analysis of PCB and PBDE, and lipid determination was performed at the Norwegian Institute of Air Research (NILU), Kjeller. Contaminant concentrations in the samples were quantified using gas chromatography (GC-HRMS Waters Autospec). Prior to quantification, samples were prepared, extracted, and cleaned using organic solvents, two silica columns, and sulphuric acid. An aliquot of the sample extract was used for gravimetric lipid determination. The samples were dried and homogenised using sodium sulphate. 12 g of each sample was weighed out, and 150 g of sodium sulphate was added before freeze drying the sample. The dried samples were weighed and separated for contaminant analyses and lipid determination.

For contaminant analyses, samples were eluted with internal standards and 150 ml cyclohexane/acetone (3:1). Prior to elution, internal standards were mixed and diluted in cyclohexane/acetone (3:1). The columns were left overnight, and the samples were evaporated to 5 ml in a turbovap evaporator before they were transferred to silica columns for extraction.

60 ml etherhexane was used as eluent. Before starting the column, 4 g of silica was added, and activated by running a column of silica, 30 ml etherhexane and sodium sulphate. After elution, the sample was again evaporated to 5 ml. The samples were then cleaned using a 4x sulphuric acid rinse. For this the sample was transferred to acid glass along with hexane, and sulphuric acid of approximately the same volume as the sample and hexane was added. After completing the acid treatment the samples were evaporated to 5 ml, and eluted in a silica column. The process was identical to the previous, but the volumes changed to 6 g silica and 40 ml etherhexane. Finally, samples were evaporated to 5 ml under nitrogen flow in a TurboVap Evaporator and transferred to smaller containers for further evaporation. The prepared samples were run through gas chromatography by NILU staff.

2.2.4 Lipid determination

Gravimetric lipid determination was performed on two replicates of each sample using an organic solvent column with cyclohexane/acetone (3:1). To ensure evaporation of the solvent the extract was left overnight, then placed in the oven at 100 degrees for an hour, and in finally in a desiccator for an hour. The evaporated samples were weighed, and lipid content calculated according to Equation 2 and 3. Final lipid content for each sample was determined as the mean of the two replicates.

$$weight\ after\ evaporation\ (g) - weight\ before\ evaporation(g) = weight\ of\ lipid\ (g) \quad \textit{(Equation 2)}$$

$$\frac{weight\ of\ lipid}{sample\ weight\ (g)} * 100 = lipid\ content\ (\%) \quad (Equation\ 3)$$

2.2.5 PFAS

Samples were analysed for PFASs by personnel at NIVA, Oslo. Two grams of homogenised sample was spiked with 6ng of mass-labelled internal standards. Spiked samples were extracted using acetonitrile and cleaned up using graphitised carbon and acetic acid. Target compounds were separated using an Acquity Ultra Performance HPLC system (Waters). The

separated samples were quantified using mass spectrometry in a Xevo G2-S Q-ToF-HRMS instrument (Waters).

2.2.6 Hg

Samples were analysed for Hg by staff at NILU, Kjeller. Prior to metal analysis the samples were digested using diluted nitric acid in an UltraClave, Milestone, Italy. After digestion, hydrochloric acid was added before determination. Hg content of the samples was quantified using an inductively coupled plasma mass spectrometer (ICP-MS). The method is developed at NILU.

2.2.7 Quality assurance and quality control

Limit of detection (LOD) and Limit of quantification (LOQ) were defined as 3SD and 10SD, respectively, of the mean blank response for all contaminants.

2.3 Data treatment

2.3.1 Data included

When using quantification methods such as mass spectrometry for detection of chemicals separated by chromatography, small signals can be disturbed by the noise created by the quantification process (D. Helsel, 2010). The limit for where the recovery cannot be separated from the instrument noise is called the limit of detection (LOD). Values below the LOD, referred to as nondetects, might not be accurate enough to give realistic representations of the analysed material. Data containing nondetects is in this text referred to as censored data.

Prior to statistical analyses, the data was treated for nondetects and censored values. In the contaminants data, congeners with high abundance of nondetects were removed. The limit for exclusion of congeners was decided after a thorough evaluation of the data. There are different recovery values for different chemicals. Therefore, the censoring level, the level for exclusion of a compound because of high amounts of nondetects, was chosen individually for

the lipophilic and non-lipophilic contaminant groups. To ensure comparability between species and matrices, the same level was set for egg and blood of both species for each group.

Several methods for replacing nondetects remaining in the data exist, and a goal when choosing a method for this purpose should be to not introduce skewed patterns to the data (D. Helsel, 2010; D. R. Helsel, 2006). Substitution of a random value between 0.5*LOD and LOD is a common method for replacement of data, but it is known to have significant drawbacks, including introduction of false patterns when creating data not drawn from the actual distribution of the dataset (D. Helsel, 2010; Singh & Nocerino, 2002). Therefore, remaining nondetects were replaced by imputation in this study. Imputation is a method in which data is filled in based on an underlying model, in this case a β distribution. The β distribution takes different shapes depending on two parameters, α and β, and can therefore be fitted to the shape of the dataset. Based on inspections of the data and repeated testing, the shape parameters were set to $\alpha = 5$ and $\beta = 1$.

2.3.2 Assessing normal distribution and homogeneity of variance

Many parametric methods assume normal distribution of the data (Altman & Bland, 1995). Normality can be assessed visually by plots, or statistically by comparing the sample distribution to a normal distribution in a significance test. To ensure good assessment, both methods were used. As a visual assessment, a frequency distribution was used because it provides information about outliers and gaps in the data in addition to the shape of the distribution. The Shapiro-Wilk test was used as significance test, because of its high power

In order to achieve normality, contaminant data were log transformed before subjection to statistical testing. All log transformed concentrations are given as log(concentration+1) in order to avoid values of 0, using the natural logarithm.

For tests such as Welch's t-tests the assumption of homogeneity of variance is important, meaning that the variance within the groups should be equal. This will be assessed using the Fligner-Killeen test. This is a non-parametric test and was chosen because it is robust against non-normal data and outliers.

2.3.3 Biometric data

An animal's body condition can be indicated by its stored energy. Mass can be used as an indicator for body condition, but the effects of structural size of the body must be accounted for. As a measure of the individual bird's body condition, and in order to control for variation in body size among individuals, a body condition index (BCI) was calculated. First, to determine which of the measured body structures was best correlated with mass, Pearson correlation tests were run on each parameter. The measured body structures were wing length, head length and bill height for Herring Gull, and wing length and head length for Common Eider. Head length was missing for three eiders. For bill height, one gull (number 13) had a value that was clearly wrong. This value was removed and replaced by NA.

The parameter with the best correlation with mass for each species was used as independent variable in a linear regression against the dependent variable mass. Standardised residuals from this regression was used as BCI for the individual birds. (Alisauskas & Ankney, 1987; Jan O. Bustnes et al., 2002; Jakob et al., 1996; Sedinger et al., 1997). For both species, head length correlated best with mass. The correlation was positive for Herring Gull and negative for Common Eider. However, because of the NAs, the predictive power of this model was low. Because of this, and in order to calculate BCI for each individual, wing was used as dependent variable for Common Eider. As a measure of BCI, the standardised residuals of each point in the linear regression was used.

2.3.4 Grouping of chemicals

Chemicals were grouped according to physicochemical properties and use. The main grouping was of lipophilic and non-lipophilic contaminants. The lipophilic contaminants are the organochlorines, HCB and PCBs, and the organobromides, PDBEs. The non-lipophilic contaminants are the organofluorines, PFASs, and mercury. Contaminants were also grouped and ranked according to their assumed fate in the environment.

2.4 Statistical analyses

2.4.1 Carbon source and trophic status

To evaluate the ecological niche of the bird species relative to the marine food web, results of stable isotope analyses for all the organisms sampled in the Urban fjord project were plotted in a scatterplot. Full descriptions of the sampled marine food web can be found in the monitoring report (Ruus et al., 2019), and in Appendix A. The stable isotope data was only used for indication of ecological niches of the birds.

Because of the low lipid content, and similar C:N ratios between the species, no lipid correction was done on the $\delta^{13}C$ of blood. To check whether the differences in lipid content between eggs of Herring Gull and eggs of Common Eider impacted the species comparison, the $\delta^{13}C$ data for eggs was corrected for lipid content. This was done using C:N ratio as a proxy for lipid content as first introduced by McConnaughey and McRoy (1979). Because of the high lipid content in the eggs, a model assuming a non-linear relationship between ^{13}C depletion and C:N ratio was preferred (Ehrich et al., 2011). The equation used was the one suggested for whole homogenate of seabird eggs by K. H. Elliott et al. (2014). The lipid correction did not result in changed interpretation of the results, and the uncorrected results were therefore used in the evaluation of ecological niche. Species comparisons of contaminant data and intercorrelation to environmental variables

Differences between contaminant concentrations in blood and eggs of Herring Gull and Common Eider were assessed using Welch's t-tests.

To evaluate differences in concentrations with regards to properties affecting environmental distribution, concentrations were visualised in boxplots with the individual congeners ordered according to these properties. These plots can be found in Appendix B. Lipophilic contaminants were ordered by lipophilicity, indicated by their octanol-water partition coefficient (K_{ow}). Partition coefficients describe the ratio of solutes between two faces in equilibrium. The octanol-water coefficient is defined as concentration in octanol divided by concentration in water, resulting in that higher numbers indicate higher lipophilicity. Because of this, K_{ow} can be used as a predictor for environmental distribution, in particular bioaccumulation potential, of contaminants. K_{ow} is usually expressed as the logarithm of the described ratio. Log K_{ow} values close to 1 are considered optimal for movement through lipophilic barriers, and chemicals with log K_{ow} greater than 4.5 are considered to have potential to bioconcentrate in organisms. PFASs were ordered by chain length of the carbon backbone, as carbon chain length has been associated with bioaccumulation (J. W. Martin et al., 2003; G. W. Olsen et al., 2009).

Multivariate analysis

To explore the structure of the contaminant concentrations and their relationships to environmental variables, multivariate analysis by ordination was performed. For easier interpretation of results, PCB congeners were grouped according to degree of chlorination into homologue groups as described in Table.

Table 1: Grouping of PCB congeners based on number of chlorine substitutions (tri=3, tetra=4, penta=5, hexa=6, hepta=7, higher=. 8,9 or 10 chlorine substitutions)

Homologue group	**PCB**
triCB	PCB 28
tetraCB	PCB 47
	PCB6
	PCB74
pentaCB	PCB 99
	PCB 105
	PCB 118
	PCB 123
hexaCB	PCB 128
	PCB 138
	PCB 153
	PCB 156
	PCB 157
	PCB 167
heptaCB	PCB 170
	PCB 180
	PCB 183
	PCB 187
	PCB 189
higherCB	PCB 194
	PCB 207
	PCB 209

Ordination is a form of dimension reduction; a set of variables are condensed into a few new dimensions, whilst maintaining as much of the variation of the original dataset as possible (Anderson, 1971; Sparks et al., 1999). The new dimensions obtained, the ordination axes, are uncorrelated to each other, and explain successively less variation (Greenacre & Primicerio,

2013). To visualise the results of the ordination, the two best ordination axes can be plotted against each other in a biplot, creating a two dimensional space capturing the greatest amount of variation in the data possible. Samples, or sites, and responses can then be placed in the biplot based on their scores on the ordination axes. Ordination is most often used in order to get an overview of the data prior to other analyses, but successful ordinations can also be interpreted and used for direct interpretation of data if there is good knowledge about the structure of the data, and assumptions are met. Ordination performed on a community data matrix, such as a pollution dataset, is called unconstrained ordination.

Ordination performed on a community data matrix, such as a pollution dataset, is called unconstrained ordination. Often a range of environmental variables thought to explain parts of the variation observed in the community is also sampled. Techniques where the dimension reduction is based on the environmental data matrix are called constrained ordination. Unconstrained and constrained ordination serve different purposes; ordination can be used as a tool for extracting gradient structure from a dataset, and to generate hypotheses about important complex-gradients. Constrained ordination on the other hand, can be used for testing hypotheses about the response of the species composition to a set of environmental variables. It can also be used for partitioning of variation in a community dataset on different sets of explanatory environmental variables.

PCA is an unconstrained ordination method that uses combinations of linear regressions of the original response variables on the sites for dimension reduction. PCA is a robust method well suited for the ecotoxicological datasets, as pollutant data tend to be linear gradients of responses. The first and second ordination axes are called PC1 and PC2. Sites are visualised as points, and responses as arrows. Length of the arrows indicate the variability of variation, and the direction reflects correlation to ordination axes and other variables. Environmental variables can be passively fitted in the biplot, but will not influence the ordination space. RDA is a constrained parallel to PCA, performing dimension reduction based on linear regressions of a matrix of environmental variables (Greenacre & Primicerio, 2013). In addition to revealing relationships between variables, RDA can also be used for model building, and to estimate the explanatory power of each environmental variable by inspecting the eigenvalues of different combinations of variables.

In this study, PCA was used to identify structure in the contaminant dataset. To determine relationships to, and explanatory power of environmental variables, multivariate forward

model selection and variance partitioning was performed. To visualise the results, the environmental variables were fitted to the PCA plot.

Forward model selection was performed stepwise using Monte-Carlo permutation tests. In the first step, all environmental variables were tested separately to identify the ones with the best explanatory power. In step two, the best explaining variable identified in step one is conditioned in the model, meaning that the remaining variables are tested with the variation due to the best variable removed from the model. If any of the remaining variables are found to be significant in step two, the best one of these will be added as a conditioning variable, and the rest of the variables are tested again. This process continues until no variables are found to be significantly explaining any of the remaining variation.

Ability to explain variation was evaluated based on permutation p-values, and relative amount of variation explained (inertia), and pseudo-F. The pseudo-F is the ratio of constrained and unconstrained total inertia, each divided by their respective ranks (df).

Explanatory power of a component was expressed as a fraction of the model inertia divided by the total inertia of the unconstrained model. The variation partitioning also used the Monte-Carlo permutation approach. Each environmental variable was tested separately with all other significant environmental variables conditioned out.

All multivariate analyses were done using the vegan package in R (Oksanen et al., 2018). Permutation tests were performed using the permutest function, and passive variables were projected onto PCAs using the envfit function.

2.4.2 Contaminant patterns

To obtain an overview of the relative distribution of contaminant groups with different properties across the species and matrices, data was visualised as barplots of the sum of concentrations of groups as a proportion of the total contaminant concentration in each species and matrix.

To understand the distribution of contaminants with different properties in the matrices of the two species, they were grouped according to contaminant groups. Relative contribution of HCB, PCBs, PBDEs, PFASs and Hg in blood was compared between the species. Since

PFAS was not analysed in eggs of Common Eider, relative contribution of HCB, PCBs, PBDEs and Hg was compared in blood and eggs of both species.

In order to assess differences in metabolism between the species, the PCBs were grouped into metabolic groups based on Cl-substitutions, according to Kannan et al. (1995). Group I-compounds lack vicinal H-atoms, making them resistant to metabolism. Group II-compounds have vicinal H-atoms in the ortho-meta position, but metabolism is inhibited due to steric hindrance by diortho Cl-substitution. Group III-compounds have the same positioning of vicinal H-atoms as group II. However, group III can be metabolised because of non-ortho or mono-ortho Cl-substitution. All the group III PCBs included in this study were mono-ortho substituted. Group IV is also metabolisable, due to vicinal H-atoms in meta-para positions. Relative distribution of the PCB groups was visualised using barplots showing sum of concentrations of each group as a proportion of ΣPCB.

Relative distribution of PFASs was also examined using barplots to evaluate metabolic differences between species. Because of the low number of PFAS compounds analysed, no grouping was done in the plots, but the distributions were discussed with regards to carbon chain length, and classification as sulfonates or carboxylates.

2.4.3 Maternal transfers

Egg laying is a source of contaminant elimination in female birds. Ratios of egg:blood concentrations of individual congeners were calculated in order to quantify the transfer of contaminants between female and egg. A ratio greater than 1 indicates higher degree of maternal transfer, assuming the blood concentrations at sampling time is similar to the egg production time. In order to investigate the relationship between maternal transfers and lipophilicity, lipophilic congeners were ordered by K_{ow}.

3 RESULTS

3.1 Contaminants included

Censoring levels were set based on visual interpretations of plots showing percentages of nondetects in individual congeners. For the lipophilic contaminants the censoring limit was set to 25% nondetects per congener. For PFAS the limit was set to 30% nondetects per compound. Hg contained only one nondetect (1.7%), and no specific censoring limit was therefore set.

A total of 68 compounds were analysed in both species. After data censoring, 35 compounds were included in the statistical analyses. The included compounds are 23 organochlorides (PCBs and HCB), 5 PBDEs, 6 PFASs, and the element mercury. In Common Eider, PFASs were only analysed in blood. PFASs were therefore only included for blood. Ordinations performed on the substituted and the imputed datasets give similar results, indicating robust data.

Table 2: Contaminants included in the analyses after removal of groups with more than 25% (lipophilic contaminants) and 30% (PFAS) nondetects.

	Herring gull						**Eider duck**					
	Blood			**Egg**			**Blood**			**Egg**		
	Mean±sd	**Median**	**Range (min-max)**	**Mean±sd**	**Median**	**Range (min-max)**	**Mean±sd**	**Median**	**Range (min-max)**	**Mean±sd**	**Median**	**Range (min-max)**
HCB	0.422±0.314	0.300	<LOD-1.27	3.66±2.61	2.52	0.72 -9.35	2.92±0.72	2.95	1.80 -4.17	0.25±0.12	0.20	0.10 -0.42
triCB	0.0876±0.0516	0.0625	<LOD -0.240	1.04±1.38	0.600	0.13 -5.75	1.89±0.995	1.630	0.88 -4.11	1.89±0.995	1.63	<LOD - 4.11
tetraCB	1.55 ±1.63	0.940	<LOD -6.18	15.8±19.5	8.93	0.61 -65.3	18.3±12.3	13.2	7.25 -4.11	18.3±12.3	13.2	<LOD -49.1
pentaCB	3.75 ± 2.88	2.81	<LOD -10.2	53.4±54.4	38.2	3.62 -215	54.9±34.1	46.3	22.3 -49.1	54.9±34.1	46.3	22.3 -138
hexaCB	10.3 ±9.48	8.98	<LOD -40.8	132±101	102	16.5 -384	104±60.6	87.5	48.8 -138	104±60.6	87.5	<LOD -263
heptaCB	2.93 ±2.07	2.27	<LOD -9.27	48.9±30.4	46.9	8.94 -120	28.8±18.6	25.0	12.2 -263	28.8±18.6	25.0	<LOD -81.1
higherCB	0.358 ±0.315	0.260	<LOD -1.30	6.06±4.28	4.98	1.36 -16.0	1.68±1.58	1.09	0.440 -81.1	1.68±1.58	1.09	<LOD -6.86
BDE	0.785±0.890	0.363	<LOD -3.49	17.0±30.3	7.00	1.16 -114	1.11±0.742	0.942	0.646 -3.67	0.149±0.0845	0.121	<LOD -0.413
PFAS	35.3±7.77	12.39	<LOD -31.31	-	-	-	20.0±10.2	16.6	<LOD -45.2	-	-	-
Hg	4.26±0.72	4.51	<LOD -5.67	3.93±0.77	4.13	2.38 -5.12	4.95±0.44	4.94	4.30 -5.79	5.15±0.50	5.03	4.54 -5.97

3.2 Biometric variables

3.2.1 Lipid content and standardisation

Lipid content was higher in egg than blood in both species (Welch's t-test: Herring Gull: t=-11.445, p<0.0001, Common Eider: t=-56.59, p<0.0001). It was also higher in eggs of Common Eider than in eggs of Herring Gull (t= -23.608, p<0.0001), and higher in Herring Gull blood than in Common Eider blood (t= 4.7642, p= 0.00026) (Figure 1). There was close to no variation in the concentrations in eider blood, except for on one outlier.

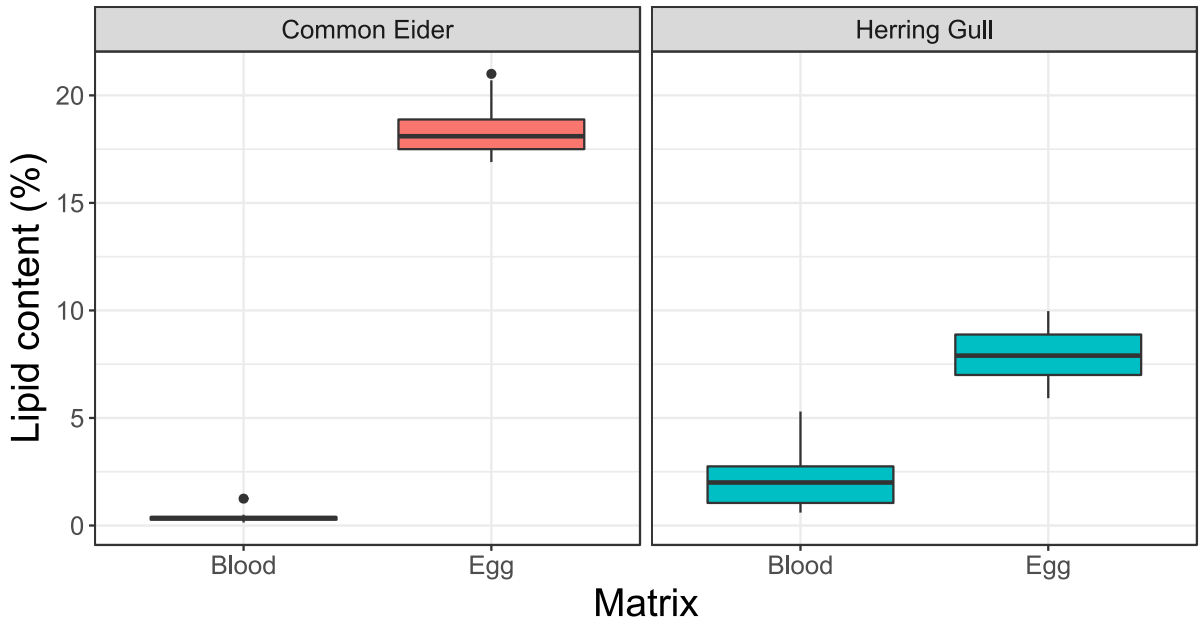

Figure 2: Lipid content in blood and eggs of Herring Gull and Common Eider. The middle line in each box represents median lipid content. The bottom and top lines represent the first and third quantiles. The whiskers represent the range of the data, and outliers are marked as individual points.

3.3 Carbon source and trophic status

There was a clear separation in $\delta^{13}C$ between Herring Gull and the marine food web, including Common Eider, with more negative $\delta^{13}C$ in Herring Gull than Common Eider and the other marine organisms (Figure 2). There was a difference in $\delta^{13}C$ between Herring Gull and Common Eider in both blood and eggs (Welch's t-test: blood: t=-9.8331, p<0.0001, eggs: t=-11.329, p<0.0001). The range of $\delta^{13}C$ values was larger for Common Eider than for the rest of the marine food web (Common Eider: range=-23.36 – -18.11, food web: range=-20.85 – -17.43). The range of $\delta^{13}C$ values was also larger for Common Eider than for Herring Gull (Herring Gull: min-max=-26.94 – -24.02).

The $\delta^{15}N$ value in Herring Gull was lower than in Common Eider (blood: t=-16.257, p<0.0001, eggs: t=-10.607, p<0.0001). Common Eider was placed in the middle part of the marine food web.

In both species, $\delta^{13}C$ had higher values in blood than in eggs (Herring Gull: t=6.2709, p<0.0001, Common Eider: t=3.4122, p<0.005), while $\delta^{15}N$ was not different between blood and eggs (Herring Gull: t=-1.4124, p=0.1704, Common Eider: t=-0.78064, p=0.4421).

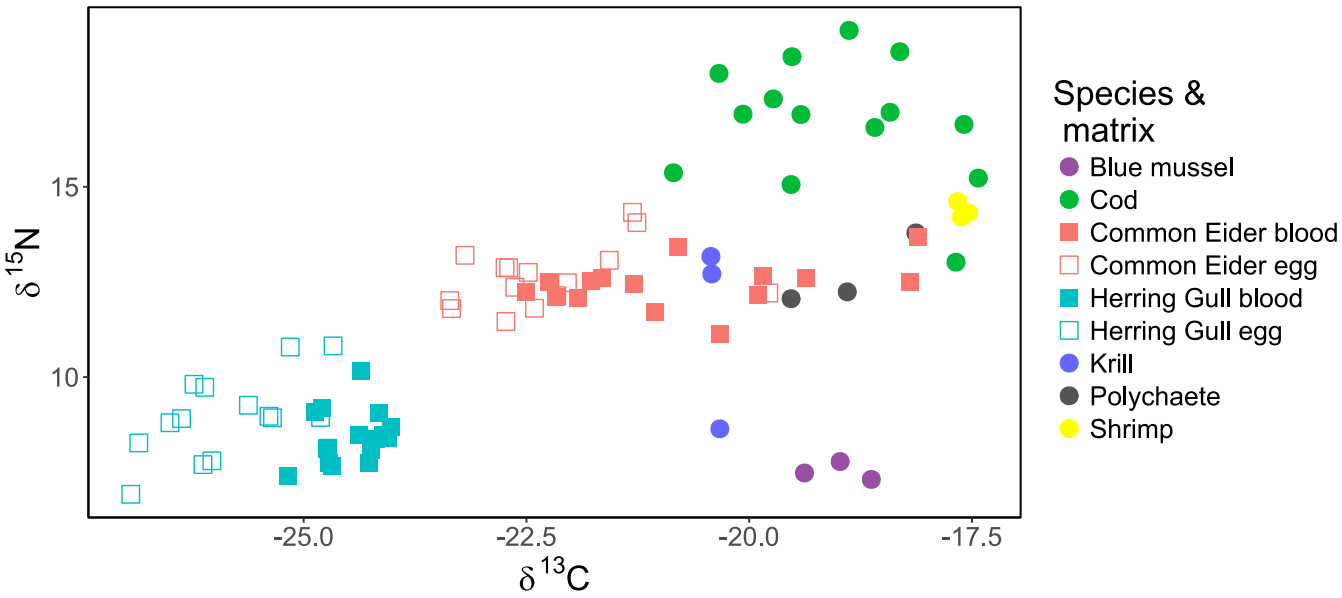

Figure 3: Scatterplot of stable isotope data. $\delta^{15}N$ is shown on the y-axis, $\delta^{13}C$ on the x-axis. Stable isotope values of blood and eggs of Herring Gull and Common Eider are plotted together with the other organisms sampled in the Urban fjord programme. Cod samples were individual samples of muscle, Blue Mussel were pooled samples

of soft body tissue, shrimp were pooled sample of soft tail tissue. Krill and polychaetes were pooled samples of whole individuals.

3.4 Contaminant concentrations and interrelationships with environmental variables

3.4.1 Species comparison of contaminant concentrations

The lipid normalised concentrations of lipophilic contaminants were higher in blood of Common Eider than in Herring Gull (Welch's two sample t-test: t=-30.626, p<0.0001). The concentrations in eggs were opposite; higher in Herring Gull than in Common Eider (Welch's two sample t-test: t=29.405, p<0.0001).

Wet weight concentrations were consistently higher in blood of Common Eider than in blood of Herring Gull (Welch's two sample t-test: t=-15.771, p<0.0001). However, the differences were smaller than for lipid normalised concentrations. Results were also corresponding for eggs in wet weight; concentrations were higher in Herring Gull than in Common Eider (Welch's two sample t-test: t=19.26, p<0.0001), but the differences were more accentuated in the lipid-normalised results.

Boxplots of species comparisons of lipid weight and wet weight contaminant concentrations ordered by K_{ow} can be found in Appendix B. There was no clear pattern of concentrations by K_{ow} in any matrix. PCB 153 is the congener with the highest lipid weight concentration in both matrices in both species.

Because PFAS was not analysed in Common Eider eggs, only blood concentrations were compared between species. When all PFASs were tested together, there was no difference in concentrations between the species (Welch's two sample t-test: t = -1.2067, p > 0.1). Concentrations of PFDA, PFUdA and PFHxS were higher in Common Eider than in Herring Gull (Welch's two sample t-test: t = -6.5874, p < 0.0001). PFTrDA was higher in Herring Gull than Common Eider (Welch's two sample t-test: t = 2.7151, p = 0.01145), and PFOS and PFDoA did not differ (Welch's two sample t-test: t = -0.73459, p > 0.1). In Hg, concentrations were higher in Common Eider in both blood (Welch's two sample t-test: t= -

3.1485, p= 0.00447) and eggs (Welch's two sample t-test: t= -5.1898, p < 0.0001). Boxplots of species comparisons of PFASs ordered by length of the carbon back bone and Hg can be found in Appendix B.

3.4.2 Interrelationships of contaminant concentrations and environmental variables

Multivariate analysis was conducted on the logarithmic contaminant concentrations to identify correlations between contaminant concentrations and environmental variables. To identify differences between species, multivariate analysis was performed for the matrices separately, and for lipophilic and non-lipophilic compounds separately.

In the PCA of concentrations of lipophilic contaminants in blood (Figure 4), PC1 explained 83.5%, and PC2 explained 9.3% of the variation in the data. With lipid content treated as a covariable, PC1 and PC2 explained 56.1% and 8.92% of the variation. Visual inspection of the PCA revealed a clear separation of the site scores for the two species along PC1. PC1 can be interpreted as a concentration gradient, increasing from left to right, revealing higher contaminant load in Common Eider than in Herring Gull. The PCB groups and HCB correlated. They were also positively correlated with $\delta^{15}N$ and $\delta^{13}C$ and C:N ratio, and negatively correlated with lipid content. Among the PCB groups, higherCB had some divergence from the other groups on PC2. HigherCB was also strongly correlated to carbon weight. The PBDES were separated from the PCBs and HCB, and the association to Common Eider was not as strong for this group. Among the PBDEs, there was a grouping of BDE47, BDE99 and BDE143, and of BDE100 and BDE154. BDE100 and BDE154 correlated with nitrogen weight. $\delta^{15}N$, $\delta^{13}C$, species and lipid content were all significant explanatory variables (RDA permutation test: $p<0.05$ for all), explaining 7.6%, 2.6%, 1.4% and 0.1% of the total variation in the dataset, respectively.

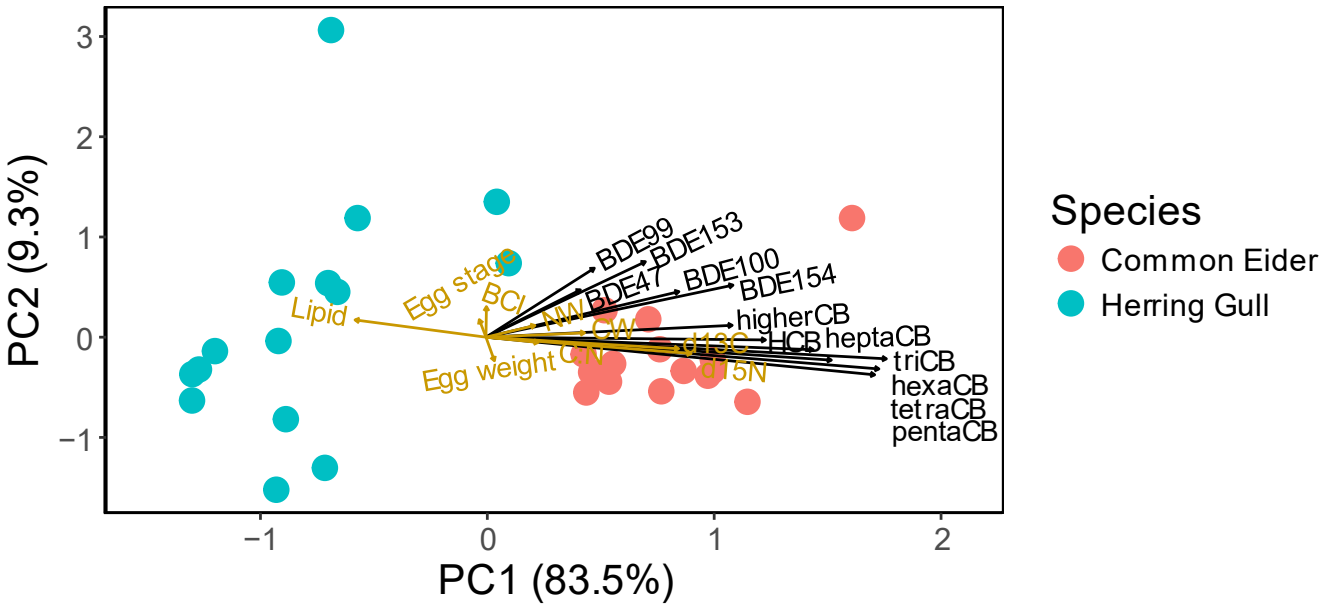

Figure 4: PCA biplot of log concentrations of lipophilic contaminants in wet weight in blood of Herring Gull and Common Eider. Individual birds (sites) are presented as points, and coloured according to the environmental variable species. Contaminant concentrations (response variables) are represented as black vectors, with length and direction of the arrows giving amount and direction of variation in the ordination space. Explanatory variables are fitted passive brown vectors. Correlation strength is given by the angle between vectors. Vectors pointing in the same direction are positively correlated, and vectors pointing in opposite directions are negatively correlated. Orthogonal vectors are uncorrelated.

For the non-lipophilic contaminants (Figure 5), PC1 explained 69.4% and PC2 explained 17.0% of the variation in the data. Based on visual inspection of the ordination diagram there was a clear separation of the species. PFTrDA and PFDoA correlated, and had higher loadings in Herring Gull than in Common Eider. Hg correlated with PFUdA, PFDA and PFHxS, and had higher loadings in Common Eider. PFOS was separated from the other PFASs, but was positively correlated with BCI and nitrogen weight, and negatively correlated with egg weight and C:N ratio. Both stable isotopes correlated with PFHxS. Carbon weight and egg stage were also positively correlated with the contaminants that had higher loadings in Common Eider. Lipid content was negatively correlated to the stable isotopes. $\delta^{15}N$, $\delta^{13}C$, species and lipid content significantly explained variation (RDA permutation test: $p<0.05$ for all), explaining 7.6%, 6.8%, 6.3% and 1.0%, respectively, of the total variation.

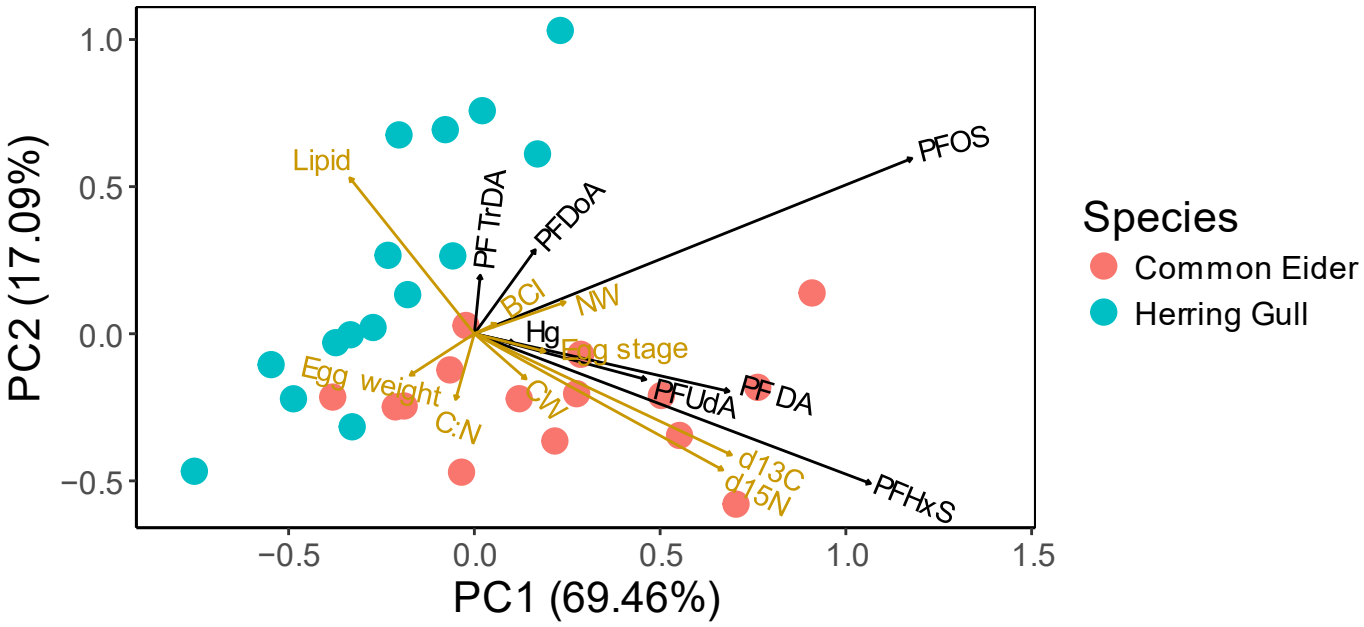

Figure 5: PCA biplot of log concentrations of non-lipophilic contaminants in wet weight in blood of Herring Gull and Common Eider. Individual birds (sites) are presented as points, and coloured according to the environmental variable species. Contaminant concentrations (response variables) are represented as black vectors, with length and direction of the arrows giving amount and direction of variation in the ordination space. Explanatory variables are fitted passive brown vectors. Correlation strength is given by the angle between vectors. Vectors pointing in the same direction are positively correlated, and vectors pointing in opposite directions are negatively correlated. Orthogonal vectors are uncorrelated.

In the PCA of lipophilic contaminants in eggs (Figure 6), PC1 explained 90.0% and PC2 explained 5.91% of the variation. Including lipid content as covariable results in PC1 and PC2 explaining 16.5% and 5.86% of the variation, respectively. Visual inspection revealed a clear separation of site sores for the two species. Lipid content is strongly correlated with PC1, and contributes to the variation explained by this axis. $\delta^{13}C$ and $\delta^{15}N$ also correlates with lipid, along with carbon weight and nitrogen weight. The contaminants were positively correlated, with higher loadings in Herring Gull. Species, $\delta^{15}N$, egg weight, WN, C:N, $\delta^{13}C$, WC and lipid content, all significantly explain variation in the data, explaining 2.7%, 1.4%, 1.3%, 1.2%, 0.7%, 0.6%, 0.6% and 0.3% of the total variation. Contaminant loadings increase with increasing nitrogen weight, and decreasing lipid content, stable isotopes, C:N ratio and carbon weight.

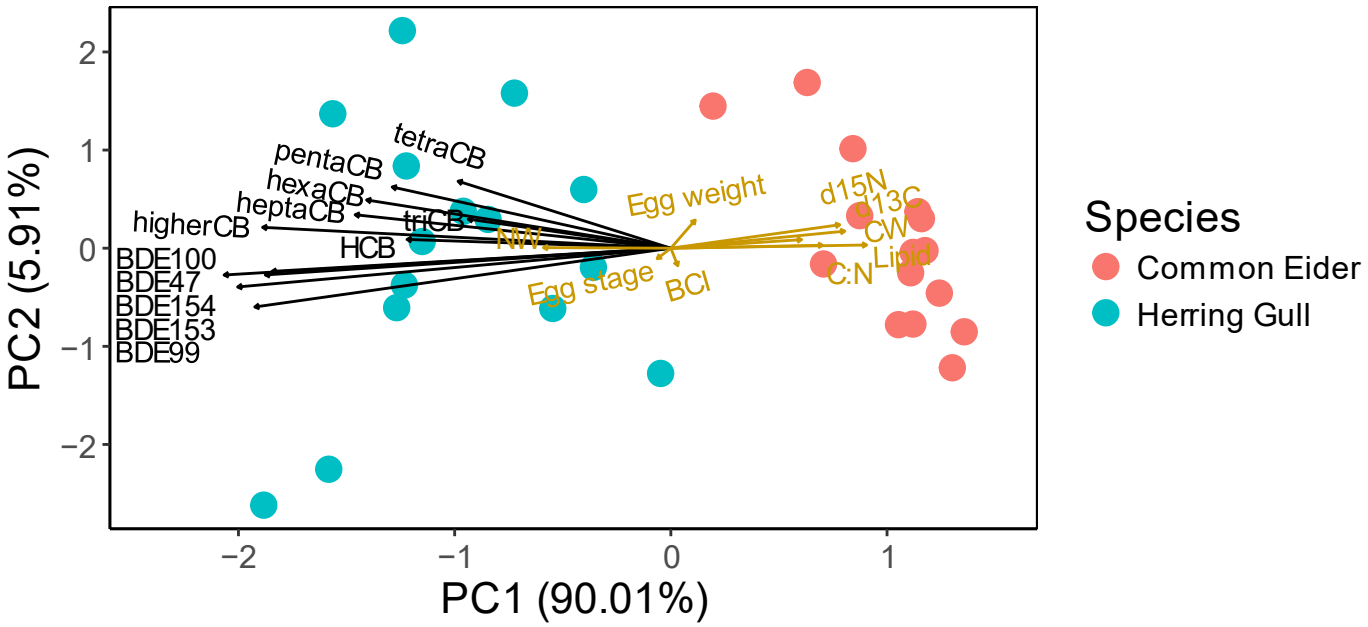

Figure 6: PCA biplot of log concentrations of lipophilic contaminants in wet weight in eggs of Herring Gull and Common Eider. Individual birds (sites) are presented as points, and coloured according to the environmental variable species. Contaminant concentrations (response variables) are represented as black vectors, with length and direction of the arrows giving amount and direction of variation in the ordination space. Explanatory variables are fitted passive brown vectors. Correlation strength is given by the angle between vectors. Vectors pointing in the same direction are positively correlated, and vectors pointing in opposite directions are negatively correlated. Orthogonal vectors are uncorrelated.

3.5 Contaminant patterns

When including all analysed congeners in blood (Figure 5), ΣPCB dominated the pattern in both species. The relative proportion of ΣPFAS and Hg was higher, and the relative proportion of ΣPCB and ΣPBDE was lower in Herring Gull than in Common Eider. The relative proportion of HCB was low in both species.

As PFAS was not analysed in eider eggs, the relative contribution of contaminants was plotted excluding PFAS in blood (Figure 6a), to make it comparable to the pattern of analysed contaminants in eggs (Figure 6b). ΣPCB was dominating in both eggs and blood. In Herring Gull Hg constituted a higher relative proportion in blood than in eggs, while in Common

Eider this was opposite. The relative proportion of ΣPBDE was higher in eggs than in blood of Herring Gull, while in Common Eider this was not the case.

In the relative contribution of PCB, HCB, PBDE and Hg in blood and eggs of Herring Gull and Common Eider (Figure), the species had opposite patterns in blood and eggs. The contribution of Hg in blood was higher in Herring Gull, while in eggs it was higher in Common Eider. The contribution of PCB was opposite, in blood it was higher in Common Eider and in eggs it was higher in Herring Gull. The PBDE contribution was similar between the species, with higher contribution in Herring Gull in both matrices, and the HCB contribution was similar in both matrices of both species.

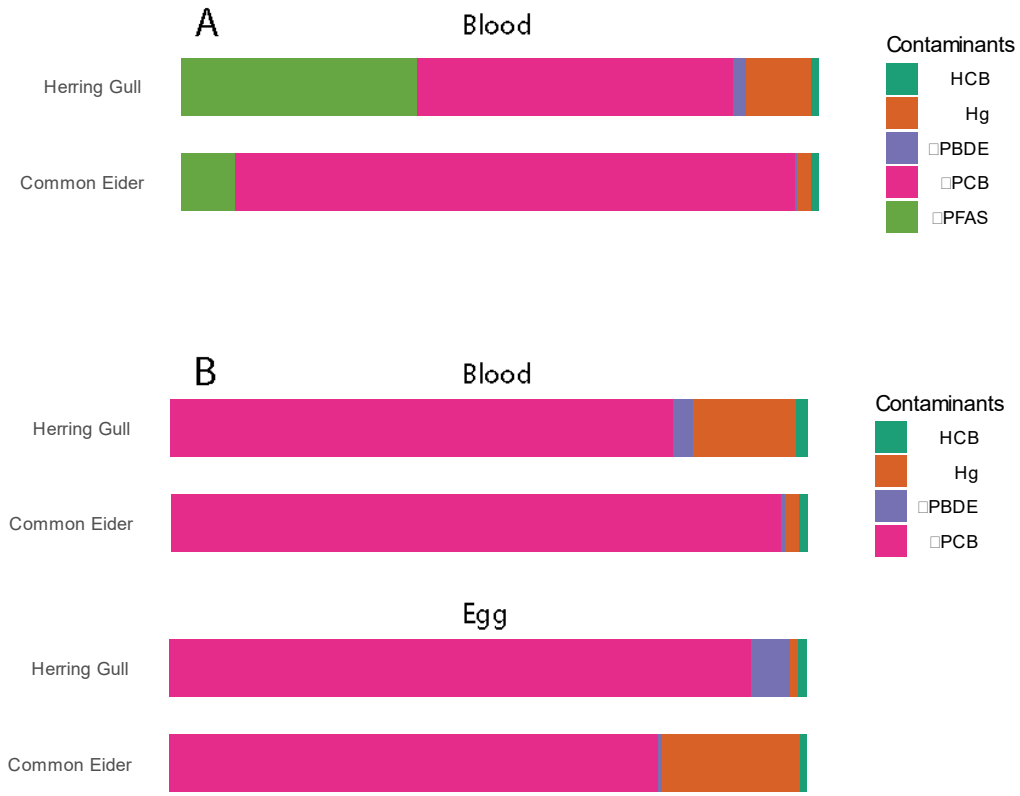

Figure 7: Relative contribution of all contaminant groups to the sum of these groups in blood (a) and relative contribution of HCB, Hg, ΣPBDE and ΣPCB to the sum of these groups in blood and eggs (b).

The relative distribution of PCB groups between blood and eggs within each species was similar (Figure 8). The contribution of persistent PCB groups is higher in Herring Gull, while contribution of the metabolisable groups is higher in Common Eider.

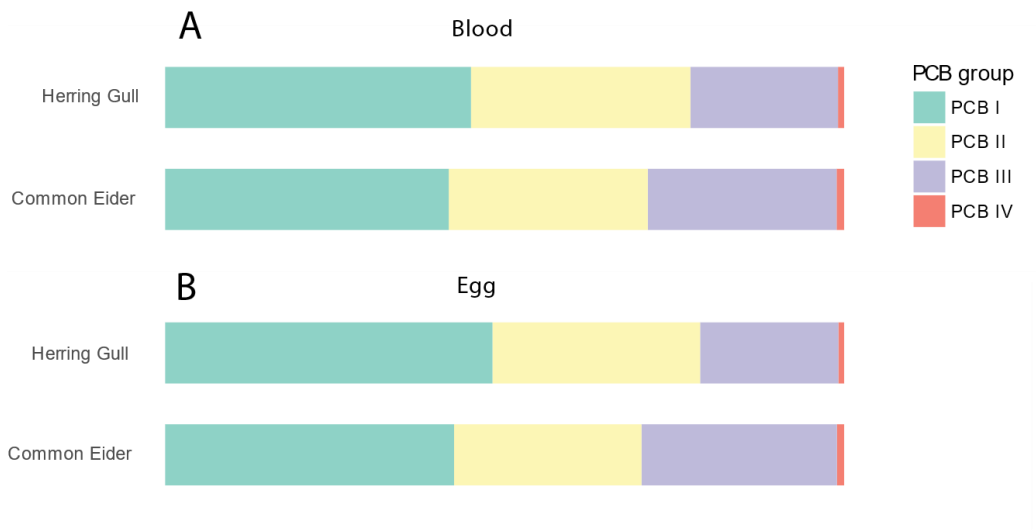

Figure 8: Distribution of metabolic PCB groups relative to the sum of PCBs in blood (a) and eggs (b).

For PFAS, there was notably higher contribution of the sulfonate PFOS in Herring Gull, and of sulfonate PFHxS and carboxylate PFDA in Common Eider. (Figure 9).

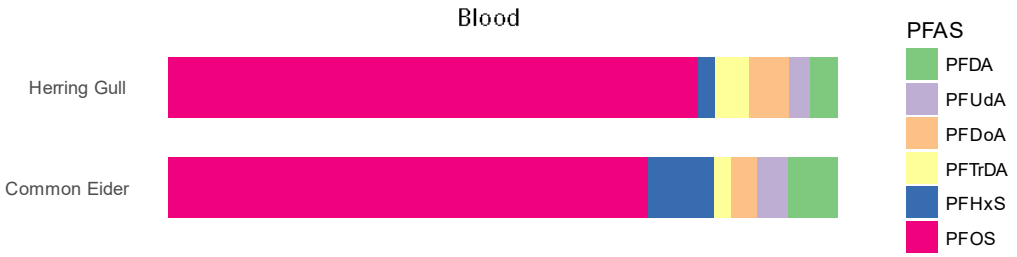

Figure 9: Distribution of PFASs relative to ΣPFAS

3.6 **Maternal transfer**

Ratios between lipid weight contaminant concentrations in eggs to blood were calculated for the individual compounds (Figure 10). In Herring Gulls all congeners had mean values above 1. In Common Eiders, all congeners were clearly below 1. There was no relationship between the maternal transfer ratios and lipophilicity as indicated by K_{ow}.

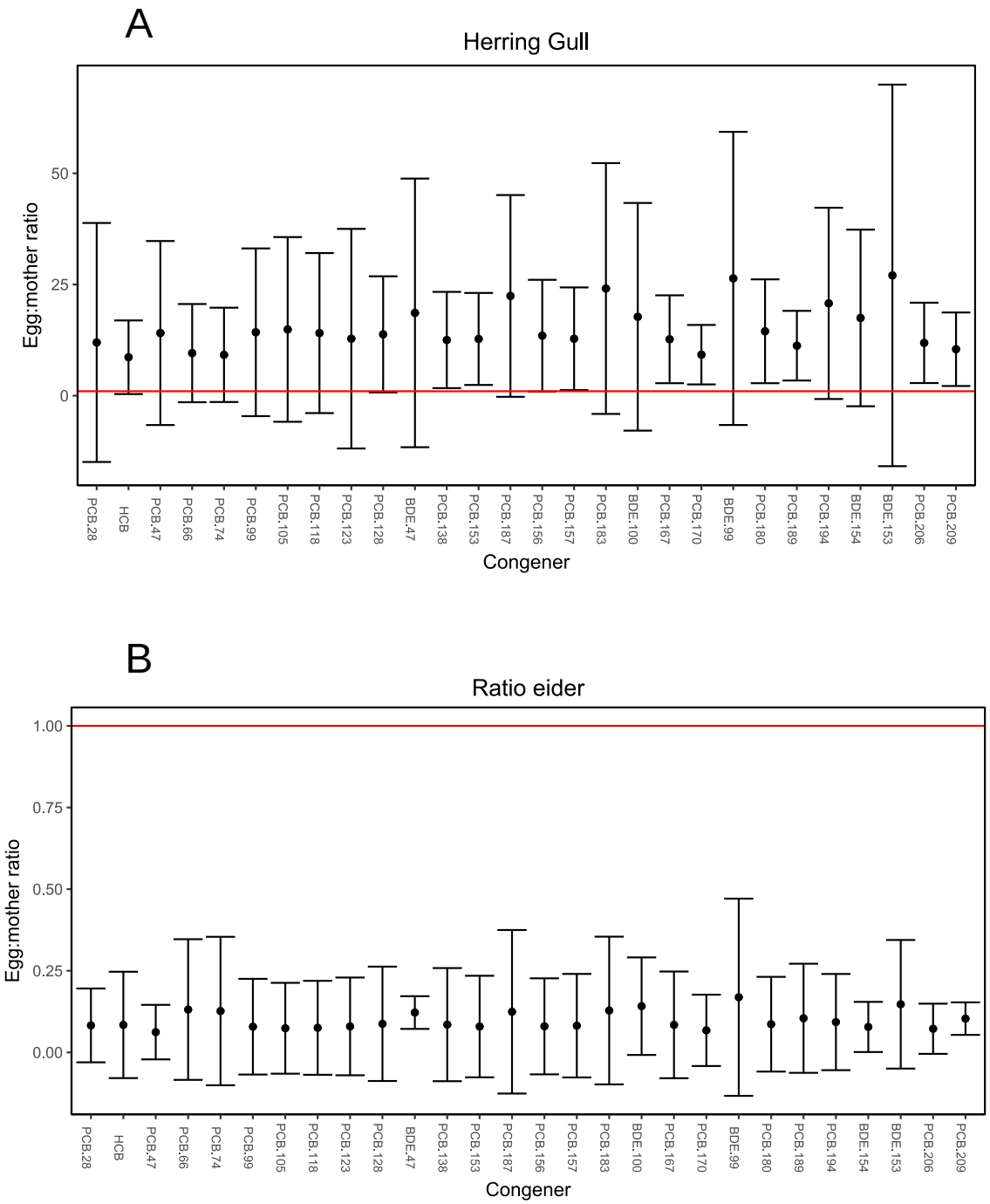

Figure 10: ratio of (mean concentration in eggs):(mean concentration in blood) in lipophilic contaminants for Herring Gull (a) and Common Eider (b). Error bars show standard error. Ratio >1 indicate maternal transfer. Congeners are sorted by K_{ow} value, increasing left to right.

4 Discussion

The overall aim of this study was to examine the suitability of Herring Gull and Common Eider as indicators of the contamination status of marine food webs in urban environments. Herring Gull is a widely used monitoring species, however, the individual opportunistic feeding habits make it hard to trace diet sources and link this to contaminant occurrence. This study investigated whether another seabird, the Common Eider, would be a better indicator of the contamination status of fjords in urban areas.

Common Eider breed on the Norwegian coastline (Huber et al., 2015). Its distribution is however not as wide as the Herring Gull, and this is a reason why it might not be as good indicator as the gull. Because of relatively low contaminants levels, Common Eider might be less suited as a contaminant monitoring species than other seabird species status (Mallory et al., 2004).

In the present study, stable isotopes were analysed to assess ecological niche of the two species in the urban environment of the Oslofjord, and occurrence of lipophilic and protein binding contaminants were compared between the species. This was done to establish whether the data met the expectations that the Oslofjord Herring Gulls are not part of the marine food web, but also feed from terrestrial sources. This might result in higher contaminant concentrations in the marine feeding Common Eider due to higher exposure in the marine food web than the terrestrial. To understand the impact of environmental factors on contaminant accumulation in the two species, relationships between contaminant patterns and environmental variables were identified using model selection and variance partitioning. Finally, maternal transfers of contaminants in the two species were assessed, and possible implications of maternal transfers on monitoring were discussed. The implications of matrix differences for contaminant studies and monitoring give a comprehensive assessment of the suitability of Herring Gull and Common Eider as monitoring species of contaminants status in urban environments.

4.1 Biological variables

Lipid content

The differences in lipid content in eggs and blood between the two species can be explained by differences in their ecology. Common Eiders are more precocial birds than Herring Gulls, and follow the mother to sea immediately after hatching (Ehrlich et al., 1988). The more altricial Herring Gull chicks spend some time around the nest before leaving (Ehrlich et al., 1988; O'Conner, 1984). This might explain a higher lipid percentage in the eider eggs, as chicks could require more lipid-rich eggs to reach a more developed stage before hatching.

Body condition index

There was a negative correlation between weight and the body size parameters in Common Eider. This could be related to their fasting behaviour, and the fact that the sampling date was late in the breeding period. Large individuals with initially sufficient fat reserves to last during the breeding period might not have left the nests to feed during the period, while smaller individuals might have fed regularly, sustaining the body condition. Individual differences in breeding strategy within the species could also lead to variation in contaminant concentrations when food is acquired at different times.

4.2 Trophic status and carbon source

The first objective was to assess the suitability of Herring Gull and Common Eider as representatives of the marine food web of the inner Oslofjord using stable isotopes.

Herring Gulls had lower $\delta^{13}C$ stable isotope values than the organisms of the marine food web, including Common Eider. This indicates a more terrestrial carbon source for Herring Gull compared to the marine food web (Peterson & Fry, 1987). Common Eider have a similar $\delta^{13}C$ signal to the other marine organisms, fitting well in the marine food web. Hence, the $\delta^{13}C$ results are in agreement with hypothesis 1.1, suggesting that Herring Gull is not part of the marine food web which Common Eider is part of.

Although the data support that eiders are members of the marine food web, the carbon signal of the Common Eider has a larger spread than that of the other marine organisms. In addition, the $\delta^{13}C$ values were lower in eggs than in blood of both species. This is likely related to

matrix properties. The lower $\delta^{13}C$ values in eggs compared to blood is likely an artefact of high lipid content in the eggs. Lipid is a tissue primarily made up of carbon, resulting in high C:N ratios in lipid rich tissues. The ^{13}C fraction is depleted in carbon found in lipids (DeNiro & Epstein, 1977). This introduces a bias that is increasing with increasing lipid concentrations (Post et al., 2007), and complicates the interpretation of stable carbon isotope values in lipid-rich matrices such as bird eggs. It also complicates the comparison of stable carbon isotopes between matrices with differing lipid content. For example, even if the carbon is derived from the same source, the $\delta^{13}C$ will have more negative values in lipid rich tissue (McConnaughey & McRoy, 1979). This bias could be omitted by performing lipid extraction prior to stable isotope analysis, but lipid extraction can cause washing out of nitrogenous compounds, leading to altered $\delta^{15}N$ ratios (Sweeting et al., 2006). Ideally, carbon and nitrogen stable isotope analyses should be run separately to ensure the most accurate results (Sweeting et al., 2006).

As an alternative to laboratory lipid extraction, correction for lipid content can be performed mathematically, using C:N ratios as a proxy for lipid content (Fry, 2002; McConnaughey & McRoy, 1979; Post et al., 2007; Sweeting et al., 2006). The use of such mathematical approaches is however not unproblematic, and suggestions for improvements and specialisations of the (McConnaughey & McRoy, 1979) approach have been suggested (Ehrich et al., 2011; Kiljunen et al., 2006; Post et al., 2007). The relationship between $\delta^{13}C$ and C:N is not consistent, and varies among taxa (Kiljunen et al., 2006; Logan et al., 2008). Therefore, species-or taxa-specific correction models are required (K. H. Elliott et al., 2014). According to (Post et al., 2007) accounting for lipids is necessary when working with matrices with high lipid content, or when comparing species with variable lipid content. When lipid content is consistently low, lipid correction is not necessary (Post et al., 2007).

The lipid correction of $\delta^{13}C$ in eggs resulted in more negative values, but did not change the interpretation of the species comparison. The correlation between corrected and non-corrected $\delta^{13}C$ was close to 1, indicating little impact of the correction. Because of the differing lipid content between eggs of Herring Gull and Common Eider, using the same parameters in the model might not give accurate results. Furthermore, mathematical lipid correction is associated with uncertainty when the birds are acquiring resources for egg production from different sources (Ehrich et al., 2011; Oppel et al., 2010). K. H. Elliott et al. (2014) warns about using this approach for coastal bird species.

Other possible explanations of the larger spread of the $\delta^{13}C$ values in Common Eider compared to the other marine organisms are more individual foraging preferences for the Common Eiders, and that nutrients for egg production may originate from varied sources. This will be further discussed in the maternal transfer discussion.

$\delta^{15}N$ was used to indicate trophic status, and by the positioning in the Urban fjord food web, the Oslofjord Common Eiders seem to fit well the common description as a mid-trophic predator (Huber et al., 2015). Common Eider has a higher position in the food web than the Blue Mussel, which is thought to be a main food item of the species (Öst & Kilpi, 1999). Herring Gull have lower $\delta^{15}N$ values than Common Eider. If Herring Gull was foraging mainly from the marine food web, a higher $\delta^{15}N$ would be expected for Herring Gull than Common Eider, as Herring Gull then would feed on a higher trophic level in the food web. The observed lower $\delta^{15}N$, combined with higher $\delta^{13}C$ therefore support the hypothesis of Herring Gull having a different food web baseline.

From these results it can be concluded that in the inner Oslofjord ecosystem, Common Eider is a part of the marine food web, while Herring Gull inhabits a different ecological niche. The Herring Gull is likely influenced by terrestrial and anthropogenic sources. The Herring Gull is however also known to forage from marine sources like fish in other habitats (Fox et al., 1990). Although the results show that fish is not a dominating part of the diet during the breeding season, exact feeding habits cannot be described by the results of this study. This implies a need for good knowledge base about the local habitat and ecological niche of populations when using seabirds for environmental monitoring in order to make correct conclusions about contaminants in an ecosystem.

4.3 Concentrations and patterns of contaminants

The second objective was to compare concentrations and patterns of contaminants in the two species, and evaluate the importance of species differences in ecological niche, metabolism and matrix differences for environmental monitoring.

4.3.1 Concentrations

All the contaminant groups included are known to accumulate in marine food webs (Borgå et al., 2006; Borgå et al., 2004; Borgå et al., 2001; Gregg T. Tomy et al., 2004). Based on this, and the expectation that the benthic-feeding Common Eider would have a more marine diet than the opportunistic Herring Gull in the urban environment of the Oslofjord, hypothesis H 2.1 stated that contaminant concentrations were expected to be higher in Common Eider than in Herring Gull. These expectations were strengthened by the stable isotope results, which suggested that Herring Gull was not linked to the marine food web to the same extent as Common Eider.

In accordance with hypothesis H2.1, blood concentrations of all contaminants were higher in Common Eider than in Herring Gull, except for three of the six PFASs investigated. The strong correlation of both stable isotopes to PCBs supports the theory that the concentration differences between the species can be explained by ecological niche. The PCB groups with the highest degree of chlorination were the ones with the lowest concentrations and the weakest correlations to the stable isotopes. This is consistent with the higher chlorinated PCBs being less available to organisms, and found in lower levels in the environment (Fisk et al., 1998; McFarland & Clarke, 1989). PCB 153 was the single congener with the highest concentration, which is consistent with PCB 153 being one of the most persistent PCB congeners, and the one which is found in the highest concentrations in the environment (Boon et al., 1987).

In addition to ecological niche, the observed higher contaminant concentrations of lipophilic contaminants in Common Eider blood can also be due to differences in breeding strategy between the species. In order to protect the eggs, female eiders rarely leave the nest during the breeding period. To maintain this strategy, they have developed the ability to store lipids prior to breeding, and fast during the breeding period (Korschgen, 1977; Milne, 1976). This results in remobilisation of contaminants from the lipid reserves (Jan O. Bustnes et al., 2012; Jan O. Bustnes et al., 2010). Body condition of female gulls also declines during the breeding period (Burger & Schreiber, 2001), but the Herring Gulls do not have the same extreme breeding strategy as the eiders, and the effects of lipid mobilising are not as strong (Cramp, 1983). Therefore, comparing contaminant concentrations in only females between the two species

during the breeding season might not reflect the feeding alone. To get a better understanding of consequences of contaminants in diet, it would be useful to also sample males.

There was no clear trend of differences in the PFAS concentration between the two species. The compounds which had higher concentrations in Common Eider were correlated with the stable isotopes, indicating that the species differences in concentrations of these PFASs could be related to a marine diet. The PFASs that had higher concentrations in Herring Gull were negatively correlated to C:N ratio, likely because of the protein binding properties of PFASs.

PFOS had notably higher concentrations than the other PFASs in both species. The PFOS concentration was in the same range of values as the wet weight concentration of lipophilic congeners with low K_{ow} -value. One explanation of similar concentrations of lipophilic congeners and PFOS is that PFOS is known to bind to lipoproteins (Armitage et al., 2013; Ng & Hungerbuhler, 2014). However, the lipid content in blood was low. A better explanation of the high concentration is that PFOS binds to albumin (P. D. Jones et al., 2003), which is an important constituent of whole blood in birds. Moreover, PFOS is one of the most used PFAS historically, and the release to the environment has been high for many years (Kallenborn, 2004). The contamination levels of this contaminant might therefore still be high even if the use is restricted today.

Of the PFASs with higher concentrations in Common Eider, PFHxS is a sulfonate, whilst PFDA and PFuDA are carboxylates. Both PFASs with higher concentrations in Herring Gull are carboxylates. However, because of the low number of PFASs, these observations do not give indications about trends in the data, and drawing conclusions on the distribution of sulfonates and carboxylates in the two species from only six included compounds is not possible. Sulfonates and carboxylates are considered long-chained, with potential of bioconcentration with carbon-chains longer than 6 and 7, respectively (Jonathan W. Martin et al., 2003). All the PFASs included in this study are considered to be long-chained. The boxplots of PFAS concentrations sorted by carbon chain length (Appendix B) revealed no pattern between chain length and concentration. Again, six compounds is not a sufficient sample size to observe trends in the data.

Results of studies assessing effects of trophic position and diet on concentrations of Hg in marine food webs are not always consistent. Studies have shown that Hg accumulate and biomagnify in food webs (Atwell et al., 1998; Borgå et al., 2006; Campbell et al., 2005), indicating that trophic position would be of importance in explaining concentration differences. Studies have also found that factors such as diet source, expressed by $\delta^{13}C$ can be of importance for explaining Hg levels (Bearhop et al., 2000; Monteiro et al., 1998)

In this study, Hg concentrations were higher in Common Eider than in Herring Gull in both matrices. In contrast to this, in a study of birds in the Barents Sea Herring Gulls had higher Hg concentrations than Common Eider (Savinov et al., 2003). This can be related to a more fish rich diet in the Barents Sea Herring Gulls than the Oslofjord Herring Gulls, as marine species are an important source of Hg (Lehnherr, 2014; Ruus, Øverjordet, et al., 2015). Herring Gulls from Hornøya, which was a study site in (Savinov et al., 2003), have been shown to be on a higher trophic level, and have a more marine diet than the Herring Gulls of Oslofjorden (Keilen, 2017). In another arctic study, also (Atwell et al., 1998) found higher $\delta^{15}N$ and higher Hg concentration in muscle tissue of the Larid species included in the study, Black-legged Kittiwake *(Rissa tridactyla)* and Glaucous Gull *(L. hyperboreus)*, than in Common Eider. This indicates that species differences of Hg concentration in Herring Gull and Common Eider can be linked to what trophic level they feed at. In the ordination of non-lipophilic contaminant concentrations, Hg was correlated with both stable isotopes, indicating importance of ecological nice for the species difference. The correlation is likely related to a more terrestrially influenced diet in Herring Gull, as concentrations of Hg in the marine environment are higher than in the terrestrial environment. Hg is known to accumulate in Blue Mussel, the main pray of the Common Eider (Ostapczuk et al., 1997; Öst & Kilpi, 1998).

Terrestrially influenced birds are expected to have lower contaminant concentrations than marine influenced birds, also in eggs. Based on the ecological niche as described by the stable isotope results, Common Eider would therefore be expected to have higher contaminant concentrations in eggs than Herring Gull in the Oslofjord. Contrary to this expectation, the results show higher contaminant concentrations in Herring Gull eggs than in Common Eider eggs. This might be explained by different maternal transfer mechanisms in the two species.

Based on these results, hypothesis H2.1 can be partly accepted. Concentrations of the lipophilic contaminants and Hg in blood were higher in Common Eider than in Herring Gull, and these differences can be explained by ecological niche of the species, as well as breeding strategy. It can however not be excluded that also other factors can contribute explanations. Trends and species differences in the PFAS concentrations are not as clear. For different PFASs, explanations include distribution in the environment as well as ecological niche.

4.3.1 Contaminant patterns

Hypothesis H2.2 stated that the contaminant pattern would be influenced by differences in metabolic abilities between the species as well as environmental distribution of the contaminants.

As legacy POPs and Hg are known to accumulate in the marine environment, recalcitrant contaminants were expected to be dominant in the pattern of the more marine influenced Common Eider. On the other hand, Herring Gull is expected to have more efficient contaminant metabolism than Common Eider (Borgå & al., Unpublished data), which would lead to an expectation of more recalcitrant congeners dominating the pattern in Herring Gull.

The contaminant composition of a species depends on uptake, distribution in body tissues and elimination, as well as distribution of contaminants in the environment (Borgå et al., 2001; Serafin, 1984). Toxicokinetics, i.e. the uptake, metabolism and elimination of a chemical in the body might differ between species (Walker, 1981). Contaminant patterns dominated by recalcitrant congeners could indicate more effective contaminant metabolism of less recalcitrant congeners (Borgå et al., 2001). For example, if one species has a pattern more dominated by PCB I than the other species, that species might have a more effective PCB-metabolism than the other.

Metabolic ability is dependent on metabolic rate of the species (Livingstone, 1992). Thus, if two species have different metabolic ability, this should be reflected in their contaminant pattern. In this study, the relative distribution of the metabolic PCB groups show higher share of metabolizable PCB groups II and III in Common Eiders in both blood and eggs, supporting the theory of better metabolic ability in Herring Gull than Common Eider.

The relative distribution of PFAS was dominated by PFOS for both species. This is in accordance to PFOS being the dominating PFAS in all biota (Kallenborn, 2004). According to (Haukås et al., 2007), high relative contribution of PFOS may be related to seabirds having an ability to biotransform and excrete less persistent fluorochemicals. Additionally, (G. T. Tomy, W. Budakowski, et al., 2004; G. T. Tomy, S. A. Tittlemier, et al., 2004) suggests that PFOSA and other precursors to PFOS may be biotransformed, increasing the relative amount of PFOS. In addition to PFOS being recalcitrant, the occurrence of the compound in the environment can explain the high contribution of this compound, as PFOS is a chemical which has been widely used and discharged over the years, even if it is restricted today. The observed high relative contribution of PFAS is thus in accordance with expectations.

The contribution of each contaminant group to the sum of contaminants in blood can be related to feeding habits in combination with distribution of the contaminants in the environment. PCBs are legacy contaminants. Even though they are now phased out and banned, they often dominate the contaminant pattern in wildlife, due to earlier high production and use combined with bioaccumulative properties and persistence (Dietz et al., 2015). The use of PFAS is still widespread, leaving the expectation of PFAS to be found in anthropogenic waste (Kallenborn, 2004). Herring Gulls are known to be feeding from waste dumps (Coulson, 2015), possibly being exposed to high PFAS concentrations. The PFAS with the highest concentration is PFOS, which is now also restricted. However, PFOS was restricted far later than the PCBs. Thus, the lower relative proportion of OC and higher of PFAS in Herring Gull could be related to terrestrial foraging.

The relative contribution of PCB and Hg in blood and eggs was opposite in the species. This could be related to matrix affinity and egg production, which will be addressed later.

4.1 Maternal transfer

Objective III was to evaluate the influence of diet and breeding strategy on maternal transfer of contaminants, and their implications for monitoring. From hypothesis H3.1 it was expected that as a result of higher concentrations in the mother, a more marine diet will also lead to higher contaminant concentrations transferred to eggs.

Differences in maternal transfer between species can be linked to differences in feeding ecology and breeding strategy (Drouillard & Norstrom, 2001). The composition of contaminants transferred to eggs is dependent on compound properties (Verreault et al., 2006). As protein is thought to be the limiting nutrient in egg production, maternal transfers are expected to be stronger for lipophilic than for protein-binding contaminants (Bolton et al., 1993; Hitchcock et al., in review). Thus, the the egg:mother ratio is in this study expected to be higher for the lipophilic contaminants than for Hg.

All maternal transfer ratios of lipophilic congeners were greater than 1 in Herring Gull, and smaller than 1 in Common Eider, indicating that maternal transfer of lipophilic contaminants is occurring to a higher degree in Herring Gull than in Common Eider. However, another explanation of the observed ratios is that during the fast, the eiders remobilise lipophilic contaminants to the blood stream, resulting in higher concentrations in blood at sampling than at egg laying. Exemplifying this, (Jan O. Bustnes et al., 2010) found a 3.6-fold increase in wet weight concentration of PCB 153 in blood of eiders during the breeding period. As a result, a good comparison of maternal transfer between the species would in addition require blood sampling of females prior to breeding.

Lipophilic congeners were ordered by K_{ow} to assess whether lipophilicity was of importance for maternal transfer. (Verreault et al., 2006) found that congeners of lower K_{ow}-values were easier transferred from blood to eggs in Glaucous Gull. The results of the present study, however, showed no apparent relationship between ratio and lipophilicity as indicated by K_{ow}.

In contrast to the lipophilic congeners, the egg:mother concentration ratio of Hg was smaller than 1 for Herring Gull, and greater than 1 for Common Eider. This is in accordance with the results of the lipophilic contaminants being explained by remobilisation of contaminants along with lipids to the blood stream, since Hg is not lipid associated and thus probably not remobilised into the blood stream (ref). However, both ratios were close to 1, indicating equilibrium in Hg concentration between the blood and egg.

The female investment and different strategies for acquiring resources for reproduction could be important in explaining the observed species differences in maternal transfer. Species investing higher amounts of lipids in a clutch has been found to have higher egg:mother ratios for lipophilic contaminants than species investing lower amounts of lipid (Drouillard & Norstrom, 2001). Further, females with large clutches relative to body mass transfer a greater

proportion of lipophilic contaminants to eggs than females producing smaller clutches (Hitchcock et al., in review). Common Eiders, using a more precocial strategy, invest more lipids in their clutch (Ehrlich et al., 1988; O'Conner, 1984), and produce bigger clutches than Herring Gulls (ref). Following this, a higher egg:mother ratio would be expected for Common Eider than Herring Gull. Contrastingly, this was not observed in the results. Again, this could be due to the remobilising of contaminants to the blood stream in Common Eider resulting in different blood concentrations in the female at egg laying and at sampling.

Lipophilic contaminant concentrations have been shown to increase with laying order in Herring Gulls (Pierre Mineau, 1982). Mercury concentrations, on the other hand, decline with laying sequence in both Herring Gull and Common Eider (Akearok et al., 2010; Becker, 1992; Becker & Sperveslage, 1989). Laying order was not controlled for in this study, introducing some uncertainty. However there are also studies which did not find differences in concentrations of lipophilic contaminants between eggs of a clutch, and that differences were bigger between mothers than within a clutch (Verreault et al., 2006), indicating that the random sampling do not create bias.

4.2 Considerations for monitoring

From this study, it is evident that the design of a monitoring programme should be carefully considered. Monitoring of urban areas will have different requirements than non-urban areas, and even small local changes can be of importance for the outcome of the study (Keilen, 2017; Knudtzon, 2019). The choice of monitoring species and matrix is dependent on the purpose of the monitoring. If the goal is to evaluate the contaminant status of an urban marine food web specifically, opportunistic species such as the commonly used Herring Gull might not be a good choice, as indicated by the results of this study. In this case, using an exclusively marine feeding bird as indicator of contaminant status is preferred. However, for assessing contaminant status of an urban area in general, Herring Gull can be a good choice, assuming sufficient knowledge about environmental factors such as feeding habits are also obtained. Herring Gull can also be good in cases of studying a specific response to

environmental change such as pollution, as was the case in the original Great Lakes monitoring programme.

Blood and eggs are effective matrices which can give pictures of the contaminant status of birds. However, the results of the matrices can vary depending on breeding strategy, compound properties of the contaminants, and lipid dynamics of the species. Therefore the matrices should be used in combination, and the mentioned factors should be accounted for in the interpretation of the results.

Herring Gull has been used in monitoring programs as an indicator high in the marine food web. In this study, Herring Gull was not found to be a high-trophic marine bird. Because of the marine niche of the Common Eider, it has potential of being a good indicator species for the marine environment. However Common Eider is a mid-trophic predator, and therefore might not completely fill the same role as the Herring Gull. Also, factors such as fasting during the breeding period can make the results of contaminant concentrations of blood and eggs in this species difficult to interpret.

As different contaminants have different affinities for lipids and proteins, matrix composition will affect the occurrence of contaminants. This has implications both for data treatment and interpretation.

When comparing contaminant patterns and concentrations between species, it is important to keep in mind that even though the same matrices are sampled in the species, they do not necessarily have the same nutrient composition, and therefore are not directly comparable. For lipophilic contaminants lipid content of the matrix have to be accounted for, while protein content is of importance in evaluation of protein binding contaminants. Further, care must be taken when evaluating of lipophilic and non-lipophilic contaminants together, as they might not be directly comparable.

When using seabird eggs in monitoring of contaminant status of the environment, origin of the egg constituents must be considered. Depending on life history, females can invest stored energy (capital breeding), or energy recently acquired (income breeding) in reproduction (Stephens et al., 2009). It is likely that eggs of species with a capital breeding strategy can be influenced by allochthonous contaminants, i.e. contaminants with origin outside of the breeding area, while eggs of birds with an income breeding strategy might reflect the contamination status of the breeding area. While placing species strictly in one of the two

categories is not realistic, Common Eiders can be considered to be closer to a capital breeding strategy than Herring Gull (Sénéchal et al., 2011) . This means that contaminant status of eider eggs can represent contaminant status of the mother and thus the environment over a longer timescale than Herring Gull eggs. Differences in breeding strategy could therefore be important to consider when designing monitoring programs. In the Oslofjord, the eiders arrive early, and also resources required prior to breeding could be expected to reflect local contamination. The origin of nutrients in eggs is therefore not likely to be important in explaining the results observed in this study. However, in other areas, where birds might be more influenced by allochthonous nutrients and contaminants during egg production, Herring Gull eggs might be expected to represent the contaminant status of the breeding area better than Common Eider eggs. Thus, local migration patterns should also be considered in combination with breeding strategy when choosing indicator species for contaminant monitoring.

Nutrient composition of the egg can also be of importance when discussing species differences in maternal transfer. It cannot just be assumed that the whole contaminant composition of an egg derives from allochthonous or autochthonous sources based on the breeding strategy of the species. The contaminants in different compartments of an egg will probably not derive from the same source, as the constituents of the eggs are not formed in the same time frame (Astheimer & Grau, 1990). In eiders, egg-lipids are found to be derived from stored lipid, while non-lipid constituents are derived from recent dietary sources (Sénéchal et al., 2011), and endogenous reserves play a bigger role in yolk than albumen production (Hobson et al., 2015). Yolk, the lipid-rich compartment where most lipophilic contaminants are stored (kilde) is formed in around 1-2 weeks (Astheimer & Grau, 1990), while albumen, which is the major Hg-binding constituent of eggs (Brasso et al., 2012; Magat & Sell, 1979), is formed in a few days (kilde). Differences in formation time of these compartments might indicate that lipophilic contaminants in the egg reflect dietary uptake over a longer period, and from a greater area than Hg. Furthermore, these effects could be expected to be stronger in Common Eider than in Herring Gull because of its capital breeding strategy.

5 Conclusion

The aim of this study was to examine the suitability of two seabird species as indicators of the contamination status of marine food webs in urban environments, and evaluate the role of ecological niche, metabolism and maternal transfer in use of blood and eggs of these species as monitoring matrices.

In the Oslofjord, the opportunistic Herring Gull is not part of the marine food web, and contaminant levels in this species might be influenced by terrestrial, urban and anthropogenic sources. As a consequence, use of Herring Gull as indicator species in environmental monitoring of urban marine food webs could confound the outcome of the monitoring.

Concentrations of lipophilic contaminants and Hg were higher in blood of Common Eider than blood of Herring Gull, indicating higher levels of these contaminants in the marine environment than in the terrestrial. Multivariate analysis indicated that higher concentrations in the marine feeding species could be related to their different ecological niche. However differences in breeding strategy could also be of importance, indicating the need for thorough knowledge about life history traits of indicator species.

From the pattern investigations it is clear that the ability to metabolise contaminants can be of importance for observed contamination status of the species. Maternal transfer results indicate that nutrient composition affect the contaminant composition of eggs, and that breeding strategy can affect the results of comparisons between mother and eggs. This further implies that to evaluate the results of a monitoring program appropriately, knowledge about the metabolism, and breeding strategy of the indicator species is important.

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6 Appendices

Appendix A: Biological variables

Table A1: Biometric data for Herring Gull (N=15) and Common Eider (N=15).

Species	Colour ring	Location	Wing length (mm)	Weight (g)	Head length (mm)	Bill height (mm)	BCI	Egg stage	Egg weight
Herring Gull	J5549	S. Skjælholmen	432	930	117.4	16.8	0.830	1	88.20
Herring Gull	JCL23	S. Skjælholmen	418	870	120.5	17.8	-1.63	4	62.43
Herring Gull	JCL59	S. Skjælholmen	427	770	110.9	16.3	-1.33	3	79.97
Herring Gull	JCL67	S. Skjælholmen	427	870	115.7	17.2	-0.0842	2	86.52
Herring Gull	JCL68	S. Skjælholmen	437	890	120.9	18.9	-1.28	2	86.66
Herring Gull	JCL72	S. Skjælholmen	430	990	117	18.3	2.39	4	85.00
Herring Gull	JCP52	S. Skjælholmen	422	930	117.8	18.4	0.707	4	68.39
Herring Gull	JJP01	S. Skjælholmen	426	885	118.8	16.85	-0.682	3	74.21
Herring Gull	JJP03	S. Skjælholmen	434	910	118.4	17.6	0.044	4	80.75
Herring Gull	JJP05	S. Skjælholmen	436	965	120.8	16.7	0.659	4	72.86
Herring Gull	JJP06	S. Skjælholmen	437	860	117.2	16.7	-0.783	3	91.48
Herring Gull	JJP07	S. Skjælholmen	438	950	120.4	17.7	0.404	4	69.47
Herring Gull	JJP18	S. Skjælholmen	429	830	113.6	NA	-0.428	4	74.52
Herring Gull	JJP19	S. Skjælholmen	429	900	117.4	17.7	0.112	2	97.78
Herring Gull	JJP21	S. Skjælholmen	415	900	115.8	17.1	0.613	5	61.80
Common Eider	NN	Husbergøya	294	1695	124.2	NA	0.307	1	105.30
Common Eider	NL	Husbergøya	308	1465	127	NA	-1.72	2	91.20

Common Eider	NK	Husbergøya	311	1660	130	NA	-1.03	1	105.60
Common Eider	KS	Husbergøya	300	1850	NA	NA	0.608	2	112.40
Common Eider	KR	Husbergøya	313	2290	NA	NA	1.89	1	106.70
Common Eider	MX	Husbergøya	306	2170	NA	NA	1.67	1	113.20
Common Eider	KT	Raudskjæra	304	1770	123.4	NA	-0.0434	3	94.00
Common Eider	KU	Raudskjæra	305	1660	124.6	NA	-0.617	4	91.80
Common Eider	KV	Raudskjæra	300	1720	124.5	NA	-0.0175	2	103.20
Common Eider	No data	Raudskjæra	314	2080	128.1	NA	0.809	3	111.10
Common Eider	NB	S. Skjælholmen	306	1525	128.1	NA	-1.30	4	119.90
Common Eider	NC	S. Skjælholmen	308	1875	127.7	NA	0.185	4	105.20
Common Eider	NE	S. Skjælholmen	315	1820	125.5	NA	-0.577	4	112.80
Common Eider	NH	S. Skjælholmen	302	1870	121.7	NA	0.558	2	107.70
Common Eider	NJ	S. Skjælholmen	302	1630	126.9	NA	-0.572	3	106.40

Table A2: Nutrient and isotope data for Herring Gull and Common eider blood and eggs (N=15 each)

Colour ring	**Species**	**Matrix**	**Lipid content**	**W%C**	**W%N**	**C/N**	**δ ^{13}C**	**δ^{15}N**
J5549	Herring Gull	Blood	5.2	58.25	11.61	5.02	-24.36	10.17
JCL23	Herring Gull	Blood	5.3	22.31	6.54	3.41	-24.71	7.74
JCL59	Herring Gull	Blood	2.3	51.12	11.38	4.49	-25.18	7.39
JCL67	Herring Gull	Blood	2.2	49.32	14.13	3.49	-24.05	8.41
JCL68	Herring Gull	Blood	1	57.68	10.88	5.30	-24.87	9.09
JCL72	Herring Gull	Blood	2.5	49.44	13.81	3.58	-24.27	7.73
JCP52	Herring Gull	Blood	2	52.22	13.06	4.00	-24.11	8.47
JJP01	Herring Gull	Blood	0.9	52.19	14.25	3.66	-24.16	9.06
JJP03	Herring Gull	Blood	0.6	52.11	13.38	3.89	-24.68	7.67
JJP05	Herring Gull	Blood	3	48.42	12.31	3.93	-24.24	8.08
JJP06	Herring Gull	Blood	0.7	56.51	11.46	4.93	-24.79	9.19

JJP07	Herring Gull	Blood	1.5	54.88	12.39	4.43	-24.38	8.48
JJP18	Herring Gull	Blood	1.9	50.36	13.57	3.71	-24.19	8.36
JJP19	Herring Gull	Blood	1.1	49.43	13.48	3.67	-24.02	8.70
JJP21	Herring Gull	Blood	4	51.04	12.87	3.97	-24.74	8.10
J5549	Herring Gull	Egg	18.76	64.01	6.61	9.68	-23.36	12.01
JCL23	Herring Gull	Egg	17.38	65.49	6.31	10.38	-21.31	14.33
JCL59	Herring Gull	Egg	18.6	64.17	6.24	10.29	-21.57	13.07
JCL67	Herring Gull	Egg	19	66.49	6.57	10.13	-22.63	12.36
JCL68	Herring Gull	Egg	19.22	64.88	6.41	10.12	-22.48	12.75
JCL72	Herring Gull	Egg	16.9	64.62	6.74	9.59	-22.41	11.81
JCP52	Herring Gull	Egg	17.5	66.26	6.52	10.16	-19.78	12.21
JJP01	Herring Gull	Egg	18.1	64.91	6.75	9.62	-22.04	12.48
JJP03	Herring Gull	Egg	21	65.71	6.53	10.07	-22.74	12.88
JJP05	Herring Gull	Egg	18.7	68.35	5.90	11.58	-22.16	12.14
JJP06	Herring Gull	Egg	17.1	65.22	7.07	9.23	-21.26	14.06
JJP07	Herring Gull	Egg	17.92	56.32	6.11	9.21	-23.34	11.80
JJP18	Herring Gull	Egg	18	64.41	6.87	9.37	-22.73	11.46
JJP19	Herring Gull	Egg	17.5	66.53	6.81	9.78	-22.70	12.87
JJP21	Herring Gull	Egg	20.7	65.45	6.70	9.76	-23.19	13.20
NN	Common Eider	Blood	0.35	52.57	14.62	3.60	-20.33	11.13
NL	Common Eider	Blood	0.5	52.11	14.61	3.57	-18.11	13.67
NK	Common Eider	Blood	0.29	57.95	11.14	5.20	-21.77	12.53
KS	Common Eider	Blood	0.45	55.87	12.06	4.63	-21.65	12.61
KR	Common Eider	Blood	0.36	55.69	12.81	4.35	-21.06	11.70
MX	Common Eider	Blood	0.35	50.07	13.67	3.66	-19.90	12.17
KT	Common Eider	Blood	0.29	56.69	13.83	4.10	-18.19	12.49
KU	Common Eider	Blood	0.33	50.27	13.73	3.66	-19.36	12.61
KV	Common Eider	Blood	0.36	51.35	14.46	3.55	-19.84	12.66
No data	Common Eider	Blood	0.14	61.87	10.58	5.85	-22.17	12.10
NB	Common Eider	Blood	1.25	57.81	12.52	4.62	-20.80	13.42
NC	Common Eider	Blood	0.43	58.20	11.64	5.00	-22.50	12.24
NE	Common Eider	Blood	0.24	58.66	11.91	4.92	-21.92	12.07
NH	Common Eider	Blood	0.25	57.39	12.38	4.64	-21.29	12.45
NJ	Common Eider	Blood	0.2	58.34	11.93	4.89	-22.25	12.51
NN	Common Eider	Egg	9.82	61.89	7.41	8.35	-25.15	10.79
NL	Common Eider	Egg	7.9	58.04	7.31	7.94	-26.13	7.70
NK	Common Eider	Egg	8.96	60.31	6.63	9.10	-26.94	6.92
KS	Common Eider	Egg	8.12	61.58	6.95	8.86	-26.37	8.91
KR	Common Eider	Egg	9.5	61.52	6.84	9.00	-26.11	9.73
MX	Common Eider	Egg	7.7	59.87	9.17	6.53	-25.62	9.26

KT	Common Eider	Egg	7.15	59.46	7.49	7.94	-26.03	7.80
KU	Common Eider	Egg	8.1	58.38	7.15	8.17	-26.50	8.80
KV	Common Eider	Egg	6.85	58.55	9.88	5.93	-25.39	8.97
No data	Common Eider	Egg	6.77	57.71	9.14	6.32	-24.73	8.13
NB	Common Eider	Egg	8.8	56.48	8.21	6.88	-26.23	9.81
NC	Common Eider	Egg	9.97	62.64	7.08	8.85	-26.85	8.27
NE	Common Eider	Egg	7.82	59.59	9.06	6.57	-24.67	10.82
NH	Common Eider	Egg	6.82	58.81	9.44	6.23	-25.35	8.93
NJ	Common Eider	Egg	5.92	42.30	8.73	4.85	-24.81	8.94

Appendix B: Figures

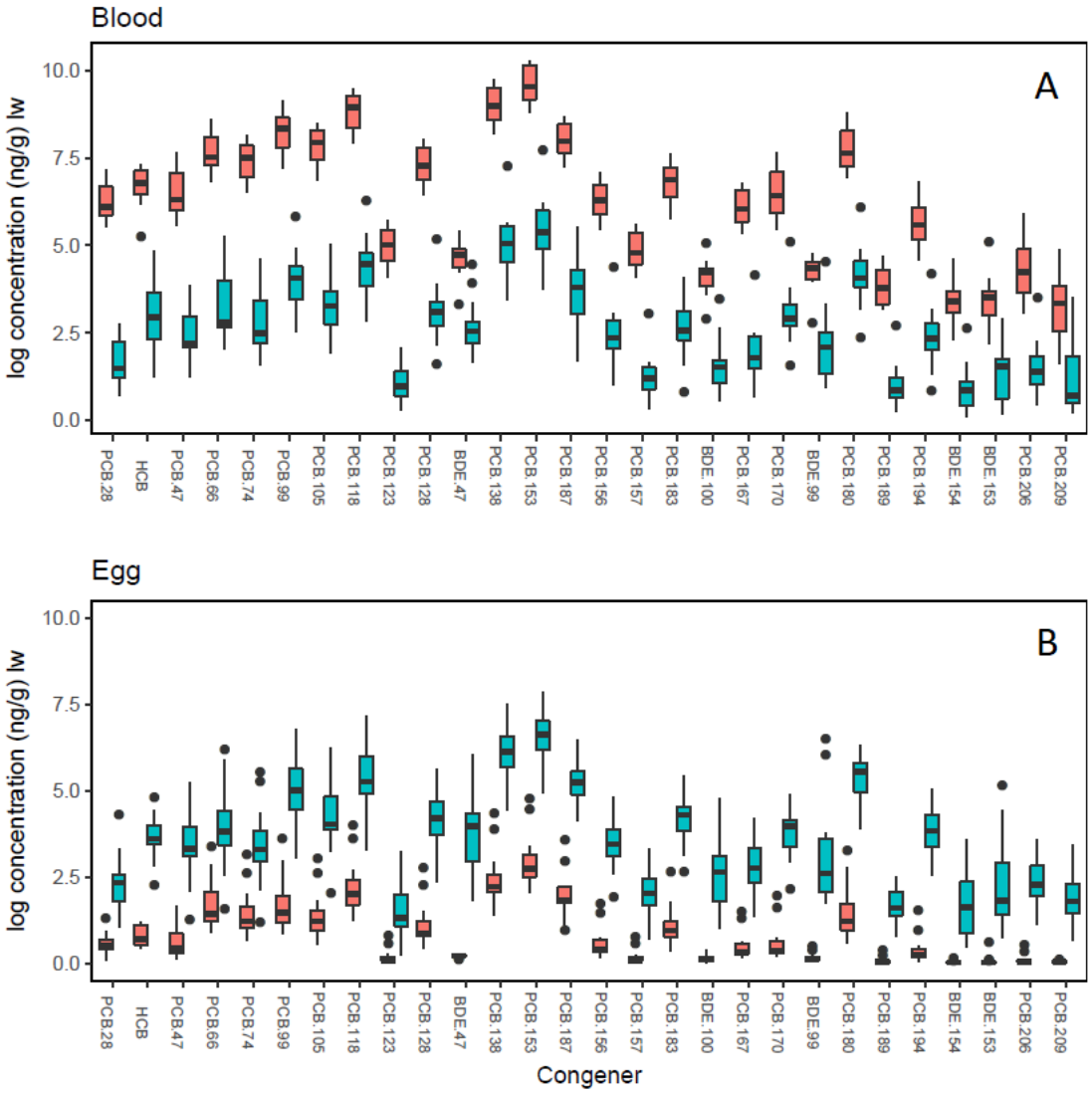

Figure 3: Log transformed lipid weight concentrations of lipophilic contaminants in blood (a) and eggs (b), ordered by K_{ow} (low – high). The middle line in each box represents median lipid content. The bottom and top lines represent the first and third quantiles. The whiskers represent the range of the data, and outliers are marked as individual points.

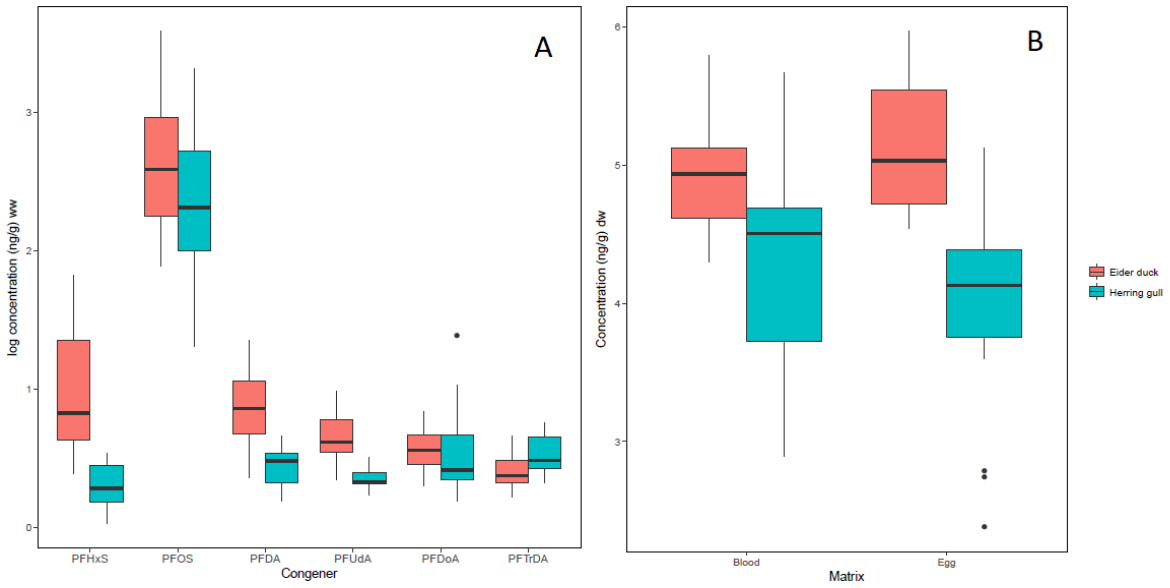

Figure 4: log transformed wet weight PFAS concentrations in blood (a) and log transformed dry weight Hg concentration (b) in blood and eggs of herring gull and eider duck. PFAS congeners are ranked by c-chain length. The middle line in each box represents median lipid content. The bottom and top lines represent the first and third quantiles. The whiskers represent the range of the data, and outliers are marked as individual points.