Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Molly Y. McPartland

DNA-double strand breaks in relation to PFASs and toxic elements in incubating female common eiders (*Somateria mollissima*) in the central Baltic Sea

Master's thesis in Cell and Molecular Biology
Supervisor: Åse Krøkje, IBI
June 2019

NTNU
Norwegian University of
Science and Technology

Molly Y. McPartland

DNA-double strand breaks in relation to PFASs and toxic elements in incubating female common eiders (*Somateria mollissima*) in the central Baltic Sea

Master's thesis in Cell and Molecular Biology
Supervisor: Åse Krøkje, IBI
June 2019

Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

NTNU
Kunnskap for en bedre verden

Acknowledgments

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

First, I would like to thank my supervisor, Åse Krøkje for the opportunity to contribute to this research, for guidance the past two years, as well as believing and pushing me towards my fullest potential. I would also like to thank my co-supervisor, Christian Sonne for the quick responses even at the busiest of times, and his endless enthusiasm for this project. Without your persistence I might have missed out on the experience of field work, and for that I am very grateful. Svend-Erik Garbus, with the help of Tomas Jessen and Peter Lyngs, also deserve a huge thank you for executing the field work and keeping this project alive. Without all of you, I never would have been able to learn and explore this topic as I have, and for that I cannot thank you enough.

I would also like to thank Chris Bingham and Syverin Lierhagen for the lab assistance and patience with my endless questions. Thank you to Brenley Noori and Randi Rodvelt who always had time to respond to my questions, no matter how simple or silly they might have been. And a colossal thank you to Louis Hunninck who was always there to fix my R code, feed me chocolate, listen to me complain, point me in the right direction, give me feedback, and provide moral support. I'm so happy we shared a hallway, and a generally misanthropic disposition.

Lastly, thanks to the family. Mara, I'll consider us even for all that chemistry help. Just kidding, really, I would have crumpled from the start without you. Megan, nobody believes in me more than you, with this thesis and generally just in life. I am so lucky to have you two. To the parents, thanks for teaching me to be curious and critical. For everything else, thanks are not enough.

June, 2019

Molly McPartland

Abstract

Per- and polyfluorinated alkylated substances (PFASs) and toxic elements have both been linked to adverse effects on genetic material which can result in severe consequences for individuals and entire populations. The aim of study was to assess the impact of blood concentrations of PFASs and toxic elements on DNA double strand breaks (DNA-DSBs) in the Common Eider (*Somateria mollissima)* breeding on Christiansø, Denmark in 2018. Furthermore, blood clinical chemistry parameters (BCCPs) were used to analyze the overall health of the incubating Eiders. The colony assessed in this study is of interest as it has experienced two mass mortality events, is part of a declining population, and has been shown poor genetic health.

Using a non-invasive approach, the same incubating female Eiders were caught and sampled once on day 5 (n=25) and once on day 20 (n=23) of incubation to provide a biological baseline for each bird enabling comparison between the early and late stages of incubation. Blood cells from whole blood were used to analyze DNA-DSBs by agaroses gel electrophoresis where the migrated fraction of total DNA (DNA-FTM) and median molecular length (MML) was quantified. Blood concentrations of chemical elements and PFASs were quantified using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) and liquid chromatography tandem-mass spectrometry (LC−MS/MS), respectively. BCCPs were provided by Aarhus University as part of a routine procedure associated with a larger research project concerning Eiders on Christiansø, Denmark.

PFASs, Cadmium (Cd), and Lead (Pb) blood concentrations increased from day 5 to day 20 of incubation most likely due to reuptake caused by lipid and protein metabolism during fasting. Zinc (Zn), Calcium (Ca), Potassium (K), Iron (Fe), Selenium (Se) and many BCCPs decreased, again likely due to fasting causing depletion of essential elements and metabolic enzymes. Strong positive correlations between body mass, total protein and albumin support the likelihood that protein catabolism is occurring meaning the Eiders have entered the third stage of fasting.

Blood concentrations of Pb were significantly elevated and well above toxicity threshold levels at which adverse effects and mortality can occur. The Pb blood levels in 2017 were significantly lower than in 2018 indicating a new point source pollution. It is possible the Christiansø Eider colony is experiencing Pb poisoning and in danger of another mass mortality event.

On day 5 of incubation DNA-DSBs were negatively correlated to PFASs while on day 20 DNA-DSBs were negatively correlated to PFASs and Mercury (Hg), but positively correlated to body mass. This indicates that as the stress of incubation increases, represented by decreases in body mass, DNA integrity decreases in tandem. However, unexpectedly, as blood concentrations of Hg and PFASs increase, DNA integrity increases in tandem. This can possibly be explained by nonlinear dose-response relationships, adaptive responses to chronic exposure, or increased rates of apoptosis and decreased rates of DNA repair.

The results of this study show the importance of continuing to monitor the Christiansø Eider colony and are applicable for both the conservation of the Christiansø Eider colony and for elucidating the complex mechanisms behind maintaining genetic homeostasis despite stress from multiple sources.

Abbreviations

Adj.	Adjusted
AIC	Akaike information criterion
AICc	Akaike information criterion corrected
AICwt	Akaike information criterion weight
ALAD	Aminolevulinate dehydratase
ALAT	Alanine aminotransferase
ALB	Albumin
ALKP	Alkaline phosphatase
AMY	Amylase
ANOVA	Analysis of variance
AP sites	apurinic/apyrimidinic site
As	Arsenic
ASAT	Asparate aminotransferase
BCCP	Blood clinical chemistry parameters
Ca	Calcium
Cd	Cadmium
CHOL	Cholesterol
Cl	Chloride
Co	Cobalt
Coeff. Value	Coefficient value
CREA	Creatine
Cu	Copper
CV	Coefficient of variation
DDE	Dichlorodiphenyldichloroethylene
df	Degrees of freedom
DNA	Deoxyribonucleic acid
DNA-DSBs	DNA double strand breaks
DNA-FTM	DNA fraction that migrated

EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
Fe	Iron
Fl	Fluorine
FRU	Fructose
g	grams
GGT	Gamma glutamyl transferase
GI tract	Gastro-intestinal tract
GLOB	Globulin
GLU	Glucose
GPx	Glutathione peroxidase
GSH	Glutathione
HCB	Hexachlorobenzene
Hg	Mercury
HNO_3	Nitric acid
HSD	Honest significant difference
HS-ICP-MS	High resolution inductively coupled plasma mass spectrometry
ID	Individual
K	Potassium
Kbps	Kilobase pairs
Kg	Kilograms
LC−MS/MS	Liquid chromatography tandem-mass spectrometry
LDH	Lactase dehydrogenase
LMPA	Low melting point agarose
LOD	Limit of detection
MeHg	Methyl mercury
mg	Milligram
Mg	Magnesium
mL	Milliliter
MML	Median molecular length
Na	Sodium

ng	Nanograms
NHEJ	Non-homologues end joining
NTNU	Norwegian University of Science and Technology
Pb	Lead
PC1	Principal component 1
PC2	Principal component 2
PCA	Principal component analysis
PCBs	Polychlorinated biphenyls
PFAS	Per- and poly-fluorinated alkylated substances
PFBS	Perfluorobutanesulfonic acid
PFCAs	Perfluoroalkyl carboxylates
PFDA	Perfluorodecanoic acid
PFDoA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptane sulfonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFOSF	Perfluorooctanesulfonyl fluoride
PFSAs	Perfluoroalkyl sulfonates
PFTrA	Perfluorotridecanoic acid
PFUnA	Perfluoroundecanoic acid
PHOS	Phosphate
POPs	Persistent organic pollutants
PPARα	Peroxisome proliferation-activated receptor- alpha
PPRE	Peroxisome proliferator hormone response
QQ	Quantile-quantile
Rf	Relative front
ROS	Reactive oxygen species

rpm	Rotations per minute
SCEs	Sister chromatid exchanges
SD	Standard deviation
SDS	sodium dodecyl sulfate
Se	Selenium
SE	Standard error
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TAs	Telomer alcohols
TBE buffer	Tris-borate-EDTA buffer
TE buffer	Tris- EDTA buffer
TP	Total protein
UDS	Unscheduled DNA synthesis
UV	Ultraviolet
ww	Wet weight
Zn	Zinc

Contents

1. Introduction

1.1. The Baltic Sea

The Baltic Sea amasses pollution from atmospheric deposition, point sources, and land-based diffusion which contribute to marine ecosystems suffering from some of the worst contamination in the world. In addition to this, the Baltic Sea has natural features that predispose it to accumulate pollutants. For example, long water residence time, expansive catchment areas, and shallowness all exacerbate the contamination load on organisms in the Baltic Sea (HELCOM, 2010). Furthermore, the Baltic Sea hosts 85 million people on its shorelines, who contribute to the pollution via traffic, shipping, energy production, agriculture, and large-scale industry (HELCOM, 2018). Per and poly-fluorinated alkylated acids (PFASs) and toxic elements are two pollutants of concern in the Baltic Sea which have been detected in organisms and are correlated to adverse health effects (Fenstad et al., 2017; Sahlin, 2017).

The Baltic Sea can be divided into seventeen sub-basins based on natural land and water features. In general, moving from north to south, these basins become more polluted (HELCOM, 2010), excluding the forty-four pollution hotspots that are still active today (HELCOM, 2018). Of these seventeen, Bornholm basin and Arkona basin are classified as moderate to poor "hazardous substance status" containing two and five industry, agricultural, and municipal pollution hotspots, respectively. In both locations biomonitoring of wildlife has demonstrated elevated levels of toxic elements and organic pollutants (HELCOM, 2010) that have been linked to a wide range of negative health effects, both in wildlife and in humans.

1.2. The Common Eider

The Common Eider (*Somateria mollissima)* hereby referred to as Eider is the largest duck in the northern hemisphere and is found along the coastlines of Scandinavia, Iceland, Svalbard, and much of northern Canada. The Eider is a migratory bird that overwinters separately from its breeding location (Waltho and Coulson, 2015). During the breeding season (April-July) female Eiders incubate for 26 days without leaving their nest and lose approximately 30% of their body fat (Korschgen, 1977). During this time lipophilic and amphipathic pollutants are mobilized in the blood stream and may add to the stress of the already fasting bird. Reuptake of compounds has

been documented in Svalbard (Fenstad et al., 2014), northern Norway (Bustnes et al. 2012), and the Baltic Sea (Fenstad et al. 2016b; 2017). The Eider is a capital breeder, meaning it prepares resources before the start of breeding, in part, at its wintering location. The main food source for the Eider is the blue mussel (*Mytilus edulis*), though if mussel stocks are low they will also eat cockles, clams, and crabs (Waltho and Coulson, 2015).

The Eider is an important organism for analyzing the effects of chemical contamination (Savinov et al., 2003; Fenstad et al., 2017; Haarr et al., 2018). Though the Eider has a low trophic level it feeds from the benthic food web taking up pollutants that have precipitated to the ocean floor (Waltho and Coulson, 2015) possibly putting them at higher risk than other pelagic feeders (Borgå et al., 2004). Furthermore, because Eiders are a long-lived species they have the capability to accumulate high levels of contaminants that have a long half-life (Savinov et al., 2003). Because Eiders do not leave their nests for 26 days, samples can be taken at the start and end of this incubation period providing a biological baseline for each bird (Fenstad et al., 2014). Additionally, colonies of Eiders are located ubiquitously throughout Arctic and sub-Arctic coastlines providing comparable data concerning source proximity (Fenstad et al. 2016b), long range transport (Fenstad et al., 2017), and the effect of multiple stressors (Dey et al., 2018).

1.2.1. The Christiansø Eider Colony

The colony of focus in this project breeds on the island of Christiansø, Denmark in the central Baltic Sea, specifically in the Bornholm basin. This colony overwinters from the western parts of the Baltic Sea to the southern Dutch part of the Wadden Sea, though exact locations are unknown (Noer, 1991). Consisting of approximately 2,000 females (10% of the breeding Danish Eiders) this colony is part of the Baltic/Wadden Flyway population which has a total of 900,000 birds ranging from Finland to Germany (Ekroos et al., 2012).

This colony is of particular interest given it has experienced drastic population decline in the last two decades with mostly unknown explanations (Ekroos et al., 2012). Additionally, two mass mortality events have occurred (2007, 2015) and are attributed to parasite infection resulting in poor body condition and starvation (Garbus et al., 2018). Furthermore, the Christiansø Eider population has an unexpectedly large decrease in body mass during incubation as compared to Arctic Eider colonies that experience much harsher conditions (Fenstad et al., 2016a). It has also been demonstrated that mussel quality and body condition are positively related, indicating that

the health and quantity of mussels play a large role in Eider breeding success (Laursen and Møller, 2016).

Blood clinical chemistry parameters (BCCPs) are non-invasive tools that have been used to evaluate liver, bone, metabolic, or renal function (Harr, 2002) and are therefore widely used as biomarkers of overall Eider health (Hollmen et al., 2001; Gabus, 2016). BCCPs will be used in this study to evaluate Eider condition. However, interpreting BCCPs can be difficult as they fluctuate greatly based on species, diet, and year (Hollmen et al., 2001; Sonne et al., 2010). BCCPs from Eiders breeding on Christiansø, Denmark have been routinely collected and used to the evaluate fasting, body condition, and susceptibility to parasites. (Hollmen et al., 2001; Garbus, 2016) Through analysis of total protein (TP), albumin (ALB), and urea population declines were linked to nutritional deficient and disease (Hollmen et al., 2001) which was later confirmed with autopsies of dead Eiders (Garbus et al., 2018).

BCCPs have been used to link chemical concentration to impacts on the liver, kidneys, bones, endocrine system, and metabolism across species (Sonne et al., 2008; 2010; 2012; 2013; Garbus et al., 2016). Analysis of certain liver enzymes such as alanine aminotransferase (ALAT) and gamma glutamyl transferase (GGT) can indicate hepatic failure (Sonne et al., 2013) which has been linked to chemical contamination exposure. Furthermore, BCCPs have been used to evaluate parasitic infection (Garbus et al., 2016) which again, is relevant to the Christiansø Eider colony given the two mass mortality events described above.

Due to the population declines and mass mortality events, the Eiders on Christiansø, Denmark are an interesting colony to study as this project may provide explanations to their declining health and overall numbers.

1.3. Per- and poly-fluorinated Alkylated Substances (PFASs)

1.3.1. Characteristics and Properties of PFASs

Invented in the 1950s, PFASs are anthropogenically produced organic chemicals that have a variety of unique properties such as high temperature resistance, friction reduction, and oil or water repellant. Because of these advantageous properties, PFASs have been used as surfacants, in fire-fighting foam and in industrial and commercial practices (Mueller and Yingling, 2017; Sahlin, 2017).

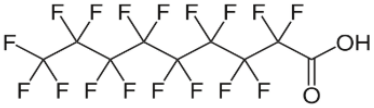
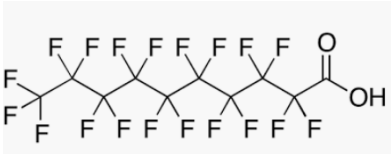
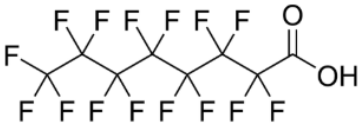
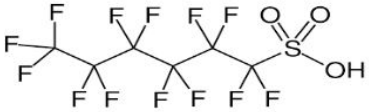
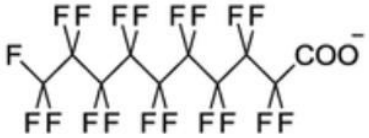
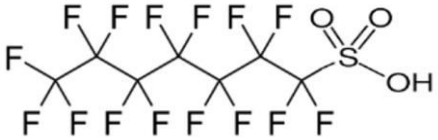
In the 1990s the scientific community began to discover the toxicity of PFASs to both organisms and their environment, through observations of occupationally exposed humans and later through experimentation on rodents (Buck et al., 2011). Since then, research concerning the detection, toxicity, transport, and fate of PFASs rapidly increased (Buck et al., 2011). In 2009, perfluorooctane sulfonic acid (PFOS), perfluorooctanesulfonyl fluoride (PFOSF), and their precursors, two of the most commonly produced and toxic PFASs, were added the Stockholm's Convention list of persistent organic pollutants (POPs) which outlawed their use as a means of "protecting human health" (UNEP, 2009).

PFASs are organic compounds where carbon atoms, have been halogenated by fluorine either in a process called electrochemical fluorination or telomerization (Dewitt, 2015). These fluorine substitutes contain partial negative charges (δ-) making them nuetrophiles that readily attack biological molecules (Vlasov, 1993). 'PFASs' is a term that encompasses per- or polyfluroalkyl acids (PFAAs) that can be further divided into perfluoroalkyl carboxylates (PFCAs) or fluorinated carbons and perfluoroalkyl sulfonates (PFSAs). PFASs are categorized by the length of their carbon chains. Long-chain PFCAs contain seven or more fluorinated carbons while long-chain PFSAs contain six or more fluorinated carbons. Conversely, PFCAs with fewer than seven fluorine substitutes are considered short-chain as are PFSAs with fewer than six fluorine substitutes (Table 1.1). The length of the carbon chain chiefly contributes to the chemical's longevity and stability (Hong et al., 2015). All the PFASs that have been outlawed by the Stockholm convention are long-chain PFASs as they are confirmed to be toxic, bioaccumulative and persistent in the environment (Krafft and Riess, 2015) while shorter chain PFASs are degraded more rapidly (Hong et al., 2015). Furthermore, long-chain PFASs persistence and stability enable transport in atmospheric and oceanic currents in a process called global distillation (Fernández and Grimalt, 2003).

While some PFASs are considered POPs, they are neither lipo- nor hydrophilic and are instead considered amphipathic, meaning they bind to proteins. This causes them to accumulate in protein-rich tissues such as the blood, liver, and eggs (Dewitt, 2015; Mueller and Yingling, 2017). PFASs have been shown to cause mortality at high doses and growth defects as well as developmental delays at lower doses in multiple species (Chen et al., 2014b; Dewitt, 2015; Nordén et al., 2016).

Furthermore, in rodents PFASs have been shown to affect the immune system by impairing T and B cell mediated immunity (Dewitt, 2015). In relation to the Christiansø Eider colony, PFASs exposure coupled with decreased immunity during incubation (Hanssen et al., 2003) could be a contributing factor to the parasitic infections causing the mass mortality events described above (Section 1.2.1).

Table 1.1. The name, acronym, structure, and chemical classification of all PFASs included in this study plus additional environmentally and toxicological relevant PFASs. Chemical classification is based on chain length and chemical structure. Modified from Wielsøe et al., 2015.

Name	# of Carbons	Structure	Classification
Perfluorooctane sulfonic acid (PFOS)	8		Long-chained sulfonic acid
Perfluorononanoic acid (PFNA)	9		Long-chained carboxylic acid
Perfluorodecanoic acid (PFDA)	10		Long-chained carboxylic acid
Perfluorooctanoic acid (PFOA)	8		Long-chained carboxylic acid
Perfluorohexane sulfonic acid (PFHxS)	6		Long-chained sulfonic acid
Perfluoroundecanoic acid (PFUnA)	11		Long-chained carboxylic acid
Perfluoroheptane sulfonic acid (PFHpS)	7		Long-chained sulfonic acid

Lastly, there have been reports of PFASs in Eider eggs (Rodvelt, 2018) showing that PFASs can be maternally offloaded. This is indicant of multi-generational effects as PFASs has been shown to be an endocrine disruptor (Tartu et al., 2014). High doses of PFASs during early development have been shown to impair organogenesis and cause malformations in zebrafish (Chen et al., 2014b). This has been observed across species with the great cormorant (*Phalacrocorax carbo sinensis),* herring gull (*Larus argentatus*) chicken *(Gallus gallus domesticus)* (Nordén et al., 2016), and mouse (Abbott et al., 2009). Therefore, it is possible reports of PFASs in Eiders breeding on Christiansø, Denmark are contributing to the observed population declines.

1.3.2. Distribution and sources of PFASs in the Baltic

PFASs have been reported globally, including in "pristine" locations supposedly untouched by human habitation, such as the Arctic (Peck et al., 2016) and Antarctica (Vecchiato et al., 2015) proving their capability of long-range transport. However, the highest levels of PFASs occur from point source pollution which is evidenced by greater contamination in water samples closer to larger cities as opposed to rural locations (Kunacheva et al., 2012). In the central Baltic Sea there are a number of pollution sources, most notably contamination from fire-fighter foam, industrial practices, and consumer products (HELCOM, 2010; Sahlin, 2017). Furthermore, wastewater treatment plants do very little to remove PFASs (Dewitt, 2015) further contributing to their contamination. Despite PFOS, PFOA, and other similar compounds being phased-out by their major global manufactures (USEPA, 2002; UNEP, 2009) PFOS is still the most common PFASs found ubiquitously in the Baltic biota (HELCOM, 2010; 2018) proving its stability and persistence in marine ecosystems.

PFOS have been found in herring gulls (*Larus argentatus*), grey seals (*Halichoerus grypus*), harbor seals (*Phoca vitulina*), and ringed seals (*Pusa hispida*) within the Baltic Sea (HELCOM, 2010). These pollution trends again support the finding that proximity to larger cities (Stockholm) reflects higher levels of PFOS, though generally the distribution is spatially homogenous throughout the Baltic Sea (HELCOM, 2010). PFNA and PFOA have also shown to be persistent in the biota as they are the next most common PFASs detected from biomonitoring of wildlife (Haukås et al., 2007). Derivatives of directly manufactured PFASs, such as PFUnA, have also been found in the biota, water, and sediment in the Baltic (Sahlin, 2017). Due to emerging concern and toxicity

reports, many companies, particularly in North America and in the European Union (EU) have chosen to phase out the use of long-chain PFAS and instead use shorter chain replacements or different halogenation (USEPA, 2002; UNEP, 2009). However, while this complies with safety regulations the new chemicals being manufactured have similar chemical structures and are therefore likely to have similar toxicological effects (Mueller and Yingling, 2017). For example, perfluorobutane sulfonate (PFBS) and perfluorohexanoic acid (PFHxA) have been used as PFOS and PFOA replacements (Dewitt, 2015) and coincidingly have been found in increasing amounts in the Baltic Sea biota (Möller et al., 2010; Sahlin, 2017).

Due to these pollution trends and evidence of toxic effects, PFASs in the Baltic Sea pose a threat to the Christiansø Eider colony. This study aims to quantify PFASs levels in Eiders both at the start and end of their incubation period as a means of contributing to the biomonitoring of PFASs in the Baltic Sea as well research concerning the adverse effects of PFASs.

1.4. Chemical Elements

1.4.1. Characteristics and Properties of Chemical Elements

As with PFASs, chemical elements have been found in elevated amounts in the Baltic Sea (HELCOM, 2010). However, different from PFASs there are existing reports of chemical elements in Eiders resulting in adverse health effects and mortality (Hollmen et al., 1998, Franson et al, 2000a; 2000b; Fenstad et al., 2016a). Therefore, chemical elements were included in this study as previous research supports the hypothesis that chemical element exposure could be a contributing factor to poor health in the Christiansø Eider colony.

Chemical elements, while naturally occurring, are considered a major pollution of concern as they have become prevalent enough to cause toxicity through smelting plants, ore processing, and combustion (Nordberg et al., 2014). Though all organisms have mechanisms to cope with chemical elements, when levels rise above background concentrations these mechanisms can be overwhelmed leading to adverse effects on the survival and health of wildlife (Koivula and Eeva, 2010).

For the purposes of this study ‘chemical elements’ refers to metals. Chemical elements are characterized by conductivity, durability, and the formation of cations in biological systems

(Nordberg et al., 2014). Chemical elements can further be divided into essential and non-essential elements (Nordberg et al., 2014). Essential elements including Calcium (Ca), Iron (Fe), Potassium (K), Selenium (Se) and Zinc (Zn) are necessary in biological systems and are taken up by organisms to maintain homeostasis. However, non-essential elements, hereby referred to as toxic elements, have the capability to mimic essential elements which can influence absorption and distribution of the element (discussed below). Furthermore, deficiencies in certain essential elements have been linked to increased levels of toxic elements which can, again, be caused by changes in toxicokinetics (see section 1.4).

The most toxicologically relevant chemical elements are Mercury (Hg), Cadmium (Cd), Lead (Pb) and Arsenic (As) both because of their toxicity and prevalence. These four elements have been extensively studied and shown to cause detrimental effects across species, organ systems, and cells. For example, Hg has been shown to cause birth defects, be neuro- and immuno- toxic (Crespo-López et al., 2009), and contribute to both reproductive and developmental toxicity (Murvoll et al., 2007; Ciesielski et al., 2017). Cd is primarily nephrotoxic though has also been the cause of severe bone deformities. Similarly, As and Pb have both been the cause of egregious human health incidences (Jaishankar et al., 2014).

Like PFASs, chemical elements can persist in the biota with the capability of biomagnification throughout the food chain (Mason et al., 1995; Lavoie et al., 2013). In aquatic environments chemical elements can bind to particles in sediments, forming an ultimate sink (Driscoll et al., 2013). This puts benthic organisms at risk as they feed and live within these sediments. For example, Hg, Cd, and Pb have been detected above toxicity thresholds in blue mussels, the Eiders preferred food, within the Baltic Sea (HELCOM, 2018). Furthermore, coastal environments with a large catchment area, such as the Baltic Sea, can collect chemical elements as they accumulate from run-off and river influxes as well as from deposition and point source pollution (Nordberg et al., 2014).

1.4.2. Distribution and sources of chemical elements in the Baltic

In the Baltic Sea there are several sources of toxic element pollution. Most prominent are large combustion plants, mines, metal smelters, and waste incinerators (HELCOM, 2010). Though steps have been taken to minimize the amount of Hg, Cd, and Pb released into the Baltic Sea, such as

industrial regulations and cleaner combustion process, toxic elements are persistent and therefore their levels reflect not just current pollution trends, but those from decades ago as well (HELCOM, 2018). As with PFASs, toxic element pollution is the result of global production due to long-range transport. In fact, it is estimated that 15%, 25%, and 50% of all Cd, Hg, and Pb respectively, in the Baltic Sea is a result of atmospheric deposition (HELCOM, 2018). Due to point sources and global pollution the Baltic Sea has measurements of Pb, Hg, and Cd above background levels in both biotic and abiotic medias. Furthermore, surface sediment levels have also shown to exceed toxicity thresholds (HELCOM, 2018) which, as mentioned before, puts benthic feeders, and organisms eating from the benthic food web, like the Eider, at particular risk as they are directly ingesting the toxic element (Chen et al., 2014a).

1.5. Toxicokinetics of PFASs and Chemical Elements

In the field of toxicology, toxicokinetics refers to the time dependent processes related to toxicants as they interact with an organism. Once exposed to a toxicant there are a series of biological processes that either increase or decrease the concentration of an ultimate toxicant at the target site (Lehman-McKeeman, 2013). Discussed below is the exposure route, absorption, distribution, storage and elimination of PFASs, As, Cd, Pb, and Hg. Research concerning the toxicokinetics of these compounds in Eiders is limited, therefore other avian species are used as examples. However, in situations where toxicokinetics in avian species could not be found, other species as well as in *vitro* experiments are referenced.

1.5.1. PFASs

It is pertinent to mention that many PFASs are not produced as such but are the derivatives of telomer alcohols (TAs), polyfluoroalkyl phosphates (PAPs), and POFS-based intermediates (Dewitt, 2015). Upon exposure to oxygen, microorganisms, or animals these compounds break down into their terminal form PFAAs (Dewitt, 2015) such as PFOS, PFOA and other congeners listed in Table 1.1.

In Eiders, exposure to PFASs is through ingestion (Haukås et al., 2007). However, in cases of maternal transfer, chicks can be born with high levels of contamination as well (Gebbink and Letcher, 2012). PFASs are then absorbed via the gastro-intestinal (GI) tract with high efficiency

given their protein-philic properties (Martin et al., 2003). Once absorbed, PFASs enter the bloodstream and binds to ALB with high affinity, which enables transport to protein rich tissues such as the liver or kidneys (Dewitt, 2015). In glacocous gulls, for example, the liver was shown to have much higher concentrations of PFOS than the brain (Verreault et al., 2005).

Eiders do not directly biotransform PFASs (Dewitt, 2015) however, they can metabolize the precursor into its terminal form (Buck et al., 2011). Though this has not been documented in seabirds directly, rats were shown to produce PFOA, PFNA, and PFHxA upon administration of TAs. Furthermore, PFASs precursors can also be metabolized to their terminal form via environmental or microbial degradation. For example, 8:2 flurotelomer alcohol is the precursor of PFOA which is biodegraded through microbial aerobic oxidation and dehydrohalogenation (Buck et al., 2011) and has been documented in black-legged kittiwakes *(Rissa tridactyla)* (Tartu et al., 2014), thick-billed murres *(Uria lomvia*) and northern fulmars (*Fulmarus glacialis)* (Braune and Letcher, 2013). Therefore, both the precursors and derivatives result in accumulation of PFASs in seabirds (Haukås et al., 2007; Haarr et al., 2018). PFASs are excreted primarily via the urine and to a lesser extend the feces with a half-life ranging from 0.5 hours to 3.5 years depending on chain length, gender, and species (Dewitt, 2015).

1.5.2. Arsenic

Eiders are exposed to As via ingestion (Mallory et al., 2004). As can exist in several different forms, the most toxic being inorganic As (As^{3+}) which is absorbed predominantly, and efficiently (80-90%) by the GI tract (Wolfe et al., 1998; Zhang et al, 2016). Alternatively, organic As is non-toxic in marine environments and is transformed to lipid or water-soluble organic forms by marine organisms (Lunde et al., 1977). Though Eiders are feeding at a lower trophic level, As^{3+} can biomagnify resulting in higher potential to cause adverse effects (Zhang et al., 2016). As has been shown to accumulate in the kidneys or liver where it is biomethylated to produce inorganic As and further biotransformed to produce methylated arsenicals, the biomarker of chronic arsenic exposure (Jaishankar et al., 2014).

1.5.3. Cadmium

Cd is produced mainly in its toxic form (Cd^{2+}) and absorbed with low efficiency by the GI tract (5-10%). However, absorption can be enhanced by dietary deficiencies in Ca, Fe and protein, putting incubating Eiders at particular risk (Wayland and Scheuhammer, 2011). Cd is taken up by the red blood cells where it interacts with proteins, primarily ALB, and is transferred to the liver and kidneys where it has a half-life of 18-30 years (Elder et al., 2014, Jaishankar et al., 2014). Once in the liver Cd can compete with Cu or Zn to bind metallothionein (MT) with higher affinity making it stable and immobile. MT-Cd is then released into the blood and filtered through the glomeruli in the kidneys where MT is degraded resulting in reactive Cd^{2+} (Elder et al., 2014). Additionally, in rats, it was shown that MT-Cd binding also disrupts Cu and Zn transport which can increase Cd toxicity (Waisberg et al., 2003).

1.5.4. Lead

Like As and Cd, Pb exposure in Eiders is predominantly through ingestion. However, where hunting of Eiders is common practice, Pb buckshot is also a source of exposure (Mallory et al., 2004). The GI tract absorbs Pb with 5-15% efficiency although dietary deficiencies of Fe, Zn, Mg and Ca can enhance absorption (Abadin et al., 2007; Skerfving and Bergdahl, 2014). This is particularly important for Eiders during incubation given they experience depletion of these essential elements during this period (Wayland and Scheuhammer, 2011).

Upon absorption 99% of the Pb in the blood is bound to hemoglobin while 1% is free for tissue distribution (Skerfving and Bergdahl, 2014). Mobile Pb in the blood inhibits delta-aminolevulinic acid dehydratase (ALAD) through Zn mimicry (Jaishankar et al., 2014) leading to improper heme synthesis and ultimately anemia. Franson et al. (2000a) observed this specifically in Eiders exposed to Pb in the Baltic Sea. Similar to PFASs, free Pb first accumulates in the liver and then in the kidneys. Pb can also mimic Ca and be incorporated into bone tissue where it accumulates with a half-life of up to thirty years (Elder et al., 2014). Pb is mainly excreted through the kidneys (Jaishankar et al., 2014; Elder et al., 2014) though has a half-life of 30 days in the blood and 40 days in soft tissue (Elder et al., 2014).

1.4.5. Mercury

Hg can exist in several forms, the most toxic being methyl Mercury (MeHg). MeHg is a concern for Eiders given it can biomagnify, is persistent in the biota, and is capable of long-range transport (Mason et al., 1995; Lavoie et al., 2013). MeHg is taken up through ingestion and absorbed 90-95% via the GI tract. Upon absorption MeHg is redistributed to all tissues within 30 hours, 10% of which has been found in the brain of birds and mammals (Wolfe, 1998). MeHg is stored in fatty tissue, however other Hg (Hg^{2+}) isotopes can be stored in the liver and kidneys as well (Björkman et al., 2007).

Mobile Hg has high affinity for sulfhydryl groups on proteins and is therefore able to sequester major antioxidants such as glutathione (GSH), leading to increased levels of oxidative stress (Koivula and Eeva, 2010). However, Hg can also be bound by the essential element Se, making it immobile. It has been shown that increased levels of Se can provide a protective effect against Hg toxicity (Kim et al., 1997). However, increased levels of Hg can also scavenge Se, depleting necessary levels for metal homeostasis (Koivula and Eeva, 2010). Unlike other toxic elements, Hg in Eiders is excreted via the feces and down of their feathers, although speciation can greatly influence mechanism of clearance (Wolfe et al., 1998; Elder et al., 2014). For example, in some avian species a main pathway of MeHg detoxification is demethylation followed by excretion of inorganic Hg (Wolfe et al., 1998).

1.6. Genotoxicity

Genetic toxicology is a field of study concerned with the impacts of toxicants on hereditary material and the processes behind its functionality and duplication. Genetic toxicologists assess genetic damage through multiple endpoints such as unscheduled DNA synthesis (UDS), sister chromatid exchanges (SCEs), DNA adducts, and DNA strand breaks (Preston and Hoffmann, 2013).

As previously mentioned, the Eider is an important organism for analyzing genotoxic effects of contamination because of its spatial distribution and limited mobility during incubation. Furthermore, the Christansø Eider colony has shown poor genetic health (Noori, 2018) meaning the results of this study could be insightful pertaining to the causes of genetic damage in seabirds.

1.6.1. DNA Damage

DNA can be damaged from chemical and physical agents. Chemical agents can be classified into three types; base modifiers, intercalating agents, and base analogues. These can cause damage through mechanisms such as covalent modification of nucleotide structure, distortion of the double helix, an incorrect incorporation into daughter strands during replication (Preston and Hoffmann, 2013). PFASs and toxic elements are classified as chemical mutagens given they can induce breakages in the phosphodiester DNA backbone causing single and double strand DNA breaks (Fig. 1.1) (Crespo-López et al., 2009; Dewitt, 2015).

Exogeneous agents can also indirectly damage the DNA through the production of reactive oxygen species (ROS). When ROS overwhelms the antioxidant defense system the cell can enter a state of oxidative stress leading to lipid and protein oxidation, single and double strand breaks, crosslinking (Lindahl, 1993), or apoptosis (Koivula et al., 2010). If the oxidative stress is large enough the cell will abondon repair mechanisms and simply undergo apoptosis (Alberts et al., 2014). In humans, ROS has been linked to aging and cancer (Tian et al., 2017) while in Eiders it has been linked to increased strand breakage (Fenstad et al., 2016b), increased chromosome abberations (Matson et al., 2004) and nest abondonment (Tartu et al., 2013).

It is important to note that endogenous agents are also responsible for much of the DNA damage occurring in cells. Simply biological processes such as oxygen consumption, heat, metabolic activity, and DNA replication produce thousands of altered DNA bases and AP sites daily (Alberts et al., 2014). Furthermore, replication is an imperfect mechanism which introduces alterations in the DNA that are called spontaneous mutations (Alberts et al., 2014).

Double stranded DNA breaks (DNA-DSBs) are particularly hazardous to the cell given that there is no template for which the cell can accurately repair the strands. Unrepaired DNA double strand breaks (DNA-DSBs) can lead to the breakdown of chromosomes, loss of genetic information, and carcinogenesis (Preston and Hoffmann, 2013). While it is difficult to extrapolate cellular effects to a population or even organismal level, new fields of research are demonstrating that changes in the genetic information can be propagated to entire populations resulting in fitness costs such as decreased immunity (Whitehead et al., 2016), or reproductive challenges (Devaux et al., 2011). Therefore, the Christiansø Eider colony's genetic health could be an indication of the overall health of the colony.

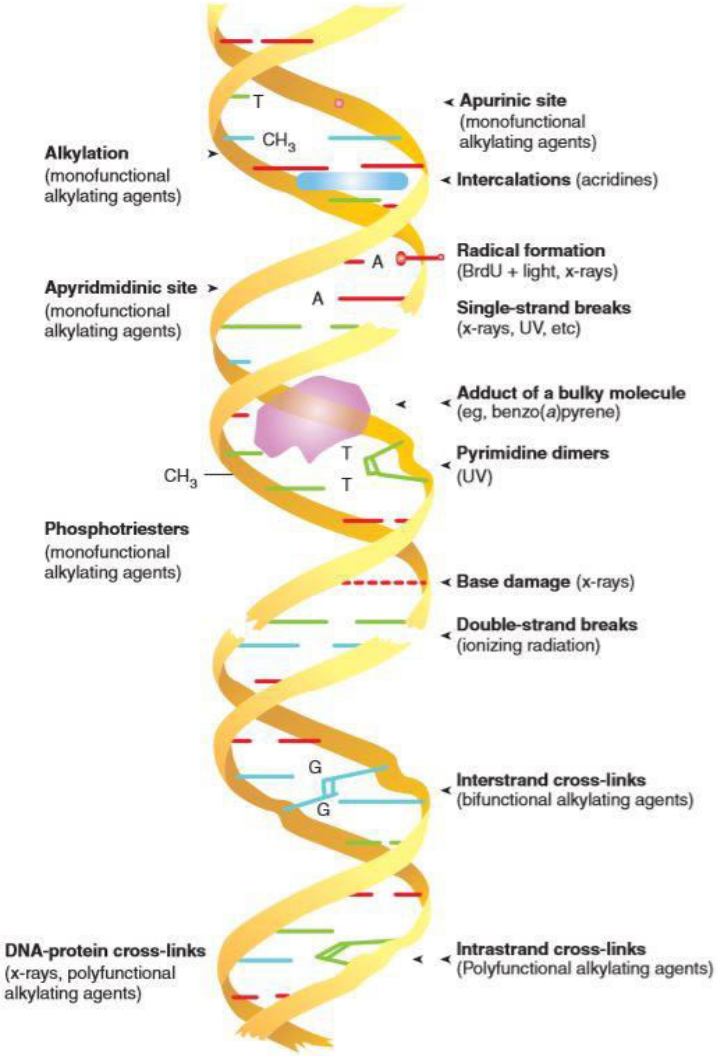


Figure 1.1. Types of DNA damage induced by physical and chemical agents. Figure from Preston and Hoffmann, 2013.

1.6.2. DNA repair and Apoptosis

These adverse population level effects do not occur until DNA damage is fixed in the genome through replication into a daughter cell and propagation to future cell generations (Preston and Hoffmann, 2013). This is where DNA repair machinery is paramount for the preservation of genetic information. Natural functions of the cell cause DNA damage, as discussed above, though only 0.02% of them become mutations because of error recognition and repair processes in the cell (Alberts et al., 2014). Double stranded DNA breaks are repaired via two mechanisms. 1) In nonhomologous end joining (NHEJ) the two ends are recognized by the three-dimensional protein Ku, which facilitates the DNA ligation. This often leads to nonessential nucleotide loss, although

occasionally it can cause aberrant chromosomes. 2) Homologous end joining is a more elegant repair mechanism though limited to newly replicated DNA. Homologous end joining uses a sister chromatid template to accurately ligase the disconnected ends (Alberts et al., 2014). However, if the DNA damage is too great to repair, the cell undergoes cell cycle arrest or apoptosis.

DNA repair is an energy costly function and if resources are limited apoptosis may be a more effective mechanism to maintain cellular of homeostasis (Nowsheen and Yang, 2013). This is particularly relevant to the Christiansø Eider colony given they deplete more resources during incubation as compared to other colonies (section 1.2.1). This could influence the cells' fate between apoptosis and DNA repair given fasting limits energy resources during the 26-day incubation period.

1.6.3. Genotoxicity of PFASs

Prior studies on the genotoxicity of PFASs have yielded mixed results. While PFASs have been shown to be genotoxic primarily through the production of ROS and disruption of the antioxidant defense system (Dewitt, 2015; Wielsøe et al., 2015) other studies have suggested there are no correlations between genotoxicity endpoints and PFASs (Sletten et al., 2016; Haarr et al., 2018)

It has been shown that higher levels of PFDA, PFNA, PFUnA, PFHxS, and PFOS are linked to chromosomal damage and oxidative stress in HepG2 cell lines (Wielsøe et al., 2015). Liu et al. (2014) showed that PFOS, PFDA, PFNA and PFOA caused increased levels of DNA instability and apoptosis in green mussels (*Perna viridis*). Furthermore, longer chain PFASs are more closely correlated to oxidative stress endpoints such as membrane and DNA damage (Nobels et al., 2010) which causes an increased risk for seabirds as long chain PFASs are also more bioaccumulative and persistent (Section 1.2).

However, research done on PFASs exposure in wildlife has been less straightforward. Antioxidant gene expression has been shown to increase with PFASs exposure in cormorants (*Phalacrocorax carbo*) (Nakayama et al., 2008), but remain unchanged in the white-tailed eagle (*Haliaeetus albicilla*) (Sletten et al., 2016). Further research has shown that PFTrA is positively correlated to metabolic rate in black-legged kittiwakes (*Rissa tridactyla*) (Blévin et al., 2017) and given that

increased metabolic rate is also associated with increased production of ROS (Jacqueline et al., 2017) this could be evidence of the genotoxicity of PFASs.

The peroxisome proliferation-activated receptor-alpha (PPARα) has also been shown to be activated by PFAA, PFOS, and PFOA (Abbott et al., 2009) which is another proposed mechanism of the genotoxicity of PFASs. Upon activation PPARα binds the peroxisome proliferator hormone response elements (PPREs) that regulates fat-cell differentiation, development of organs (Dewitt, 2015), and lipid or protein metabolism (Alberts et al., 2014). Additionally, PPARα is known to activate apoptotic pathways through the destruction of pro-apoptotic effector BCL2 family proteins (Gao et al., 2015). Though this is not a directly genotoxic mechanism it can still cause regenerative hyperplasia which has been linked to carcinogensis (Preston and Hoffman, 2013). Given these factors cumulatively there is strong evidence suggesting PFASs, directly or indirectly lead to genotoxicity in Eiders.

1.6.4. Genotoxicity of toxic elements

Unlike PFASs, there is widespread acceptance that toxic elements are genotoxic due to their production of ROS and interaction directly or indirectly with DNA or DNA repair molecules (Crespo-López et al., 2009; Jaishankar et al., 2014). Pb, Hg, and As produce ROS which is directly genotoxic through strand breakages and cross-linking (section 1.5.1). Cd however, cannot directly produce ROS though still causes oxidative stress through lipid and protein peroxidation (Koivula and Eeva, 2010). Additionally, Cd and As have been shown to inhibit DNA repair enzymes that are upregulated in response to ROS (Hartwig et al., 2003; Waisberg et al., 2003) further exacerbating genetic damages.

Many reports of genotoxicity in relation to toxic elements in wildlife exist. For example, Hg has been linked directly to DNA-DSBs in Eiders (Fenstad et al., 2016a), increasing Cd concentrations have been positively correlated with DNA strand breakages in copepod (*Acartia tonsa*) shrimp (*Palaemon varians*) and pleuronectiform fish (*Solea senegalensis*) (Pavlaki et al., 2016). Additionally, increased exposure to As, Hg, and Pb was shown to increase number of chromosome aberrations in silver rasbora fish (*Rasbora tornieri*) (Tengjaroenkul et al., 2017).

It is also important to note the effect of toxic elements on cellular defense mechanisms such as antioxidants and antioxidant enzymes. Cd, Hg, Pb, and As have all been shown to decrease levels of vitamin E and glutathione (GSH), indicating elevated oxidative stress (Koivula and Eeva, 2010). Furthermore, antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which catalyze reactions to sequester free-radicals, have been shown to decrease with increasing exposure to toxic elements (Świergosz-Kowalewska et al., 2006; Koivula and Eeva, 2010). However, it has been suggested that some species, such as long-lived birds, have increased antioxidant capabilities to cope with oxidative damage as a mechanism associated with longevity (Holmes and Martin, 2009).

Insult from multiple sources (PFASs and toxic elements) is particularly hazardous given that repair mechanisms and antioxidant capabilities can be overcome leading to widespread damage of the genome. Furthermore, interactive effects between multiple contaminants have been shown to cause additive or synergistic effects in *vitro* studies (Østby et al., 2005). As there is a strong link between genotoxicity and carcinogenesis (Preston and Hoffmann, 2013) whole populations could be put at risk. Genotoxicity is linked to population level changes in genetic diversity and fitness costs associated with those mutations (Matson et al., 2006; Reid et al., 2016). This cumulatively, makes PFASs and toxic elements dangerous both to an individuals' genetic stability and the health of whole populations.

1.7. Aim

The aim of this study was to investigate how DNA integrity was affected by circulating blood levels of PFASs, chemical elements, and body mass loss in the Eider during incubation. We measured DNA-FTM and MML as a means of quantifying DNA-DSBs in female incubating Eiders on Christiansø, Denmark during the spring of 2018.

It was hypothesized that DNA-DSBs would increase in tandem with 1) blood concentrations of PFASs, 2) blood concentrations of toxic elements and 3) body mass lost during incubation. It was also hypothesized that blood concentrations of PFASs and toxic elements would increase from day 5 to day 20 of incubation. In tandem, it was expected that essential elements would decrease. Lastly, BCCPs were investigated to analyze the systematic health of the Eiders in relation to PFASs

and chemical elements. BCCPs were also used in determining if poor health status contributed to an increase in DNA-DSBs.

2. Methods and Materials

2.1. Sampling and Sampling Location

Field sampling was conducted on the island of Christiansø (Ertholmene), Denmark (55°19'N 15°11E) in the Baltic Sea during the breeding season of 2018 (Fig 2.1). The same incubating female Eiders were sampled twice; once on day 5 (n=25) and once on day 20 (n=23) of the incubation period. The sampling was completed in conjunction with Aarhus University with the assistance of certified veterinarians and in accordance with current Danish ethical legislation.

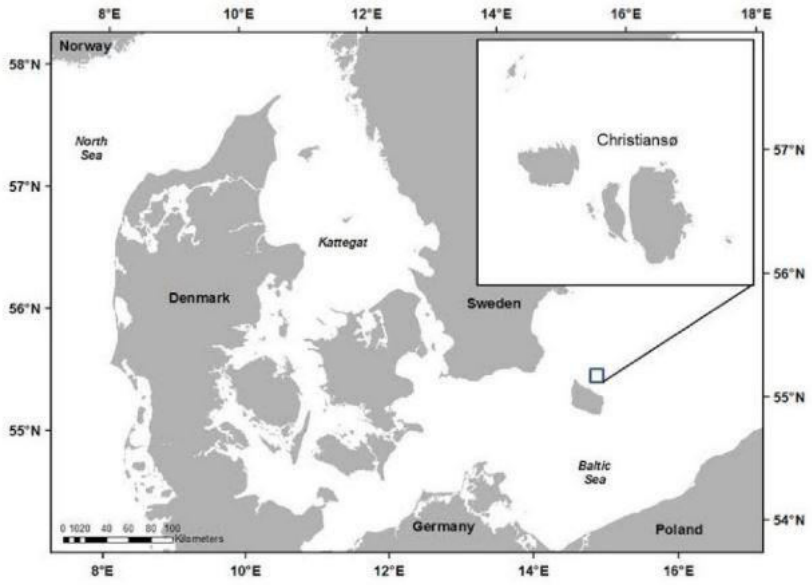


Figure 2.1. Study location in the central Baltic Sea. The Eider colony studied in this report incubates on Christiansø, Denmark (55°19'N 15°11'E). Figure from: Garbus (2016)

Prior to sampling, 50 Eider nests were located during a four-day period (April 12-April 16) and marked with yellow identification tags. The first sampling considered "day 5", took place from April 20-April 23. The second sampling (day 20) followed 15 days later from May 6-May 9. The female Eiders were caught on their nests using a fishing rod with a nylon snare at the end. For the first sampling, if the Eider had no identification ring, they were given one around the ankle. The Eiders were then weighed with a spring scale (Pesola Spring Balance) and roughly 1 mL of blood was taken per 100 g of the bird's body mass, from the brachialis vein under the wing using a heparinized syringe. 1 mL of whole blood was allocated for the DNA analysis. The whole blood was put in an eppendorf vial (1 mL), transported in aluminum foil to avoid sunlight, and placed in

liquid nitrogen within five minutes of being drawn. These samples were transported to Trondheim, Norway in liquid N_2 (-196ºC) and stored at -80ºC. Additionally, 1mL of whole blood was allocated for High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) analysis, also stored in -80ºC, and 4 mL for PFASs analysis. During the second sampling body condition parameters were also taken including; total tarsus length, wing maximum and minimum, and head size (mm).

2.2. Detection of Double Strand DNA Breaks

The analysis of double strand DNA breaks was conducted at the Department of Biology, Norwegian University of Science and Technology (NTNU) in Trondheim, Norway using agarose gel electrophoresis.

2.2.1 Principals behind Agarose Gel Electrophoresis

DNA double and single strand breaks have been previously quantified using the comet assay, which separates DNA strands from supercoiled chromatin based on degree of fragmentation (Theodorakis et al., 1994). Agarose gel electrophoresis follows the same principles (Shaposhnikov et al., 2008). As the DNA backbone is negatively charged it will travel towards an anode under applied electrophoresis in a liquid medium (Lee et al., 2012). Whole red blood cells containing DNA are first embedded in low melting point agarose (LMPA) plugs then lysed to isolate the DNA. The plugs are then placed into an agarose gel that serves as a matrix enabling separation of different sized DNA fragments under electrophoresis. Smaller molecules can travel further through the matrix while larger molecules cannot. The DNA remaining in the plugs, or wells, of the agarose gel are undamaged supercoiled chromatin that cannot travel through the gel's pores (Lee et al., 2012). Using this separation method, the amount of undamaged DNA in the wells can be compared to the fragmentation in the agarose gel (Appendix, Fig. A.5). Furthermore, the size of the DNA fragments can be determined through comparison with a known DNA size ladder (Appendix, Fig. A.3, A.4) (Krøkje et al., 2006).

DNA can be visualized through exposure to a dye or stain that binds specifically to the DNA molecules. Ethidium bromide (EtBr) is a common stain that intercalates between adenine and thymine base pairs. When exposed to UV light, EtBr will fluoresce an orange color that can be

used to quantify the amount of material in the gel and its location in respect to the standard size ladder (Melvin, 1987).

Agarose gel electrophoresis is a fast and objective method for biomonitoring because it does not require sacrificing the test organism or inconsistencies in chromosome scoring. Given that avian karyotypes have microchromosomes, it is especially useful when monitoring the genetic health of birds. Initially this method was applied by Theodorakis (1994), using whole blood cells as opposed to a single nucleoid as used in the comet assay. It was adapted by Krøkje et al. (2006) who applied the method to avian species (glaucous gulls) and further work by Fenstad et al. (2014) adapted the method for Eider blood.

2.2.2. Preparation of Plugs

6-10 µL of whole blood was suspended in 500 µL of TE buffer (10 mM Tris base and 1 mM EDTA) and mixed thoroughly. 500 µL of 1% LMPA (37 ºC) was added to the plug mixture and equilibrated to 37ºC to avoid unnecessary damage to the blood cells. The plug mixture was then pulse centrifuged up to a speed of ~8K rmp to remove possible blood coagulation. 50 µL of plug mixture was cast in plug molds and set at 4ºC for 1 hour to harden. The plugs were then placed in Theodorakis lysis buffer (NaCl (100 mM), Tris buffer (10 mM), EDTA (10 mM), SDS (5 %)) (Theodorakis et al., 1994), mixed with 1 mg/mL of freshly added Proteinase K, at 55ºC for 16 hours allowing the blood cells to lyse.

2.2.3. Gel Electrophoresis

The plugs were removed from the lysis buffer, equilibrated to room temperature, and placed in wells of 0.6% agarose electrophoresis gels. The plugs were sealed into the wells using 1% LMPA. The gels were run in TBE buffer (90mM Tris base, 90mM boric acid, 2mM EDTA) at 23V for 14 hours. The standard DNA ladder, also functioning as a positive control, was whole linearized lambda DNA digested by Hind III restriction enzymes which created fragments lengths (kpbs) of; 564, 2027, 2322, 4361, 6557, 9416, 23130 and 48337. The standard was suspended in TE buffer and mixed with xylene cyanol, bromphenol blue loading dyes as an indication of distance electrophoresed.

Two individuals were run on the same gel. Triplicates of day 5 and day 20 for each individual were placed side by side for easier visual comparisons (Appendix, Fig. A.6). Two identical gels were run simultaneously to create independence among samples.

2.2.4. Staining and Quantification

After electrophoresis was finished the gels were stained in ~10 µL of EtBr and ~500 mL of TBE buffer for two hours. The gels were then rinsed and placed in a gel documentation system (Biorad, Gel Doc 2000). The stained DNA fluoresces under trans UV light and the Gel Doc machine quantifies the photons emitted in respect to amount of DNA. The Biorad image analysis software (Quantity One-4.2.1) provides DNA intensity curves over distance migrated (relative front (rf)) which can then be quantified as follows.

The DNA ladders known fragment sizes and the corresponding rf value provided a standard curve (Appendix, Fig. A.4, A.7) which was then used to calculate the median molecular length (MML) (Krøkje et al., 2006). DNA-FTM was an additional measure of DNA-DSBs and was calculated by comparing the amount of DNA in each well to the DNA in each lane (Fenstad et al., 2014).

$$DNA - FTM = \frac{DNA\ in\ gel}{DNA\ in\ gel + DNA\ in\ well} \times 100$$

All calculations were done in Excel using a standardized spreadsheet created by Knut Rodvelt with the direction of Randi Rodvelt.

2.3. Detection of Chemical Elements

The quantification of chemical elements was conducted at the Department of Chemistry, NTNU Trondheim, Norway using HR-ICP-MS. A package of 64 chemical elements were analyzed, though for the purposes of this study only toxicologically relevant elements were analyzed, as discussed in section 2.6.

2.3.1. Preparation of samples and acid digestion

Whole blood from 48 different samples (from 25 Eiders on day 5 and from 23 of the same Eiders on day 20) were acid pretreated and diluted prior to ICP-MS. 500-1300 mg of blood were

transferred into 15 mL Teflon tubes specifically used in conjunction with the Milestone UltraCLAVE unit. 2.5 mL of Scanpure nitric acid 50% (HNO_3) was added to each sample and then digested in a high pressure (160 bar), high temperature (240°C) microwave unit (UltraCLAVE) for 2 hours. The samples were then diluted with distilled, ion-exchanged Milli-Q water to reach a final volume of 30-33 mL and placed in 15 mL vials for HR-ICP-MS quantification.

2.3.2. Chemical element quantification

HR-ICP-MS was conducted in the Thermo Finnigen model Element 2 instrument. Three blanks were run in conjunction with all samples as a measure of contamination. Quality assurance was also measured using a positive control of spiked blood (Seronorm-1191-L2-2) and a negative control of un-spiked blood (Seronorm-1191-L1-1).

The instrumental detection limit (IDL) was determined by 3 times the standard deviation of the average blank measurement (Jian-Li et al., 2015). This IDL is a representation of the instrument's detection capabilities and serves as the Lower Limit of Detection (LOD). All element concentrations are presented in the Appendix (Table A.3) and are reported in µg/kg ww.

2.4. Detection of PFASs

The quantification of PFASs was conducted at the Aarhus University in Aarhus, Denmark by Rossana Bossi as part of a research project concerning Eiders on Christiansø, Denmark which has been conducted for several years by a research group at Aarhus University. Liquid chromatography tandem-mass spectrometry (LC−MS/MS) with electrospray ionization in negative mode was used to analyze the congers listed in Table 2.1. As reported by Bossi (personal communication); serum (or plasma) samples were extracted with solid phase extraction cartridges (Oasis HLB Waters 100 mg) and control samples were human serum from AMAP intercalibration (from Intitute de Publique Sante', Quebec, Canada). The analytical method used is documented in Dassuncao et al., 2017 and will not be discussed here as the analysis was performed independently from this masters project.

Table 2.1. Acrynom, name, detection limit and quantification limit (ng g^{-1} ww) of all PFASs analyzed in this study.

Compound	Full name	Detection limit (ng g^{-1} ww)	Quantification Limit (ng g^{-1} ww)
PFBS	Perfluorobutane sulfonate	0.02	0.07
PFHxS	Perfluorohexanesulfonate	0.03	0.08
PFHpS	Perfluoroheptane sulfonate	0.04	0.11
PFOS	Perfluorooctane sulfonate	0.09	0.28
PFDS	Perfluorodecane sulfonate	0.12	0.37
PFOSA	Perfluorooctane sulfonamide	0.40	1.19
PFPeA	Perfluoropentanoic acid	0.06	0.19
PFHxA	Perfluorohexanoic acid	0.01	0.03
PFHpA	Perfluoroheptanoic acid	0.02	0.05
PFOA	Perfluorooctanoic acid	0.07	0.20
PFNA	Perfluorononanoic acid	0.09	0.27
PFDA	Perfluorodecanoic acid	0.03	0.09
PFUnA	Perfluoroundecanoic acid	0.05	0.15
PFDoA	Perfluorododecanoic acid	0.14	0.41
PFTrA	Perfluorotridecanoic acid	0.14	0.41
PFTeA	Perfluorotetradecanoic acid	0.14	0.41

2.5. Detection and quantification of BCCPs

The quantification of BCCPS was independently conducted by Thomas Jessen and Svend Erik Garbus from the Aarhus University in Aarhus, Denmark as part of a routine procedure associated with the larger research project concerning Eiders on Christiansø, Denmark being conducted by a research group at Aarhus University. As reported by Garbus (2016), all analyses were done with an automated spectrophotometric analyzer containing ion-selective electrodes (ADVIA 1800, Siemens).

The following 24 BCCPs were analyzed: ALAT, aspartate aminotransferase (ASAT), alkaline phosphatase (ALKP), GGT, amylase (AMY), lactate dehydrogenase (LDH), lipase, ALB, globulin (GLOB), TP, fructose (FRU), glucose (GLU), Fe, Ca, Na, Mg, Phosphate (PHOS), Cl, K, bile acid BILE), total bile (TB), creatine (CREA), urea, and cholesterol (CHOL). The analytical method

used is documented in Sonne et al. (2008; 2010; 2013) and will not be discussed here as the analysis was preformed independently from this masters project.

2.6. Statistical Analyses

All statistical analyses were performed with the free statistical computing and graphics software, R (R Core Team, 2018). A list of additional R packages used is included in the Appendix (Table A.5).

2.6.1 Treatment of Samples and Limit of Detection

23 Eiders sampled twice in this study; once on day 5 of incubation and once on day 20. Two additional Eiders were sampled only on day 5. These two individuals were included in DNA-FTM, MML, PFASs, BCCPs, and chemical element quantification. However, upon running statistical analysis, they were removed from the dataset to fulfill equal length vector requirements.

PFASs chosen for further analysis were PFOS, PFNA, PFDA, PFHpS, PFHxS, and PFNuA. The other PFASs congener were below the LOD (Appendix, Table A.4) in >60% of the individuals and were therefore excluded from analyses due to lack of statistical power.

The toxic elements As, Cd, Hg, and Pb were chosen for further analysis due to their toxicological relevance, biological monitoring, and previous research conducted on Christiansø, Denmark (HELCOM, 2010; Fenstad et al., 2017; Noori, 2018). Fe, Ca, Se, Zn, Co, and K were chosen due to their effects on toxic element uptake and toxicity (Section 1.4). All the listed elements were above the LOD in all individuals., as described in the Appendix (Table A.3).

The BCCPs included in this study are a routinely checked panel and included as general indications of Eider organ health. All 24 BCCPs chosen for analysis were present in >60% of the individuals. BCCPs recorded for only one day of incubation were excluded from analysis.

For calculation of DNA-FTM and MML, Significance of “gel” and “replicate” were checked to ensure “gel” did not influence the results. Parallel gels were averaged together if the CVs were <20%. Neither replicate nor gel was significant in the models indicating that the method described in section 2.2 did not influence the results.

2.6.2. Paired t-tests and correlation analyses

Paired t-tests were used to analyze the differences between means on day 5 and day 20 for body mass, PFASs concentrations, chemical elements, DNA-FTM, MML, and BCCPs. Paired t-tests were chosen over Tukey's honest significant difference (HSD) test because there were equal sample sizes for both days of sampling (n=23).

Correlation analyses were done as preliminary exploration of the data. Shapiro-Wilk's test was used prior to correlations to ensure normality of data. Log transformations were done on all PFASs, chemical elements, BCCPs, DNA-FTM and MML to create normality. Correlations were done with Pearson's moment product in cases of normal distribution, and with Spearman's rank correlation to account for non-normality after log-transformation. However, all graphs are presented in non-log transformed data.

Bonferroni's correction for multiple variables was not used as this study was interested in specific relationships between PFASs, chemical elements, DNA integrity, and BCCPs as opposed to general patterns. However, this does increase the possibility of making a type I error (Perneger, 1998).

2.6.3. Multivariate Statistics

DNA-FTM was used in multivariate statistical analyses because MML is considered a less exact measurement of DNA-DSBs than DNA-FTM, as the calculations are subject to individual discretion (Fenstad et al., 2016a). Additionally, DNA-FTM has been used in previous studies (Fenstad et al., 2016a; 2016b; Noori, 20018) concerning Eider populations and is therefore also presented in this study for comparison purposes.

All factors, dependent and independent, were log-transformed to fulfill model assumptions. Furthermore, in situations of causation (PLS and linear modelling) variables with much larger concentrations were scaled.

2.6.3.a. Partial least-squares modelling

Partial least-squares (PLS) analysis was used to model DNA-FTM as a function of ΣPFASs, toxic elements, essential elements, and body mass on day 5 and day 20 of incubation. PLS modelling

was chosen over linear regressions to account for small sample sizes and multicollinearity between body mass, ΣPFASs, and toxic elements. Furthermore, by analyzing DNA-FTM using PLS it is possible to analyze the variance in the dependent variable without penalization of increasing parameters (Carrascal et al., 2009). All data was scaled to unit variance (mean=0, SD=1) and centered before analysis. Coefficient plots were used to determine significant variables affecting DNA-FTM. Variables were considered significant if there 95% confidence intervals did not include zero.

To better isolate the predictors of DNA-FTM, analyses were run on day 5 and day 20 separately. This accounts for confounding factors unable to be included in the models but were associated with the stress of breeding. ΣPFASs was used over each individual congener to avoid multicollinearity and issues of model over fitting. Additionally, this increases statistical power as entire individuals are not being excluded based on missing data for one congener. ΔDNA-FTM between day 5 and day 20 as a function of ΔPFASs, Δtoxic elements and Δbody mass was not analyzed as this assumes that DNA integrity is deteriorating at a describable rate, linearly or exponentially for example. However, as this has not been confirmed, conclusions can only be drawn from the sampling days collected and not about the entire course of incubation.

Correlation tests between residuals from day 5 and day 20 were also performed to analyze for individual variation influencing DNA-FTM irrespective of the predictor variables.

2.6.3.b. Linear Models

Based on the coefficient plots, linear models were run to confirm the relationship between independent variables and DNA-FTM. Again, models were run on for both day of sampling separately with DNA-FTM as a response variable and mass lost, Hg, Cd, As, Pb, and ΣPFASs as predictor variables. The models were simplified until all factors were significant. The most parsimonious models with the largest significance and adjusted R^2 were used to obtain parameter estimates for day 5 and day 20 of incubation. Histograms and QQ plots of the residuals were checked for normality and assurance that all assumptions were met.

2.6.3.c. Principal Component Analysis

Presented in the Appendix (Fig. B.3), principal component analyses (PCA) were done as a means of exploring the different variables and visualizing the clustering and grouping between PFASs,

chemical elements, and DNA-DSBs. Variables included in the PCA were; 1) PFASs [PFOS, PFNA, PFDA, PFHxS, PFHpS, PFUnA], 2) chemical elements [As, Ca, Co, Cd, Cu, Fe, Hg, K, Pb, Se, Zn], 3) DNA-DSBs [MML, DNA-FTM], and 4) body mass. Day of incubation was colored and ellipsed to visualize the effect of day on the variables and individuals. Furthermore, all variables were scaled and centered to allow for accurate comparisons.

Based on the results of the PCA containing all individuals (Appendix, Fig. B.4) two outliers were removed, and correlation analyses were re-run between chemical elements, body mass, and DNA-FTM. However, as these individuals were not measured/collected incorrectly they are not true outliers and are included in all multivariate analyses.

3. Results

3.1. Body mass loss from day 5 to day 20 of incubation

There was a statistically significant decrease in body mass from day 5 to day 20 the incubation period. The mean body mass loss between day 5 and day 20 of the incubation period was 622.39 g (±23.91 g) (paired t-test, df=22, t=26.023, $p=2.2x10^{-16}$, Table 3.1). The median body mass on day 5 was 2205 g and on day 20 was 1575 g. The range for day 5 was 1935 g to 2475 g while on day 20 body mass ranged from 1225 g to 1865 g (Table 3.1).

Table 3.1. Body mass (g) for female incubating Eiders on Christiansø, Denmark. The mean, standard error, median, range, and number of observations for day 5 and day 20 of incubation for the 2018 breeding season are presented. Paired t-test results are presented with degrees of freedom, t and p values.

	day 5 of incubation					**day 20 of incubation**					**paired t-test**		
parameter	**mean**	**SE**	**median**	**range**	**n**	**mean**	**SE**	**median**	**range**	**n**	**df**	**t**	**p**
Body mass (g)	2181.39	29.87	2205	1935-2475	25	1553.60	32.12	1575	1225-1865	23	22	26.02	<0.001

3.2. PFASs at day 5 and day 20 of incubation

PFBS, PFHxS, PFHpS, PFOS, PFDS, PFOSA, PFHxA, PFHpA, PFOA, PFNA, PFUnA, PFDoA, PFTrA, PFTeA were measured in female Eiders incubating on Christiansø, Denmark. Of these PFASs, only PFOS, PFNA, PFDA, PFHpS, PFHxS, and PFNuA were above the LOD. From day 5 to day 20 of the incubation period PFOS, PFNA, PFDA, PFHpS, and PFNuA all increased significantly (Table 3.2). The ΣPFASs increased over the 15-day incubation by 52.54%. PFOS increased the most dramatically with a 51.52% increase. PFUnA and PFDA closely followed with a 49.34% and 47.00% increase, respectively. PFHsP and PFNA had an increase of 39.03% and 39.74%, respectively. PFHxS had a 3.62% increase which was not statistically significant (Table. 3.2).

On day 5 PFOS was the most abundant PFASs with a mean of 5.31 ng g^{-1} ww and a range of 2.38 - 8.50 ng g^{-1} ww. Next most common was PFHxS (2.11 ng g^{-1} ww, range: 1.01 – 6.09 ng g^{-1} ww) followed by PFHpS (0.16 ng g^{-1} ww), PFNA (1.07 ng g^{-1} ww), PFUnA (0.41 ng g^{-1} ww), and

PFDA (0.35 ng g^{-1} ww) on day 5 of incubation. The sum of PFASs was 8.31 ng g^{-1} ww ranging from 4.44 – 14.11 ng g^{-1} ww (Table 3.2).

On day 20 of incubation PFOS was still the most abundant PFASs with a mean of 8.02 ng g^{-1} ww and a range of 4.06 – 13.00 ng g^{-1} ww. Again, the next most prevalent was PFHxS (2.49 ng g^{-1} ww). PFNA (1.50 ng g^{-1} ww), PFUnA (0.61 ng g^{-1} ww), and PFDA (0.52 ng g^{-1} ww) were the next most common. PFHpS was most rare with a mean of 0.22 ng g^{-1} ww. The average sum of PFASs was 13.25 ng g^{-1} ww, ranging from 6.99 – 19.99 ng g^{-1} ww (Table 3.2).

The composition of PFASs was mostly consistent between day 5 and day 20 of the incubation period with PFOs contributing 60.53% to the ΣPFASs on day 5 and 63.90% on day 20. The next largest contributor was PFHxS contributing 25.39% on day 5 and 16.52% on day 20. PFNA made up 12.87% of ΣPFASs on day 5 and 11.32% on day 20. PFDA, PFHpS, PFOA, and PFUnA contributed the remaining percentages (Fig. 3.1). None of the remaining compounds contributed more than 5% independently.

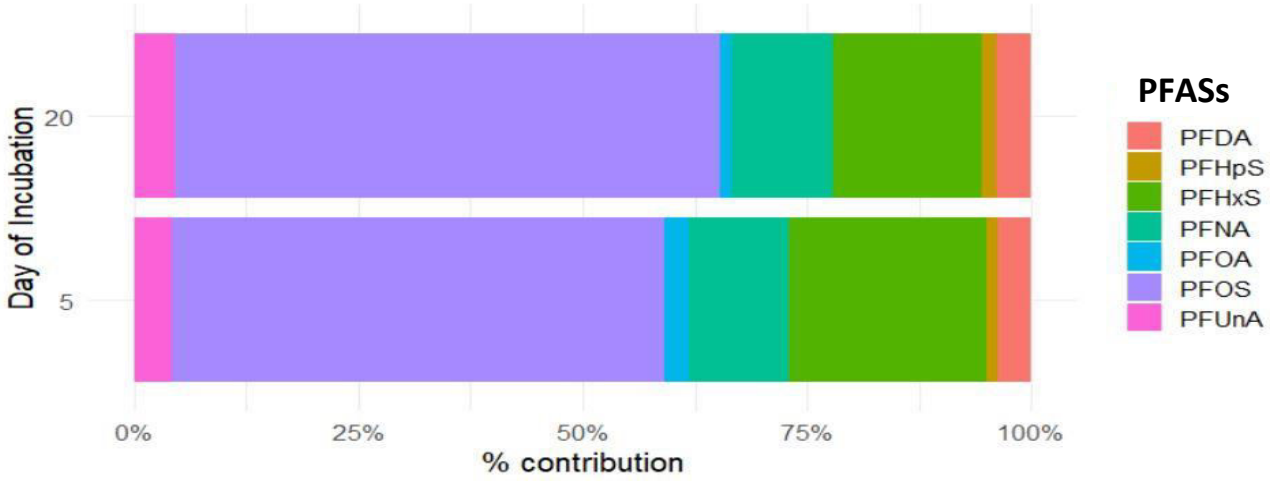


Figure 3.1. Overview of the mean contribution (%) of each congener (PFDA, PFHpS, PFHxS, PFNA, PFOA, PFOS, and PFUnA) to the total PFASs concentration in female incubating Eiders breeding on Christiansø, Denmark. Day 5 and day 20 of the incubation period for the 2018 breeding season are presented. Different colors denote different compounds.

Table 3.2. Blood concentrations of PFASs (ng g^{-1} ww) for female incubating Eiders on Christiansø, Denmark. The mean, standard deviation, median, range, and number of observations for day 5 and day 20 of incubation for the 2018 breeding season are presented. All compounds were log-transformed prior to paired t-test. Paired t-test results are presented with degrees of freedom, t and p values. Compounds that were not detected in >60% of individuals were excluded from analysis.

	day 5 of incubation				**day 20 of incubation**				**paired t-test**		
Compound	**Mean ± SD**	**median**	**range**	**n**	**Mean ± SD**	**median**	**range**	**n**	**df**	**t**	**p**
PFOS	5.31±1.41	5.25	2.38-8.05	23	8.02±2.44	7.85	4.06-13.00	23	22	-7.4965	<0.001
PFHpS	0.16±0.08	0.14	0.07-0.42	19	0.22±0.10	0.21	0.07-0.52	19	18	-6.3368	<0.001
PFNA	1.07±0.34	1.01	0.65-2.19	23	1.50±0.50	1.33	0.91-2.97	23	22	-6.6528	<0.001
PFHxS	2.11±1.22	1.76	1.01-6.09	23	2.19±1.31	1.81	0.76-5.57	23	22	-1.0828	0.2916
PFDA	0.35±0.10	0.33	0.16-0.57	23	0.52±0.17	0.50	0.27-0.83	23	22	-7.4613	<0.001
PFUnA	0.41±0.13	0.40	0.14-0.70	23	0.61±0.23	0.54	0.30-1.01	23	22	-6.315	<0.001
ΣPFASs	8.31±2.60	9.16	4.44-14.11	23	13.25±3.71	12.96	6.99-19.99	23	22	-5.89	<0.001

3.2.1. Relationship between body mass and PFASs

PFOS, PFDA, and PDUnA were all strongly negatively correlated to body mass ($p<0.001$, r_s = -0.55, -0.52, -0.49). PFHsP, PFNA were also negatively correlated to body mass ($p<0.01$, r_s = -0.42) although PFHxS was not (Fig. 3.2). This shows that as body mass decreases, blood concentrations of PFOS, PFDA, PDUnA, PFHsP, and PFNA all increase. All PFASs values were log transformed and therefore correlations were done with Pearson's product moment correlation.

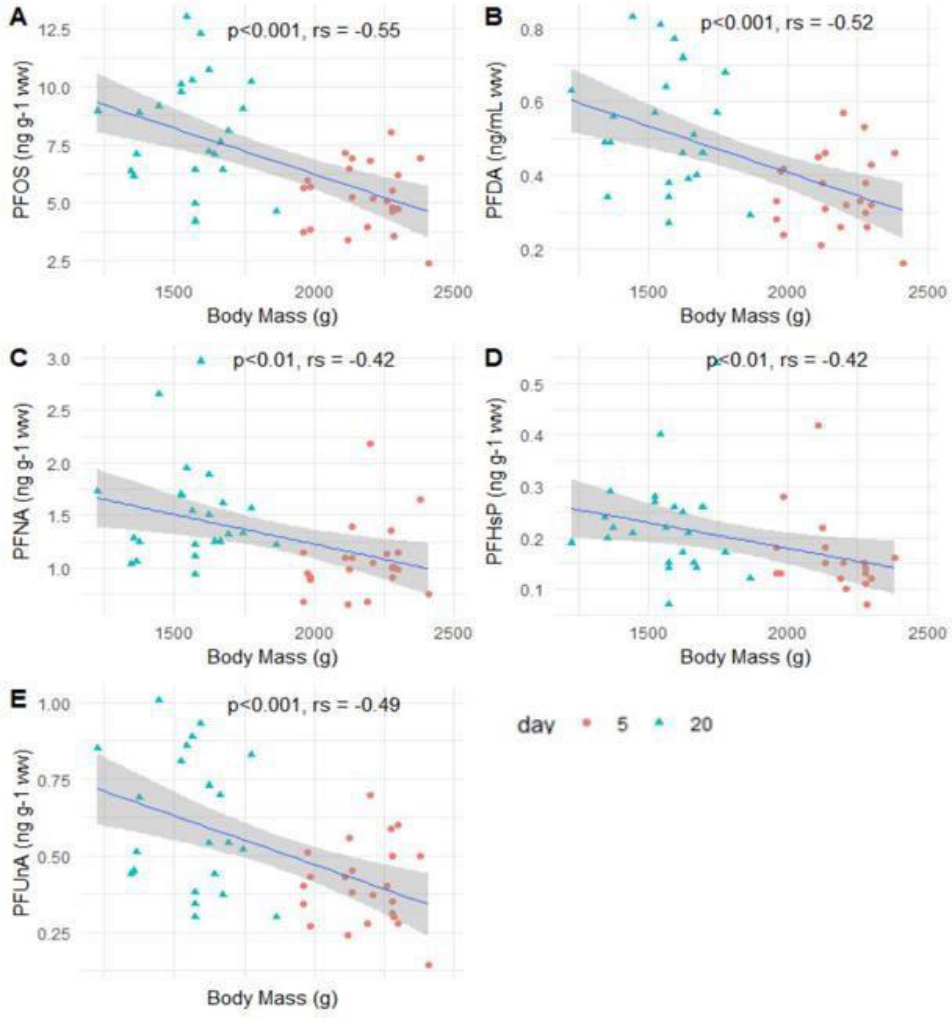


Figure 3.2. Scatter plots of significant correlation between body mass (g) and blood concentrations of (A) PFOS, (B) PFDA, (C) PFNA, (D) PFHsP and (E) PFHsP (ng g^{-1} ww) in female incubating Eiders on Christiansø, Denmark. Day 5 and day 20 of the incubation period for the 2018 breeding season are presented. Day 5 is represented by orange circles and day 20 by green triangles. Pearson's moment correlation coefficients (r_s) and p-values (p) are displayed on each graph. All PFASs have been log-transformed. Linear regression lines and 95% confidence intervals (shaded region around lines) are for presentation purposes only.

3.3. Chemical elements at day 5 and day 20 of incubation

The blood concentrations of As, Cd, Hg, Pb, Ca, Cu, Fe, Co, K, Se and Zn were all measured in female Eiders incubating on Christiansø, Denmark. Of these, the toxic elements that increased significantly from day 5 to day 20 were Cd and Pb. Hg decreased significantly by 17.08% (t=2.806, p=0.01) and As had no significant changes (t= -0.53, p=0.6) (Table 3.4). Cd increased from 0.819 ± 0.397 µg/kg ww to 1.19 ± 0.499 µg/kg ww (t= -3.047, p=0.006) while Pb increased from 221.0478 ± 311.76 µg/kg ww to 256.94 ± 237.66 µg/kg ww (t=-2.843, p=0.009) (Table 3.3). This corresponds to a 43.30% increase of Cd and a 16.24% increase of Pb. Pb was the most abundant toxic element, followed by Hg, As and lastly, Cd (Table 3.3).

All the essential elements significantly decreased from day 5 to day 20 of incubation except for Co (t=-0.76, p=0.45) and Cu (t=-1.54, p=0.137), which showed no significant change (Table 3.4). Se decreased by 21.40% (t=5.28, p=<0.0001), Zn by 7.70% (t=2.93, p=0.008), K by 12.04% (t=4.30, p=0.0002), and Fe by 12.64% (t=3.53, p=0.002) between day 5 and day 20 of incubation (Table 3.4).

Table 3.3. Blood concentrations of chemical elements (µg/kg ww) for female incubating Eiders on Christiansø, Denmark. The mean, standard deviation, median, range, and number of observations for day 5 and day 20 of incubation for the 2018 breeding season are presented. Compounds that were not detected in >60% of individuals were excluded from analysis.

	day 5 of incubation				**day 20 of Incubation**			
Compound	**Mean ± SD**	**median**	**range**	**n**	**Mean ± SD**	**median**	**range**	**n**
Toxic Elements								
As	12.91±3.18	12.25	7.26 - 21.81	25	13.41±3.52	14.01	6.73 - 20.49	23
Cd	0.82±0.397	0.720	0.46 - 2.37	25	1.19±0.499	1.08	0.55 - 2.42	23
Hg	209.15±99.89	169.950	81.57 - 443.25	25	183.33±86.17	152.76	65.05 - 388.36	23
Pb	221.05±185.43	126.30	45.16 – 875.38	25	256.94±237.66	181.81	30.08 - 1050.08	23
Essential Elements								
Ca	58878.76±14825.05	56275.17	39541.73 - 105788.55	25	51024.43±595.25	52961.21	36874.74 - 57949.65	23
Cu	345.28±71.37	316.04	258.73 - 487.50	25	370.27±67.95	365.88	161.34 - 541.73	23
Fe	488172.10±56320.51	475849.16	416348.8 - 736762.4	25	426482.9±36788.75	428805.87	286192.2 - 474340.3	23
Co	1.27±0.50	1.23	0.65 – 2.17	25	1.13±0.393	1.07	0.69 – 2.66	23
K	1945027.0±156154.7	1925566.00	1725493 - 2583159	25	1710835.0±140300.6	1746625.00	1175555 - 1850180	23
Se	2947.22±815.78	2771.00	1687 - 4807	25	2316.54±685.25	2140.50	1413 - 3893	23
Zn	5546.33±535.70	5644.22	4588.75 - 6511.58	25	5119.32 ±606.20	5165.635	2984.48 - 6001.71	23

Table 3.4. Significant differences of chemical elements blood concentrations in female incubating Eiders on Christiansø, Denmark between day 5 and day 20 of incubation during the breeding season of 2018. All compounds were log-transformed prior to paired t-tests. Paired t-test results are presented with degrees of freedom (df), t and p values. Bold font indicates significant difference between the days of incubation while a + indicates an increase from day 5 to day 20, while a - indicates a decrease from day 5 to day 20.

Compound	t	p	df	Trend during incubation
Toxic Elements				
As	-0.53	0.600	22	
Cd	-3.047	**0.006**	22	+
Hg	2.806	**0.01**	22	-
Pb	-2.843	**0.009**	22	+
Essential Elements				
Ca	3.05	**0.006**	22	-
Co	-0.76	0.45	22	
Cu	-1.54	0.137	22	
Fe	3.53	**0.002**	22	-
K	4.30	**0.0002**	22	-
Se	5.28	**<0.0001**	22	-
Zn	2.93	**0.008**	22	-

3.3.1. Relationship between body mass and chemical elements

None of the toxic elements were correlated to body mass. However, when individuals F26 (day 20) and F27 (day 5) were removed from the dataset (Appendix, Fig. B.3, B.4), there was a correlation between Cd and body mass ($p=0.01$, $r_s= - 0.35$) (Table 3.5).

The essential elements Ca ($p=0.01$, $r_s= 0.42$), Cu ($p=0.01$, $r_s= - 0.30$), Fe ($p=0.01$, $r_s= 0.54$), K ($p=0.01$, $r_s= 0.60$), Se ($p=0.01$, $r_s= 0.5$), and Zn ($p=0.01$, $r_s= 0.48$) were all significantly positively correlated to body mass (Table 3.5). Co was the only essential element not correlated to body mass. However, when individuals F26 (day 20) and F27 (day 5) are removed Cu was also no longer correlated, though the other essential elements maintained correlations to body mass (Table 3.5).

Table 3.5. Correlations between body mass and chemical elements in female Eiders breeding on Christiansø, Denmark during the breeding season of 2018. All data was log-transformed to create normality. Pearson's moment r_s correlation coefficient values are presented with asterisks (*,**,**) denoting a p-value of <0.05, <0.01 and <0.001, respectively. The third column (Body mass w/o ID 27.1 and 26.2) lists the significant correlations between chemical elements and body mass when individual F27 on day 5 and F26 on day 20 have been removed.

Compound	Body Mass	Body Mass w/o ID 27.1, 26.2
Toxic Elements		
As		
Cd		-0.35*
Hg		
Pb		
Essential Elements		
Ca	0.42**	0.39*
Co		
Cu	-0.30*	
Fe	0.54***	0.52**
K	0.63***	0.60***
Se	0.35*	0.39**
Zn	0.48**	0.47**

3.3.2. Relationship between toxic elements and essential elements

Analysis of all correlations between toxic elements and essential elements can be found in the Appendix (Table B.6). Discussed here will only be significant correlations relevant for the aim of this study and relevant for the toxicokinetics of Hg, Pb, Cd and As.

There we no significant correlations between Hg and Se, Zn, or Ca. Furthermore, there were no significant correlations between Cd and Fe, Ca, or Zn. Lastly, Pb was not correlated to Fe or Ca (Appendix, Table B.6). However, Cu was significantly negatively correlated to both Cd (r_s=0.68, p=<0.0001) and Pb (r_s=0.51, p=<0.001), both with and without individuals F27 (day 5) and F26 (day 20). Additionally, Fe was correlated to Cd (r_s= - 034, p=0.014) when individuals F27 (day 5) and F26 (day 20) had been removed (Fig. 3.3).

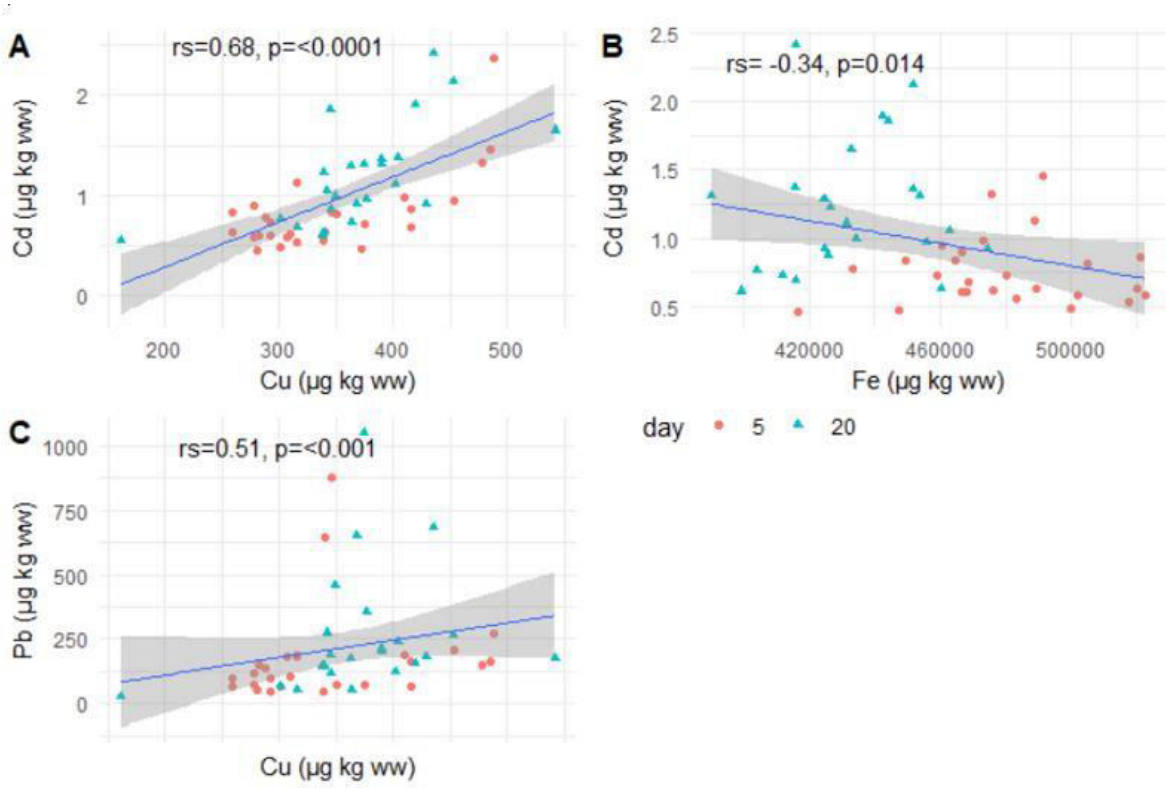


Figure 3.3. Scatter plots of significant correlation between blood concentrations of (A) Cu and Cd, (B) Fe and Cd, and (C) Cu and Pb (µg/kg ww) in female incubating Eiders on Christiansø, Denmark. Day 5 and day 20 of the incubation period for the 2018 breeding season are presented. Day 5 is represented by orange circles and day 20 by green triangles. Pearson's moment correlation coefficients (r_s) and p-values (p) are displayed on each graph. All chemical elements have been log-transformed. Linear regression lines and 95% confidence intervals (shaded region around lines) are for presentation purposes only.

3.4. DNA-DSBs at day 5 and day 20 of incubation

There was a significant increase between DNA-FTM on day 5 and day 20 of incubation (paired t-test, df=22, p=0.0026). On day 5 the mean DNA-FTM was 14.83%, on day 20 the mean DNA-FTM was 16.36%. For day 5 the median was 13.16% and ranged from 9.89 - 26.11%. On day 20 the median was 14.75% with a range from 11.24 - 30.23 (Table 3.6). The mean CV from all replicates for every individual was 18.51% on day 5 and 19.23% on day 20. There was no significant difference in MML throughout the incubation period (paired t-test, df=22, p=0.221). The mean MML was 396.60 kbps on day 5 and 388.67 kbps on day 20 (Table 3.6). The median MML on day 5 was 388.67 kbps and 394.45 kbps on day 20. The MML ranged from 238.20 - 528.26 kbps on day 5 and 212.82 - 497.21 kbps on day 20. The mean CV from all replicates for

every individual was 10.70% and 12.90% for day 5 and day 20, respectively. The mean CV for DNA-FTM for replicate gels was 14.40% and between replicate rounds was 18.85%. For MML the mean CV between replicate rounds was 11.74% and between replicate gels was 8.79% (Appendix, Table B.10).

Table 3.6. DNA-FTM (%) and MML (kbps) for female incubating Eiders on Christiansø, Denmark. The mean, standard deviation, median, range, and number of observations for day 5 and day 20 of the incubation period for the 2018 breeding season are presented. DNA-FTM was log-transformed prior to paired t-tests. Paired t-test results are presented with degrees of freedom (df), t and p values.

	day 5 of incubation				**day 20 of incubation**				**paired t-test**		
Parameter	**Mean ± SD**	**median**	**range**	**n**	**Mean ± SD**	**median**	**range**	**n**	**df**	**t**	**p**
DNA-FTM (%)	14.83±4.13	13.16	9.89-26.11	25	16.36±5.04	14.75	11.24-30.22	23	22	-3.38	0.0028
MML (kbps)	396.6±69.30	388.67	238.20-528.26	25	388.67±68.51	394.45	212.82-497.21	23	22	1.26	0.221

3.5. Relationship between DNA-DSBs, PFASs, chemical elements, and body mass

3.5.1. Partial least-squares modelling

Partial least-squares (PLS) modeling was done to analyze the best predictor variable for DNA-FTM on day 5 and day 20 of the incubation period. Blood concentrations of PFASs, Hg, As, Cd, Pb, Ca, Cu, Fe, Se, Zn, and body mass were used as predictor variables while DNA-FTM was the response variable. On day 5 only ΣPFASs was significant in explaining DNA-FTM (t= -2.3, p= 0.031). ΣPFASs had a negative effect on DNA-FTM, indicating that, early in incubation, as ΣPFASs increased, DNA-FTM decreased (Fig. 3.4).

On day 20, both Hg (t= -2.1, p=0.046) and ΣPFASs (t= -2.2, p=0.043) were significantly and negatively related to DNA-FTM (Fig. 3.4), indicating that, late in incubation, as ΣPFASs and Hg increased, DNA-FTM decreased.

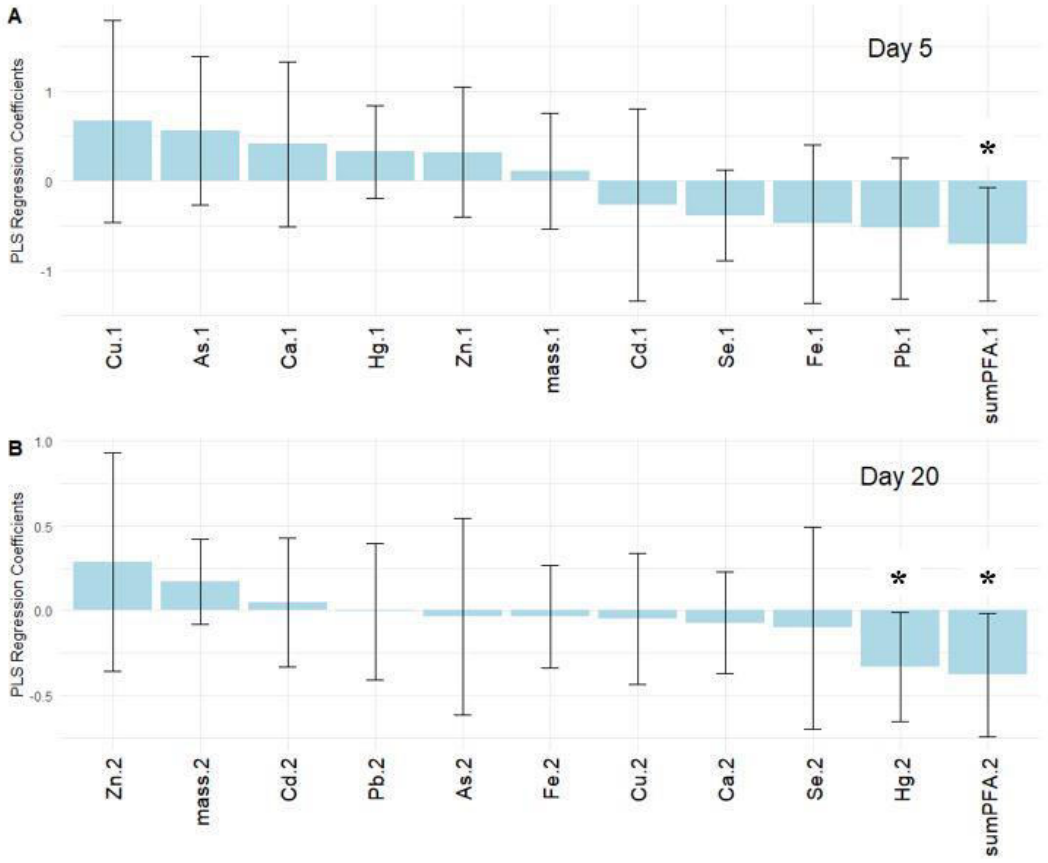


Figure 3.4. Coefficient plots from the PLS model with DNA-FTM (%) in female incubating Eiders on Christiansø, Denmark as a response variable and PFASs, Hg, As, Cd, Pb, Ca, Cu, Fe, Se, Zn, and body mass as predictor variables. Day 5 (A) and day 20 (B) of the incubation period for the 2018 breeding season are labelled and presented separately. A positive relationship to DNA-FTM is depicted by boxes above zero while negative relationships are represented by boxes below zero. When the 95% confidence intervals do not cross zero the coefficients are significant, as denoted by asterisks (*,**,**) representing a p-value of <0.05, <0.01, and <0.001, respectively.

3.5.2. Linear Modelling

Linear models were run to confirm the relationship between DNA-FTM and the contaminants identified in the PLS analysis. Again, day 5 and day 20 were seperated with 1 model selected for each day.

The best model for day 5 has an overall p=0. 0.013 and adj. R^2= 23.43% in confirmation with the PLS analysis and includes only ΣPFASs. This model shows a significant negative effect of ΣPFASs on DNA-FTM, indicating that as blood concentrations of ΣPFASs increase, DNA-FTM decreases (Fig. 3.5). A 10% increase in ΣPFASs results in a 4.25% decrease of DNA-FTM on day

5. On day 20 concentrations of Hg, ΣPFASs, and body mass were included in the best model (p=0.00017, adj. R^2= 66.62%) (Table 3.7). This is in conjunction with the PLS analysis, although mass was significant in the linear models, but not the PLS. However, the ΣPFASs is not significant in the linear model, though the interaction between mass and ΣPFASs is. These two examples could be an indication of collinearity between ΣPFASs and mass in the linear models. Hg had a negative effect on DNA-FTM; a 10% decrease in Hg resulted in a 1.91% increase in DNA-FTM. Similarly, a 10% increase in ΣPFASs results in a decrease of DNA-FTM by 9.10%, though depending on body mass, this effect changes. Lastly, body mass had a significant, positive effect on DNA-FTM. A 10% increase in body mass, resulted in a 13.42% increase in DNA-FTM (Fig. 3.6). Histograms of the residuals as well as quantile-quantile plots for both models can be found in the Appendix (Fig. B.5).

Table 3.7. Model estimates from the best linear models for DNA-FTM (%) in female incubating Eiders breeding on Christiansø, Denmark. Day 5 (n=22) and day 20 (n=23) of incubation for the breeding season of 2018 are presented. DNA-FTM, ΣPFASs and chemical elements have been log-transformed to account for non-normality in the data. Mass lost was also scaled when obtaining parameter estimates to allow for relative comparisons between predictors.

DNA-FTM	**Coefficients**	**Coeff. value**	**St. error**	**t-value**	**p-value**	**Adj. R^2**
day 5						
DNA-FTM ~ ΣPFASs	Intercept	3.6085	0.3514	10.268	p<0.001	23.43%
	ΣPFASs	-0.4305	0.1580	-2.725	p=0.013	
day 20						
DNA-FTM ~ Hg + ΣPFASs + mass + mass: ΣPFASs	Intercept	3.922	0.386	10.148	p<0.001	66.62%
	Hg	-0.193	0.124	-2.636	p=0.017	
	ΣPFASs	-0.955	1.408	-0.765	p=0.455	
	Mass	1.26	0.334	3.774	p=0.0016	
	ΣPFASs:mass	-0.473	0.133	-3.545	p=0.0027	

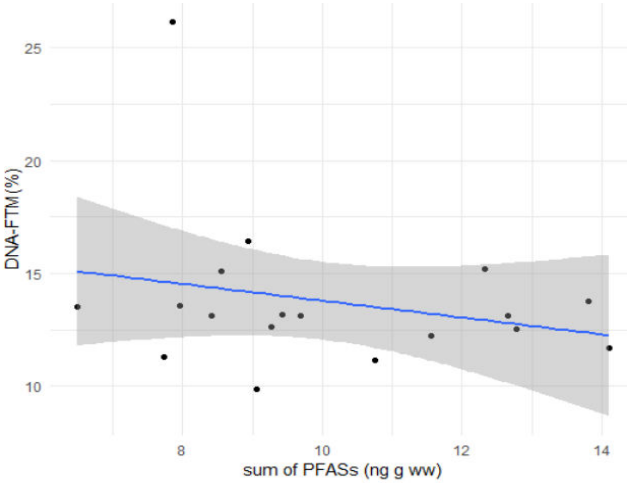


Figure 3.5. Linear regressions of the best models for day 5 explaining DNA-FTM (%) in female Eiders incubating on Christiansø, Denmark in 2018. DNA-FTM (%) was best explained by ΣPFASs. DNA-FTM and ΣPFASs have been back transformed and are presented in their original scale. Table 3.7 provides the linear regression line parameter estimates. The shaded regions indicate 95% confidence intervals.

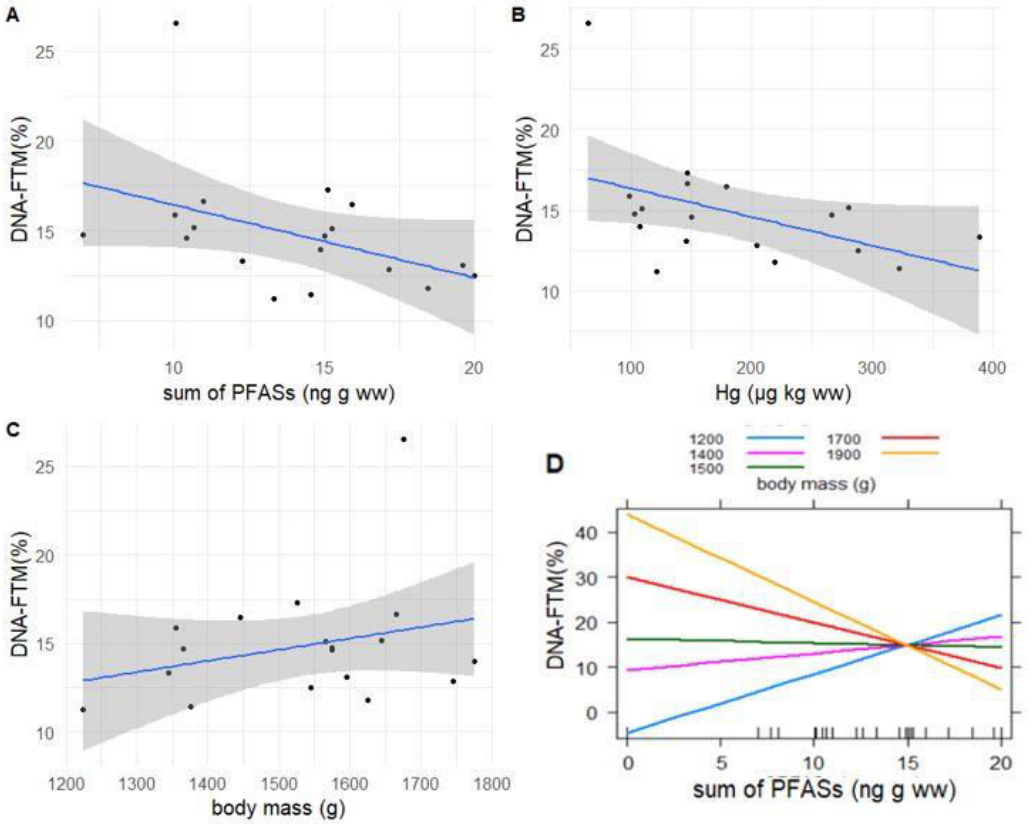


Figure 3.6. Linear regressions of the best model for day 20 explaining DNA-FTM (%) in female Eiders incubating on Christiansø, Denmark in 2018. DNA-FTM (%) was best explained by (A) ΣPFASs, (B) Hg, (C) body mass and (D) the interaction between ΣPFASs and body mass. DNA-FTM, ΣPFASs, and Hg have been back transformed and are presented in their original scale. Table 3.7 provides the linear regression line parameter estimates. The shaded regions indicate 95% confidence intervals.

3.6. Biochemical Response

In total, 24 BCCPs were measured in the female incubating Eiders on Christiansø, Denmark (methods). The mean, median, ranges, and paired t-test from day 5 and day 20 can be found in the Appendix along with percent changes between day 5 and day 20 (Appendix, Table B.4, Fig B.1).

3.6.1. Correlation Analyses with BCCPs

Correlation analyses were run between BCCPs and ΣPFASs, As, Cd, Hg, Pb, and body mass. For correlations between specific PFASs congeners and BCCPs see Appendix (Table B.7). ΣPFASs was weakly ($0.05 < p\text{-value} < 0.001$) correlated to ALKP ($r_s = -0.35$), Ca ($r_s = -0.33$), and FRU ($r_s = 0.38$). It was moderately ($0.001 < p\text{-value} < 0.0001$) correlated to AMY ($r_s = -0.43$) and Fe ($r_s = -0.43$) and strongly ($p\text{-value} < 0.0001$) correlated to CHOL ($r_s = 0.66$) and bile ($r_s = 0.57$). As was only weakly correlated to Mg ($r_s = 0.33$) while Cd was weakly correlated to GLU ($r_s = -0.30$) and CREA ($r_s = 0.29$). Hg was weakly correlated to GLU ($r_s = -0.30$) while Pb was weakly correlated to both FRU ($r_s = 0.30$) and moderately correlated to CREA ($r_s = 0.39$).

Body mass was strongly correlated to ALAT ($r_s = 0.74$), ALKP ($r_s = 0.53$), AMY ($r_s = 0.84$), Fe ($r_s = 0.86$), Ca ($r_s = 0.80$), PHOS ($r_s = 0.73$) and ALB ($r_s = 0.76$). Lastly, body mass was moderately correlated to ASAT ($r_s = 0.48$) and TP ($r_s = 0.44$) (Table 3.8).

Table 3.8. Significant correlations between BCCPs and ΣPFASs, As, Cd, Hg, Pb, and body mass for female incubating Eiders from Christiansø, Denmark during the breeding season of 2018. All contaminants and BCCPs data was log-transformed to create normality. If data was still not normal, Spearman's rank correlation test was performed. The r_s correlation coefficient value is presented with asterisks denoting significance ($p<0.05$*, $p<0.001$**, $p<0.0001$***).

BCCPs	ΣPFASs	As	Cd	Hg	Pb	Body Mass
Liver/digestive Enzymes						
Alanine aminotransferase (ALAT)						0.74***
Aspartate aminotransferase (ASAT)						0.48**
Alkaline phosphatase (ALKP)	-0.35*					0.53***
Amylase (AMY)	-0.43**					0.84***
Electrolytes/Minerals						
Iron (Fe)	-0.43**					0.86***
Calcium (Ca)	-0.33*					0.80***
Chloride (Cl)						
Phosphate (PHOS)						0.73***
Magnesium (Mg)		0.33*				
Potassium (K)						
Protein/Carbohydrate groups						
Albumin (ALB)						0.76***
Fructosamine (FRU)	0.38*				0.30*	
Glucose (GLU)			-0.30*	-0.30*		
Creatine (CREA)			0.29*		0.39**	
Total Protein (TP)						0.44**
Metabolic products						
Cholesterol (CHOL)	0.66***					
Bile	0.57***					

4. Discussion

The present study investigated how DNA integrity was affected by circulating blood levels of PFASs and toxic elements in female Eiders incubating on Christiansø, Denmark during the spring of 2018. This study uses both a cellular and biochemical approach to evaluate the health of female Eiders in relation to chemical contamination. The unique study design employed in this research, allows for direct comparison within test subjects from the early and late stages incubation. This provides a baseline control for each bird, which minimizes confounding factors associated with individual variation. To our knowledge this is the first study that analyzed PFASs in relation to genotoxic damage in the central Baltic Sea. The results of this study provide insight into current pollution trends in the Baltic Sea, the cellular impacts of that pollution, and the effects of chemical contamination on a declining population.

4.1. Body mass and health status

Body mass was measured as a means of analyzing overall body condition and health status of Eiders during incubation. Severe body mass loss can result in nest abandonment (Waltho and Coulson, 2015) or death (Korschgen, 1977) making this measure an important indicator of health as well as reproductive success.

During the 15-day incubation period body mass of female Eiders significantly decreased (Table 3.1). This body mass loss can be attributed to the fasting behavior that occurs during incubation as a means of egg protection (Waltho and Coulson, 2015) and is consistent with previous research on Eiders (Bustnes et al., 2010; Fenstad et al., 2014).

Though there has been evidence that female Eiders leave their nests to forage during this incubation period (Garbus, 2016), it is apparent that fasting is still the cause of the body mass decrease as reflected in the BCCPs and essential elements. From the start to the end of the 15-day incubation period ALAT, ASAT, ALKP, GGT, Na, Cl, PHOS, Ca, Fe, ALB, TP, lipase and LDH all significantly decreased (Appendix, Table B.1). Similar results have been shown to occur due to fasting in geese (Robin et al., 1987) and penguins (Robin et al., 1988). The Eider also had similar decreases in enzymatic activity in Finland (Hollmen et al., 2001) and in previous years on Christiansø, Denmark (Garbus, 2016). This is further supported by significant positive correlations

between body mass and TP, AMY, ALKP, ASAT, ALAT, and ALB (Table 3.8). Decreases in these parameters have previously been demonstrated as indicators of fasting in Eiders (Hollmen et al., 2001; Garbus, 2016).

These results differ from previous research as the percent body mass loss in the present study is much greater than that reported on Svalbard (19-21%) by Fenstad et al. (2014) and in northern Norway (18-23%) by Bustnes et al. (2012). However, these results are consistent across years as the same Christiansø Eider colony had a 31% body mass decrease during the 2017 breeding season (Noori, 2016) (Table 4.1), indicating this is not a yearly anomaly. It has been shown that harsher conditions, most notably temperature, are attributed to larger mass lost during incubation (Bustnes et al., 2012). However, as the central Baltic Sea has milder temperatures than Svalbard this cannot explain the large mass loss. Dehydration has also been presented as a contributing factor to poor body condition (Garbus, 2016). However, K and urea, both biomarkers of dehydration (Campbell et al., 2004), remained stable throughout the 15-day sampling period, indicating dehydration did not contribute to poor body condition.

Table 4.1. Body mass lost (g) and percent body mass lost (%) for female incubating Eiders on Christiansø, Denmark. The mean, standard error, median, range, and number of observations for the 2017 and 2018 breeding season are presented. Paired t-test results are presented with degrees of freedom (df), t and p values. 2017 data from Noori, 2018.

	2017				2018				t-test		
Parameter	**mean**	**SE**	**range**	**n**	**mean**	**SE**	**range**	**n**	**df**	**t**	**P**
Body mass lost (g)	734	34.64	420-1125	28	622.39	23.917	365-815	23	46.04	2.6513	0.01096
Percent body mass lost (%)	31	1.21	16.6-43.6	28	28.53	1.07	17.29--37.97	23	48.98	1.5279	0.133

Given the unusually large mass loss observed in the Christiansø Eider colony, another aim of this study was to determine if the Eiders sampled had entered into the third stage of fasting as described by Korschgen, (1977) and marked by a shift from lipid to protein catabolism. Furthermore, given the unique proteinphilic properties of PFASs, this is informative concerning reuptake of compounds.

Urea is an indicator of third stage fasting and is expected to increase, as it is the final nitrogenous biproduct of protein catabolism (Campbell et al., 2004). However, that is not observed in this study. Similarly, Garbus (2016), studying the same colony, did not find an increase in urea despite decreases in protein levels throughout incubation. However, strong positive correlations between

body mass, TP and ALB support the likelihood that protein catabolism is, in fact, occurring (Table 3.8) (Hollmen et al., 2001; Garbus et al., 2018). Additionally, highly significant decreases in TP (10.07%) and ALB (19.34%) (Appendix, Table B.4) further provide support that the Eiders fasting on Christiansø, Denmark have begun protein catabolism by the 20th day of the incubation period.

The Baltic/Wadden Flyway population has been shown to be in poor health (Desholm et al., 2002; Garbus et al., 2018; Laursen et al., 2019a) which can explain the large decrease in body mass exhibited in 2017 and 2018. There have been two mass mortality events (2007, 2015) attributed to parasite infection resulting in poor body condition and starvation (Garbus et al., 2018). Parasitic infections have been linked to changes in nutrient absorption as shown by decreases in ALAT (Hollmen et al., 1999), TP and ALB (Hanssen et al., 2017), all three of which significantly decreased from day 5 to day 20 of incubation (Appendix, Fig. B.1). These results could be an indication of parasitic infections resulting in large body mass loss and the possibility of another mass mortality event.

Food limitation, specifically the blue mussel, is also a factor affecting the Baltic/Wadden Flyway population's health (Larsson et al., 2014; Laursen et al., 2019a). Limitations in mussel availability lower the starting body weight when entering incubation (Larsson et al., 2014; Laursen et al., 2014). This can lead to general overall decreases in health and therefore a faster rate of body mass lost caused by the combined pressures associated with incubation (Korschgen, 1977). Furthermore, if has recently been shown that, prior to migration, Eiders allocate resources mainly on building the size of the gizzard (Laursen et al., 2019b). Upon arrival to the breeding grounds body condition is then built up favorably over gizzard size (Laursen et al., 2019b). This indicates that fluctuation of blue mussel stock at the breeding grounds can affect the body condition of Eiders prior to incubation. Toxic algae blooms also decrease the abundance of blue mussels (Larsson et al., 2014) and are becoming increasingly frequent in the Baltic Sea (ESA, 2019). These factors combined could explain the differing ranges between body mass loss in 2017 and 2018 as well as the consistently large body mass lost for the female Eider breeding on Christiansø, Denmark.

4.2. PFASs

4.2.1. PFASs contamination on day 5 and 20 of incubation

A primary goal of assessing PFASs in this research was to analyze their impact on Eiders in the central Baltic Sea during incubation. With the present study design, it is possible to observe changes in bioavailability of PFASs caused by the stress of breeding and incubation within the model organism. Due to pollution trends in the Baltic Sea it was expected that Eiders breeding on Christiansø, Denmark would have higher blood concentrations of PFASs when compared to the Canadian Arctic (Butt et al., 2007; Braune et al., 2011) and northern Norway (Verreault et al., 2005). Blood concentrations of PFASs were also expected to increase from day 5 to day 20 of the incubation period due to body mass loss causing remobilization of PFASs.

Because exposure to PFASs is through diet (Haukås et al., 2007) it is expected that birds feeding at a higher trophic level than the Eider would have higher levels of PFASs. This is reflected in our findings, as apex predators such as glaucous gulls on Svalbard have levels 20x that of the Christiansø Eider colony (Verreault et al., 2005). Furthermore, the black-legged kittiwake, who are not apex predators, though still feeds at a higher trophic level than the Eider, have PFASs levels double those reported in the present study (Contantini et al., 2019). However, it is important to remember species differences play a large role in uptake and excretion and therefore can create difficulties when drawing conclusions across species lines (Haarr et al., 2018).

PFOS was the most abundant PFASs detected in the Eiders breeding on Christiansø (Table 3.2). This is to be expected as PFOS was the most produced PFASs between 1940-1990 and is most wide-spread in the environment (Mueller and Yingling, 2017). PFOS was found to be the most prevalent PFASs detected in glaucous gulls in the Norwegian Arctic (Verreault et al., 2005), in tree swallows (*Tachycineta bicolor*) in central Minnesota (Custer et al., 2012), and in black guillemot (*Cepphus grylle mandtii)* in Svalbard (Eckbo et al., 2019). There are few studies reporting PFOS levels in Eiders and none could be found regarding Eiders in the central Baltic Sea. However, Eiders breeding on Kongsfjorden, Svalbard were found to have lower overall PFASs concentrations (1.7±1.5 ng/g) than those reported in this study (Haarr et al., 2018). This is to be expected as PFASs levels are much lower in the Arctic biota (Hung et al., 2016) as compared to the Baltic biota (HELCOM, 2018).

PFHxS was the next most common PFASs in the female incubating Eiders on Christiansø. This is reflected in usage patterns as PFHxS is a common, shorter chain, replacement for PFOS (UNEP, 2017). glaucous gulls on Svalbard reflect the same contamination pattern (Verreault et al., 2005) although PFNA was reportedly the second most abundant PFASs in tree swallows in Minnesota (Custer et al., 2012) while PFUnA was second most prevalent in black-legged kittiwakes and black guillemot from Svalbard (Costantini et al., 2019; Eckbo et al., 2019). Because of PFOS's prominence in the environment (HELCOM, 2018) it is likely that the patterns of contamination in this study are due to elevated levels of exposure as opposed to greater bioavailability or affinity.

These results show that despite regulations on long chain PFASs and "phasing out" efforts (Section 1.1), wildlife in the central Baltic Sea are still suffering from chronic contaminant exposure. Furthermore, these results are a testament to the dangers of continued production of shorter chain replacements, as they share the same bioaccumulative and persistent properties as their predecessors.

4.2.2. Biochemical response to PFASs

While this study looked primarily at the genetic impacts of PFASs, BCCPs were analyzed to provide some insights to overall organ system health and body condition in relation to PFASs. This provides systematic information that is otherwise lacking in the field of genetic toxicology. Although POPs have been linked to changes in BCCPs, it is not possible to determine the mode of action or mechanisms of toxicity (Sonne et al., 2010; 2013).

There is little research on PFASs in relation to BCCPs and none could be found specifically concerning Eiders. However, BCCPs in relation to POPs have been studied (Sonne et al., 2010; 2013; Jacobsen et al., 2017) which will be used here as a reference. In the present study PFASs were strongly correlated to bile and CHOL (Table 3.8) which is reflected in research that found positive correlations between POPs, bile, and CHOLlevels in raptor chicks (Sonne et al., 2010) and in sled dogs (Sonne et al., 2008). However, in contrast Hoff et al. (2005) found a negative relationship between serum CHOL and PFOS levels in great tits (*Parus major*) and blue tit (*Parus caeruleus*) nestlings. These correlations have been linked to impacts on hepatic and metabolic function, though no effect has been specifically identified given BCCPs fluctuate greatly based on diet, sex, and species (Hoff et al., 2005; Sonne et al., 2010; 2013; Jacobsen et al., 2017).

Relevant to this study is the negative correlation between ALKP and PFASs which can be indicative of hepatic effects and supported by the results presented by Sonne et al. (2010). A possible mechanism of toxicity to explain this hepatic disfunction is the activation of PPARα associated pathways by PFASs resulting in vacuole formation and liver hypertrophy (Dewitt, 2015). Furthermore, decreasing ALKP levels could be an indication of disrupted Ca metabolism and bone morphometry (Andrews, 1989), which is supported by the negative correlation between Ca and PFASs. The relationships presented in this study could be indicative of impaired hepatic functionality caused by PFASs exposure possibly contributing to observed population declines in the Baltic/Wadden Flyway Eider population (Desholm et al., 2002).

4.2.3. PFASs reuptake

Total PFASs concentration increased during the incubation period by 43.15%. The cause of this increase is likely the catabolism of proteins as a result of third stage fasting. This catabolism releases the previously bound PFASs, enabling reuptake of compounds into the bloodstream. Supporting this is the negative correlations between body mass and PFASs and the clear distinction between day 5 and day 20 of incubation (Fig. 3.2). There have been no reports of PFASs reuptake in the Eiders, but, reuptake of Hg, *p'p*-DDE, PBCs, HCB, and a number of chlordanes during incubation has been documented by Bustnes et al. (2012) and Fenstad et al. (2016a). However, these compounds are lipophilic meaning any degree of fasting leads to reuptake while PFASs are amphipathic and preferentially bind proteins (Dewitt, 2015). Therefore, lipid reserves must be completely depleted for protein reserves to be metabolized (Korschgen, 1977) meaning PFASs reuptake is dependent on preexisting fat reserves, and the extent of fasting. Consequently, this makes PFASs reuptake comparisons between species and even within species difficult. However, as discussed above, it is possible the Eiders in this study have entered the third stage of fasting during incubation (Garbus, 2016) meaning they are catabolizing proteins and therefore remobilizing any protein sequestered compounds, such as PFASs.

4.3. Chemical Elements

A primary goal of assessing chemical elements in this study was to analyze spatial and temporal trends of toxic element blood contamination in the central Baltic Sea. As with PFASs, it was hypothesized that from day 5 to day 20 of the incubation blood concentrations of toxic element would increase as lipid and protein metabolization released previously immobile elements.

Furthermore, essential element blood concentrations were analyzed as a means of understanding possible trends in toxic element absorption and distribution. It was hypothesized that essential element blood concentrations would decrease over the 15-day incubation period.

Noori, (2018), reported on toxic element concentrations from Eiders breeding on Christiansø, Denmark in 2017. Additionally, Fenstad et al. (2017) reported on toxic element concentrations from the northern Baltic Sea (Tvärminne, Finland) in Eiders at the end of incubation which will also be used as a comparison discussed below.

Table 4.2. As, Cd, Pb and Hg blood concentrations (µg/kg ww) in incubating female Eiders breeding on Christiansø, Denmark (2017, 2018) and Tvärminne, Finland (2011) on day 5 and day 20 (or day 25 for 2017 on Christiansø, Denmark) of incubation. Data from Noori (2018) and Fenstad et al. (2017).

Year and location	day	As	Cd	Pb	Hg
2018, Christansø,	5	12.91±3.18	0.82±0.397	209.15±99.89	221.05±185.43
Denmark	20	13.41±3.52	1.19±0.499	256.94±237.66	183.33±86.17
2017, Christansø,	5	13.62 ± 3.84	0.82 ± 0.30	41.65 ± 66.29	175.40 ± 63.72
Denmark	25	15.53 ± 4.68	1.27 ± 0.46	55.65 ± 65.60	179.59 ± 62.89
2011, Tvärminne,	5	NA	NA	NA	NA
Finland	20	16.24 ± 5.82	2.4 ± 0.7	45.06 ± 17.07	174.22 ± 66.59

4.3.1. Arsenic

Arsenic blood concentrations for Eiders breeding on Christiansø, Denmark in 2018 did not significantly change from day 5 to day 20 of incubation (Table 3.4, 4.2). These results are comparable to the breeding season of 2017, (Table 4.2) which also reported no significant change from the start to the end of incubation (Noori, 2018). Furthermore, these results reflect levels reported in the northern Baltic Sea (Table 4.2) (Fenstad et al., 2017), indicating As pollution is relatively constant spatially between the northern and central Baltic Sea as well as temporally within the Bornholm Basin. HELCOM (2010) reports on World War II dumping sites within the Bornholm Basin which could be a possible source of As pollution, but based on Noori, (2018) and the results presented here, the Eider does not appear to be at high risk of As toxicity nor does there appear to be new sources of As exposure. Furthermore, HELCOM (2018) does not classify As as a mandatory parameter to be reported, and is not considered a primary pollutant in the Baltic Sea (HELCOM, 2018) and will therefore not be discussed further in this report.

4.3.2. Cadmium

Cd levels in the present study significantly increased (Table 3.3, 3.4) from day 5 to day 20 of incubation. This is again consistent with the breeding season of 2017 (Table 4.3) indicating no new sources of Cd exposure. Though there is no avian threshold for Cd toxicity (Wayland et al., 2008a) the levels reported in this study are below those in the northern Baltic Sea (Table 4.2) where it was concluded that the Eiders were not at risk of Cd toxicity (Fenstad et al., 2017). Furthermore, there were no correlations between mortality and Cd levels in North American King Eiders (*Somateria spectabilis*) which had Cd levels well above what is reported in this study (Wayland et al., 2008a). This indicates the population declines observed in the Baltic/Wadden Flyway Population are not likely a result of Cd toxicity.

It should be mentioned that Cd levels in this study were acquired from blood samples and Cd accumulates primarily in the liver and kidneys (Scheuhammer, 1987). Therefore, it is possible the levels reported in this study do not reflect the actual contamination of this colony. However, chronic Cd exposure has been shown to be linked to reduced Ca uptake (Koivula and Eeva, 2010) which is not reflected by the BCCPs in this study (Table 3.8). Furthermore, Cd is not correlated to any liver or kidney BCCPs (Table 3.8) again indicating this colony is not experiencing Cd toxicity.

The significant increase from day 5 to day 20 is likely caused by fasting, as reflected in the significant correlation between body mass and Cd. This is supported by the results of the 2017 breeding season (Noori, 2018) as well as Cd increases due to fasting in female King Eiders in the Canadian Arctic (Wayland et al., 2008b). However, the correlation is masked when individuals F26 (day 20) and F27 (day 5) are included in the analysis.

There is an equilibrium between Cd levels in the organs and blood, with organ samples reflecting chronic exposure while blood samples reflect short term exposure (Wayland et al., 2001). This indicates the Cd levels reported in this study are remobilized from the liver and kidneys as a result of fasting (Wayland et al., 2005). However, it is also possible dietary exposure during incubation could result in the Cd levels observed here. Eiders in worse body condition at the end of incubation (Table 3.1, 3.8) have been shown to leave their nests more often to forage (Garbus, 2016) which could explain the increased levels of Cd on day 20. This is supported by the 6.87% increase of FRU from day 5 and day 20 of incubation which is a possible indication of increased digestion (Campbell et al., 2004) from recent foraging. Coupled with this is the significant decrease in Ca

from start to finish of incubation (Appendix, Table B.4). As Cd mimics Ca (Wayland and Scheuhammer, 2011), it is possible that the Eiders absorb more Cd towards the end of incubation as their need for the essential element Ca increases.

However, Cd was correlated only to Cu and Fe (Fig. 3.3) and not Ca. A significant positive correlation between Cd and Cu was reported in 2017 as well (Noori, 2018) and explained by the possibility of Zn deficiencies causing increased levels of Cu and Cd absorption. In the present study Zn did significantly decrease from day 5 to day 20 of the incubation period, although was not significantly correlated to either Cu or Cd (Appendix, Table B.6). There is little research concerning the effects of Cu on Cd toxicokenetics and therefore the outcome of this correlation is not well understood.

4.3.3. Lead

Pb levels in the present study significantly increased (Table 3.3) from day 5 to day 20 of the incubation period. This is inconsistent with the 2017 breeding season on Christiansø, Denmark (Table 4.2) as there has been a 361.70% increase between the two years. Furthermore, these Pb blood concentrations are 470.22% above those from the northern Baltic Sea (Table 4.2) (Fenstad et al., 2017). As Pb inputs into the Baltic Sea have temporally decreased (HELCOM, 2018) it is more likely the increased exposure in 2018 is a result of a new, point source pollution. Further research should continue to monitor Pb levels in Eiders and attempt to identify this source.

The Pb blood levels reported in this study are above toxicity threshold levels (200 µg/kg) at which adverse effects and mortality can occur (Franson and Pain, 2011) both on day 5 and day 20 of incubation. Although the median is below this threshold for both days (Table 3.3), 15% and 22% of the Eiders are above or approaching the sub-lethal but toxic threshold of 500 µg/kg (Franson and Pain, 2011) on day 5 and day 20 respectively. Furthermore, 40 mg/dL and 60 mg/dL (400-600 µg/kg) are considered grounds for removal from wild for rehabilitative treatment while blood levels of Pb >60mg/dL are potentially lethal (Fallon et al., 2017). There have been reports of buckshot explaining elevated levels of Pb in Eiders (Hollmen et al., 1998), however as almost all the individuals had elevated levels of Pb, and buckshot would affect only a few significantly, this is likely not the newest exposure source.

Two decades ago Pb concentrations were also reported to be above threshold toxicity levels in Eiders breeding the Baltic Sea (Franson and Pain, 2000) resulting in mortality caused by Pb poisoning (Hollmen et al., 1998). Since then, Pb levels have significantly declined in the biota (HELCOM, 2018) which has been reflected in Eiders (Franson and Pain, 2011; Fenstad et al, 2017; Noori, 2018). However, Pb blood concentrations of Eiders in the present study indicate many individuals are in danger of Pb poisoning.

This Pb source could be responsible for the mass mortality events recorded in 2007 and 2015 that killed over 200 Eiders collectively. While autopsies were preformed on dead Eiders in 2015 (Garbus et al., 2018), Pb levels were not recorded, and could therefore have been an unknown contributing factor. The cause of death was documented as emancipation exacerbated by high parasitic load (Garbus et al., 2018) however, Pb has been shown to decrease immune function (Koivula and Eeva, 2010) and body condition (Fallon et al., 2017). Furthermore, two clinical signs of Pb intoxication are lethargy and poor body condition, both of which were documented in the mass mortality event of 2015 as many of the Eiders were found dead on their nests, in a state of starvation. These events taken together should be warning of the possibility of another mass mortality event for the Christiansø Eider colony.

While Pb did significantly increase from the start to the end of incubation it was not correlated to body mass. This is consistent with results from Fenstad et al. (2017) in the northern Baltic Sea, though inconsistent with the 2017 results (Noori, 2018). As body mass is not correlated to Pb in this study it seems likely that the increased levels of Pb are due to exposure on the breeding grounds as opposed to remobilization of stored Pb, further supporting the dangers of another mass mortality event. As with Cd, it is possible the Eiders leave their nests more to forage towards the end of incubation (Garbus, 2016) resulting in increased exposure and absorption of Pb. This is again, supported by the increased levels of FRU indicating recent digestion (Campbell et al., 2004), as well as the significant positive correlation between Pb and FRU (Table 3.8).

It was expected that Pb would be correlated to Zn, Ca, or Fe as it is known to mimic both Zn and Ca (Skerfving and Bergdahl, 2014) and inhibit ALAD activity leading to anemia (section 1.4.2). However, as with Cd, Pb was only significantly correlated to Cu which could again be a result of increased absorption due to simultaneously decreasing Ca levels (Fig 3.3). Research suggests Pb blood concentrations over 200 µg/kg are associated with decreasing ALAD capacity (Franson et

al., 2000), however, without further analysis it is impossible to confirm if the mechanism of action of Pb poisoning is associated with ALAD activity.

4.3.4. Mercury

In 2018 blood concentrations of Hg were comparable to the 2017 results as well as levels reported in the northern Baltic Sea at the end of incubation in 2011 (Table 4.2) (Fenstad et al., 2017). This shows that the bioavailability of Hg has remained constant in the Baltic Sea and there are no new sources of exposure. These results are also consistent with the hypothesis that Eiders in the Baltic Sea experience higher levels of contamination than their Arctic counterparts. Hg concentrations in Nova Scotia, Canada reported in the Eider are approximately half (80.0 µg/kg) of those reported in the Baltic (Pratte et al., 2015).

The Hg concentrations reported in this study are well below critical avian toxicological thresholds (2100 µg/kg to >6700 µg/kg) reviewed by Fuchsman et al. (2017). However, it should be noted these reference values are for MeHg, which cannot be distinguished from other forms of Hg in this study. Furthermore, as Hg and MeHg are filtered in the liver, it is possible these results do not reflect total contamination in the model organism. Although, Wayland et al. (2001) found that blood concentrations of Hg were related to corresponding levels in the kidneys and liver indicating these results do, in fact, accurately reflect the Hg body burden in Eiders.

From day 5 to day 20 of the incubation period Hg significantly decreased by 12.34% (Table 3.4). This is contrary to the 2017 results in which Hg concentrations remained unchanged throughout incubation (Noori, 2018). It was expected that Hg would be negatively correlated to Se as research has shown an interaction between the toxic and essential element (Kim et al., 1996). However, in the present study Hg and Se were not correlated (Appendix, Table B.6) indicating the decrease in Hg seen over incubation is not due to a Se binding. Supporting this are the normal ranges of Se required for homeostasis in avian species (Harry and Gary, 2011) both at the beginning and end of incubation. Considering this it seems likely the decrease in Hg observed over the incubation period is due to excretion, most commonly via the feces or feathers. As blood Hg reflects recent dietary exposure (Wayland et al., 2011), we can conclude that the blood concentrations of Hg in the Christiansø Eider colony are not due to exposure on their breeding grounds.

4.4. DNA-DSBs

The unique contribution of this study was to analyze DNA damage in relation to PFASs, chemical elements, and the stress of incubation. The primary goal of this analysis was to determine if DNA integrity was affected by the stress of incubation and if circulating levels of contaminants contributed to its degradation. It was hypothesized that both the stress of incubation and contaminant blood concentrations would increase DNA-DSBs in the Eiders breeding on Christiansø, Denmark in 2018.

In this study DNA-FTM and MML were both analyzed as indicators of DNA integrity. It is expected that as DNA-FTM increases, MML decreases. However, while DNA-FTM showed a significant increase from day 5 to day 20, there was no significant difference in MML between the two sampling days. It is possible these measurements did not reflect the same conclusion because MML is considered a less exact measurement of DNA-DSBs than DNA-FTM as the calculations are subject to individual discretion (Fenstad et al., 2014). Furthermore, MML is a measurement of the median molecular length of the *peak*, which corresponds to the band that has traveled through the agarose gel matrix (Appendix, Fig. A.5, A.6). The MML of this peak is then used to represent the length of the DNA fragments in the entire sample excluding the many different sized fragments in the sample which have not migrated as far through the matrix. DNA-FTM however compares the entire amount of DNA in the well compared to that in the gel making it a more sensitive measure of DNA-DSBs (Fenstad et al., 2014). This could explain why DNA-FTM detects a significant difference between sampling days while MML does not.

There was a significant increase in DNA-FTM between day 5 and day 20 of incubation (Table 3.6). This indicates that female Eiders have an increase in DNA instability from day 5 to day 20 of incubation. This is consistent with Fenstad et al. (2016) who found female Eiders in the northern Baltic Sea to have a DNA-FTM of 14.26% at the end of incubation (~day 26). In comparison to Eiders breeding on Svalbard, those in the Baltic Sea have lower levels of DNA damage, as expected given the harsher Arctic conditions during the incubation period (Fenstad et al., 2016a).

Table 4.3. DNA-FTM on day 5 and 20 of incubation for incubating female eiders in Christansø, Denmark (2017, 2018), Kongsfjorden, Svalbard (2008, 2009), and Tvärminne, Finland (2011). However, DNA-FTM was measured on day 25 of incubation in 2017 in Christansø, Denmark, which is noted by (25). Percent increase during incubation is also provided. Data is from the present study, from Noori (2018), Fenstad et al., (2014), and Fenstad et al., (2016a).

Year and location	DNA-FTM day 5	DNA-FTM day 20 (25)	DNA-FTM % increase during incubation
2018, Christansø, Denmark	14.83 ± 4.13	16.63 ± 5.04	10.32%
2017, Christansø, Denmark	63.4 ± 10.6	79.1 ± 7.9	27.4%
2008, Kongsfjorden, Svalbard	37.4 ± 19.8	60.2 ± 19.7	61.0%
2009, Kongsfjorden, Svalbard	16.7 ± 9.4	27.8 ± 17.6	66.5%
2011, Tvärminne, Finland	N/A	14.26 ± 4.9	N/A

However, on Christiansø, during the breeding season of 2017 Eiders had much larger DNA-FTM (Table 4.3) (Noori, 2018). This corresponds to an increase between day 5 and day 20 that is more than double the increase seen in the present study (Table 4.3). It should be noted that in 2017 samples were taken on day 25 of incubation, where in 2018 the second sampling was on day 20. These additional days of incubation likely contributed to some of differences in DNA-FTM increase seen between years. As the Eiders are in their poorest body condition at the end of incubation (Korschgen, 1977) DNA integrity likely decreasing more rapidly in these 5 days than it would at the start of incubation. However, without additional sampling days over the course of the incubation period it is impossible to draw conclusions. Furthermore, as the DNA-FTM was elevated on day 5 in 2017 as compared to 2018, it is likely that, in addition to sampling inconsistencies, yearly variation is a large contributing factor to these differences. Fenstad et al. (2014) also found that Eiders incubating on Svalbard experienced a much larger increase in DNA-FTM throughout incubation than seen in the present study (Table 4.3). These discrepancies indicate that the 2018 Christiansø Eiders either; (1) experience less DNA insult, (2) have increased rates of DNA repair, or (3) have increased rates of apoptosis during the incubation period.

One of the main sources of DNA insult is body mass loss (Fenstad et al., 2014; Fenstad et al., 2016a; Noori, 2018) which is exacerbated by poor weather conditions (Bustnes et al., 2012; Fenstad et al., 2014) and limited food availability (Larsson et al., 2014; Laursen et al., 2014). However, as body mass lost was consistent between sampling years (Table 4.1.), the differences

in DNA-FTM are more likely caused by exogeneous factors such as heat energy, UV-light, free radicals, ionizing radiation, or chemical contamination.

Individual variation accounts for some of the differences seen in year and population (Fenstad et al., 2016a; 2016b). Biological and non-biological parameters such as metabolization capacity (section 1.5.3) and external stress could also result in differences in DNA-FTM (Fenstad et al., 2014). For example, harsher conditions such as storms or colder temperatures during the incubation period causes the Eiders to allocate more energy towards body heat (Bustnes et al., 2012) limiting energy for DNA repair. This could cause higher levels of DNA damage as a result of yearly weather fluctuations.

Without further testing of DNA repair or apoptotic markers, it is impossible to disentangle other molecular and cellular processes that determine levels of DNA damage. However, high levels of DNA damage have been shown to induce apoptosis (section 1.5.2.) which would result in lower levels of reported DNA damage but higher rates of cell death. As the Christiansø Eider colony has shown to lose such large percentages of body mass it seems more likely they are favoring apoptotic pathways over DNA repair towards the end of incubation as apoptosis is the less energy costly action (Nowsheen and Yang, 2013).

4.4.1. Relationship between DNA-DSBs, PFASs, chemical elements, and body mass

This study aimed to investigate the relationship between genetic instability, PFASs, and toxic elements to assess the cellular health of the Eider colony breeding on Christiansø, Denmark. PFASs (Liu et al. 2014; Wielsøe et al., 2015) and toxic elements (Koivula and Eeva, 2010; Fenstad et al., 2016b) have been shown to be genotoxic, both primarily through the production of ROS. Given PFASs capability of bioaccumulation and long-range transport, it is important to investigate their toxicity in the biota. It was expected that as PFASs and toxic element concentrations increased DNA-FTM would increase in tandem. Furthermore, based on previous research, it was expected that Hg and body mass loss would be implemented in increasing DNA-FTM (Fenstad et al., 2014; 2016a; Noori, 2018). Lastly, essential elements were expected to be negatively correlated with DNA-FTM given their protective properties against toxic elements (Section 1.4).

From both the PLS and the linear models it is shown that ΣPFASs are related to DNA-FTM on day 5 and day 20 of incubation. Additionally, on day 20, both Hg and body mass had a significant

effect on DNA-FTM (Fig. 3.4). This indicates that environmental exposure to PFASs and Hg effects DNA-FTM along with the pressures of fasting.

On day 20 Hg and body mass become significant predictors of DNA damage. These results are supported by correlation analysis (Appendix, Table B.9) as well as previous research finding mass loss to be the best predictor variable of DNA-FTM in the female Eider at the end of incubation (Fenstad et al., 2014). On day 5 of incubation the Eiders are still in good body condition (Korschgen, 1977) and therefore DNA integrity is not effected by body mass. However, as the Eiders enter into third stage fasting (discussed above and by Garbus, 2016) resources are allocated elsewhere (Laursen et al., 2019b) resulting in decreased antioxidant response and DNA maintenance (Fenstad et al., 2014). Coupled with this is the decrease in essential elements (Table 3.4) which disrupts metal homeostasis and hinders the Eiders ability to deal with oxidative stress (Koivula and Eeva, 2010) resulting in increased levels of DNA damage.

There was a significant interaction between body mass and PFASs on DNA-FTM showing that at a lower body mass, ΣPFASs begin to have a positive effect on DNA-FTM (Fig. 3.6). This highlights the central question; if poor body condition leads to higher levels of contamination (Wayland et al., 2002) or if high levels of contamination lead to poor body condition (Fenstad et al., 2014). In the present study, as body condition worsens, blood concentrations of PFASs increase leading to increased DNA-FTM, which supports the former statement (Wayland et al., 2002).

Even though Hg blood concentrations decrease throughout incubation, Hg becomes a significant predictor of DNA-FTM on day 20. Hg is not correlated to body mass or ΣPFASs, so its' significance is not a statistical artifact or the result of multicollinearity. In *vitro* studies have shown that PFASs decrease the total antioxidant capacity (Wielsøe et al., 2015) which could result in the increased production of ROS from Hg which was previously scavenged by antioxidants. However, this is speculative given interaction effects are complex and not well understood.

In this study, both Hg and ΣPFASs were negatively related to DNA-FTM (Fig. 3.4, 3.5, 3.6) indicating that as blood concentrations of Hg and PFASs increased, DNA-FTM decreased. This is an unexpected result as both Hg and PFASs have been shown to produce ROS (Wielsøe et al., 2015; Costantini et al., 2019) which is a known mechanism of genotoxicity (Preston and Hoffmann, 2013).

This can be explained by limited concentration ranges covered in the data. Previous research has described a non-linear dose response relationship that included a threshold between PFASs and physiological status in black guillemot (*Cepphus grylle mandtii*). This could explain why both Wielsøe et al. (2015) and Lui et al. (2014) found DNA damage to increase signficicantly only after a certain exposure concentration. Given this, it is possible that the negative relationship between PFASs and DNA-FTM is due to exposure concetrations being below the above mentioned threshold, which could result in a weak, but negative relationship.

It has been suggested that chronic exposure to DNA-damaging contaminants can result in the upregulation of defense mechanisms ultimately leading to a reduction of DNA damage (Maness and Emslie, 2007; Collins, 2009). This has been observed in both royal terns *(Sterna Maxima)* (Maness and Emslie, 2007) as well as polar bears *(Ursus maritimus)* (Gilmore, 2015) and is ascribed to adaptive responses to contaminant exposure. Adaptative responses to ionizing radiation are well documented both in *vitro* (Maeda et al., 1999) and in *vivo* studies (Galvan et al., 2014). For example, free-ranging birds residing around Chernobyl have increased antioxidant responses, decreased oxidative stress levels, and decreased DNA damaged with increased levels of background radiation (Galvan et al., 2014). Supporting this are the higher levels of Radiocaesium (HELCOM, 2017) and Radon (Gravesen and Jakobsen, 2016) recorded in the Baltic Sea. In fact, Bornholm hosts three of the five most radioactive surface rocks measured in Denmark (Gravesen and Jakobsen, 2016). This radioactive exposure could be providing protective adaptation and an increased oxidative stress response to contaminant exposure in the Christiansø Eider colony. An interesting avenue for further research would be to investigate the link between radioactivity and DNA damage within the Bornholm basin.

Another mechanism of adaptation is fixation of adaptive alleles within a population (Bickham, 2011; Lee et al., 2017; Whitehead et al., 2017). Contaminant exposure has shown to change population-wide genetic diversity to favor adaptive alleles such as the reduction of biotransformation enzymes in Atlantic Killifish (*Fundulus heteroclitus*) (Whitehead et al., 2017) or faster developmental times in *Caenorhabditis elegans* (Dutilleul et al., 2017). However, for this to occur there needs to be large populations with preexisting genetic diversity (Whitehead et al., 2017). If the Eiders in the present study are undergoing contemporary evolution due to selection pressure caused by genotoxins, they may favor the upregulation of DNA repair machinery which

would result in an inverse correlation between DNA damage and contaminant blood concentrations. However, it is impossible to confirm this hypothesis without genome sequencing and analysis of multiple, interconnected cellular pathways.

A last explanation for the unexpected results in this study is the possibility that DNA-FTM is not an accurate indicator of genetic stability or cellular health. As mentioned above, when discussing body mass and DNA-FTM it is possible that increased rates of apoptosis are masking levels of DNA damage. It has been shown that PFASs (Liu et al., 2014; Wielsøe et al., 2015) and toxic elements (Koivula and Eeva, 2010) increase apoptosis, often through the production of ROS which has multiple mechanisms of genotoxicity. For example, activation of P53 via strand breakage or lipid and protein oxidation will cause the cell to abondon repair mechanisms and simply undergo apoptosis (Alberts et al., 2014). Following this mechanism, on day 5 of incubation repair machinery was predominating. However, as PFASs and toxic element concentrations increased, it is possible the oxidative stress was too great and apoptosis became the main mechanism to maintain genetic homeostatis resulting in a negative trend between DNA damage and chemical concentrations (Fig 5.6). Furthermore, the decreases in Ca reported thorughout incubation can contriubte to endoplasmic reticulum Ca disturbances leading to the induction of apoptosis (Nowsheen and Yang, 2013). Lastly, activation of PPARα, the main target of PFASs, is known to upregulate apoptosis through the degradation of BCL2 family proteins (Gao et al., 2015). These factors cumulatively show the necessity of further investigation into additional cellular and genetic processes explaining the link between DNA damage, oxidative stress, and apoptosis.

4.5. Future Prospects

It is imperative that further research continues to monitor the Christiansø Eider colony. The results of this study highlight the extreme yearly variation in DNA integrity and chemical contamination, especially in regard to Pb exposure. As it is possible that Pb is contributing to the mass mortality events on Christiansø it is paramount to identify this Pb source, so appropriate action can be taken. Furthermore, radioactivity within the Bornholm basin should be monitored and analyzed in respect to DNA integrity and antioxidant responses as it is possible Radon and Radiocesium exposure are instigating adaptive responses to ROS within this Eider colony.

It is also recommended to investigate cellular responses to PFASs and toxic element exposure. To determine if the genotoxicity (DNA-DSB) observed in Eiders is being propagated cell-wide,

biomarkers of DNA repair, apoptosis and cell senescence could be utilized. This would provide information concerning if DNA damage is too great to repair, causing the cell to undergoes cell cycle arrest or apoptosis (Alberts et al., 2014). Elucidating the mechanisms behind cell fate will contribute to the knowledge concerning genotoxic responses to environmental pollution. This provides a broader understanding of how genetic damage can propagate into cellular alterations and ultimately affect the overall health of an organism.

This study further displays the interest of the Christiansø Eider colony both as a species for biomonitoring, but also as a test organism for molecular and cellular responses to environmental pollution. Large yearly differences between 2017 and 2018 show that it is crucial to continue monitoring this colony as it has the potential to elucidate some of the factors dictating DNA integrity and cellular health.

5. Conclusion

The main goal of the present study was to analyze DNA stability in relation to blood concentrations of PFASs and toxic elements. This study used the non-invasive approach of collecting blood and recording body mass from 25 and 23 female Eiders (*Somateria mollissima*) on Christiansø, Denmark on day 5 and day 20 of incubation, respectively. Additionally, BCCPs were obtained and analyzed in relation to body mass, PFASs and toxic elements.

Body mass decreased by 28.53% from day 5 to day 20 of the incubation period. This is in conjunction with the hypothesis and attributed to fasting and minimal foraging that occurs during this time period. Confirming the state of fasting are decreases observed in liver and metabolic enzymes as well as essential elements. It is difficult to draw concrete conclusions concerning weather the Eiders have entered the third stage of fasting, however, decreases in TP content and ALB indicate protein catabolism.

Concentrations of PFASs were found to be increasing from day 5 to day 20 of the incubation period, though a much smaller increase was observed in this study as compared to POPs increases in Eiders incubating on Svalbard and northern Norway. However, it is difficult to compare as POPs and PFASs have different chemical properties. PFOS was the most abundant PFASs followed by its shorter, chain replacement PFHxS. Hg, Cd, and As levels were comparable to those recorded in female Eiders in 2017 on Christiansø, Denmark and in the northern Baltic Sea in 2011. However, Pb concentrations had increased dramatically which can be explained by a new source of exposure.

The best explanatory variable for DNA-FTM on day 5 was ΣPFASs while on day 20 it was ΣPFASs, body mass, and Hg. The importance of body mass and Hg on DNA-FTM is reflected in previous years (Christiansø, Denmark (2017) and the northern Baltic Sea (2011). However, in this study the trend between DNA-FTM, ΣPFASs and Hg was negative while in the past it has been positive. This can possibly be explained by nonlinear dose-response relationships, adaptive responses to chronic exposure, or increased rates of apoptosis and decreased rates of DNA repair. Of course, without further research into the genome and cellular processes none of these hypotheses can be confirmed. This research is relevant for both conservation purposes of the Christiansø Eider colony and for elucidating the complex mechanisms behind maintaining genetic homeostasis despite stress from multiple sources.

References

Abadin, H., Ashizawa, A., Stevens, Y.W., Llados, F., Diamond, G., Sage, G., Citra, M., Quinones, A., Bosch, S., and Swarts, S. (2007). *Toxicological Profile for Lead*. (pp. 1-414). Agency for Toxic Substances and Disease Registry (US), Atlanta, GA, 1st edition.

Abbott, B. D., Wolf, C. J., Das, K. P., Zehr, R. D., Schmid, J. E., Lindstrom, A. B., Strynar, M. J., Lau, C. (2009). Developmental toxicity of perfluorooctane sulfonate (PFOS) is not dependent on expression of peroxisome proliferator activated receptor-alpha (PPARα) in the mouse. *Reproductive Toxicology*, *27*, 258–265.

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., and Walter, P. (2014). DNA Replication, Repair, and Recombination. In *Molecular Biology of the Cell 6e*. (pp. 237-298). Garland Science, New York, NY, 6th edition.

Andrews, J. E. (1989). Polychlorinated Biphenyl (Aroclor 1254) Induced Changes in Femur Morphometry calcium metabolism and nephrotoxicity. *Toxicology*, *57*, 83–96.

Bickham, J. W. (2011). The four cornerstones of evolutionary toxicology. *Ecotoxicology 20*(3), 497-502.

Björkman, L., Lundekvam, B. F., Lægreid, T., Bertelsen, B. I., Morild, I., Lilleng, P., Lind, B., Plam B., Vahter, M. (2007). Mercury in human brain, blood, muscle and toenails in relation to exposure: An autopsy study. *Environmental Health: A Global Access Science Source*, *6*(30), 1–14.

Blévin, P., Tartu, S., Ellis, H. I., Chastel, O., Bustamante, P., Parenteau, Gabrielsen, G. W. (2017). Contaminants and energy expenditure in an Arctic seabird: Organochlorine pesticides and perfluoroalkyl substances are associated with metabolic rate in a contrasted manner. *Environmental Research*, *157*, 118–126.

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

Braune, B. M., and Letcher, R. J. (2013). Perfluorinated sulfonate and carboxylate compounds in eggs of seabirds breeding in the Canadian Arctic: Temporal trends (1975-2011) and interspecies comparison. *Environmental Science and Technology*, *47*(1), 616–624.

Braune, B. M., Trudeau, S., Jeffrey, D. A., and Mallory, M. L. (2011). Biomarker responses associated with halogenated organic contaminants in northern fulmars (*Fulmarus glacialis*) breeding in the Canadian Arctic. *Environmental Pollution*, *159*(10), 2891–2898.

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

Bustnes, J. O., Moe, B., Hanssen, S. A., Herzke, D., Fenstad, A. A., Nordstad, T., Borgå, K., Gabrielsen, G. W. (2012). Temporal Dynamics of Circulating Persistent Organic Pollutants in a Fasting Seabird under Different Environmental Conditions. *Environmental Science and Technology*, *46*(18), 10287–10294.

Bustnes, J. O., Moe, B., Herzke, D., Hanssen, S. A., Nordstad, T., Sagerup, K., Gabrielsen, G. W., Borgå, K. (2010). Strongly increasing blood concentrations of lipid-soluble organochlorines in high arctic common eiders during incubation fast. *Chemosphere*, *79*(3), 320–325.

Butt, C. M., Mabury, S. A., Muir, D. C. G., and Braune, B. M. (2007). Prevalence of long-chained perfluorinated carboxylates in seabirds from the Canadian arctic between 1975 and 2004. *Environmental Science and Technology*, *41*(10), 3521–3528.

Campbell, T. W. (2006) Clinical Chemistry of Common Nondomestic Mammals, Birds, Reptiles, Fish, and amphibians. In *Veterinary hematology and clinical chemistry*. (pp. 569-607). Blackwell Publishing, Iowa, USA.

Carrascal, L.M., Galvan, I. and Gordo, O. (2009) Partial least squares regression as an alternative to current regression methods used in ecology. *Oikos*, *118*, 681–690.

Chen, C. Y., Borsuk, M. E., Bugge, D. M., Hollweg, T., Balcom, P. H., Ward, D. M.,Mason, R. P. (2014a). Benthic and pelagic pathways of methylmercury bioaccumulation in estuarine food webs of the Northeast United States. *PLOS ONE*, *9*(2), 1–11.

Chen, J., Tanguay, R. L., Tal, T. L., Gai, Z., Ma, X., Bai, C., Dong, Q. (2014b). Early life perfluorooctanesulphonic acid (PFOS) exposure impairs zebrafish organogenesis. *Aquatic Toxicology*, *150*, 124–132.

Ciesielski, T. M., Hansen, I. T., Bytingsvik, J., Hansen, M., Lie, E., Aars, J., Styrishave, B. (2017). Relationships between POPs, biometrics and circulating steroids in male polar bears (*Ursus maritimus*) from Svalbard. *Environmental Pollution*, *230*, 598–608.

Collins, A. R. (2009). Investigating oxidative DNA damage and its repair using the comet assay. *Mutation Research - Reviews in Mutation Research*, *681*(1), 24–32.

Costantini, D., Blévin, P., Herzke, D., Moe, B., Gabrielsen, G. W., Bustnes, J. O., and Chastel, O. (2019). Higher plasma oxidative damage and lower plasma antioxidant defences in an Arctic seabird exposed to longer perfluoroalkyl acids. *Environmental Research*, *168,* 278–285.

Crespo-López, M. E., Macêdo, G. L., Pereira, S. I. D., Arrifano, G. P. F., Picanço-Diniz, D. L. W., Nascimento, J. L. M. d., and Herculano, A. M. (2009). Mercury and human genotoxicity: Critical considerations and possible molecular mechanisms. *Pharmacological Research*, *60*(4), 212–220.

Custer, C. M., Custer, T. W., Schoenfuss, H. L., Poganski, B. H., and Solem, L. (2012). Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reproductive Toxicology*, *33*(4), 556–562.

Dassuncao, C., Hu, X. C., Zhang, X., Bossi, R., Dam, M., Mikkelsen, B., and Sunderland, E. M. (2017). Temporal Shifts in Poly- and Perfluoroalkyl Substances (PFASs) in North Atlantic Pilot Whales Indicate Large Contribution of Atmospheric Precursors. *Environmental Science and Technology*, *51*(8), 4512–4521.

Desholm, M., Christensen, T. K., and Ens, B. (2002). Status of the Baltic/Wadden Sea population of the Common Eider (*Somateria mollissima*). *Wildfowl*, *53*, 167–203.

Devaux, A., Fiat, L., Gillet, C., and Bony, S. (2011). Reproduction impairment following paternal genotoxin exposure in brown trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*). *Aquatic Toxicology*, *101*(2), 405–411.

Dewitt, J. C. (2015). Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. *Molecular and Integrative Toxicology*. (pp. 203-218). NY, USA. Humana Press.

Dey, C. J., Semeniuk, C. A. D., Iverson, S. A., Richardson, E., McGeachy, D., and Gilchrist, H. G. (2018). Forecasting the outcome of multiple effects of climate change on northern common eiders. *Biological Conservation*, *220*, 94–103.

Driscoll, C. T., Mason, R. P., Chan, H. M., Jacob, D. J., and Pirrone, N. (2013). Mercury as a Global Pollutant: Sources, Pathways, and Effect. *Environmental Science and Technology*, *47*, 4967–4983.

Dutilleul, M., Réale, D., Goussen, B., Lecomte, C., Galas, S., and Bonzom, J. M. (2017). Adaptation costs to constant and alternating polluted environments. *Evolutionary Applications*, *10*(8), 839–851.

Eckbo, N., Bohec, C. Le, Planas-Bielsa, V., Warner, N. A., Schull, Q., Herzke, D., Borgå, K. (2019). Individual variability in contaminants and physiological status in a resident Arctic seabird species. *Environmental Pollution*, *249*, 191–199.

Ekroos, J., Fox, A. D., Christensen, T. K., Petersen, I. K., Kilpi, M., Jónsson, J. E., Green, M., Laursen, K., Cervencl, A., De Boer, P., et al. (2012). Declines amongst breeding eider (*Somateria mollissima*) numbers in the Baltic/Wadden Sea flyway. *Ornis Fennica*, *89*(2), 81-90.

Elder, A., Nordberg, G., and Kleinman, M. (2014). Routes of Exposure, Dose, and Toxicokinetics of Metals. In *Handbook on the Toxicology of Metals*, (pp. 45-70). Elsevier, Oxford, UK, 4th edition.

ESA (2019*). Algal Bloom in the Baltic Sea - Enviornment Hazards.* Earth Watching. earth.esa.int. Accessed on May 14, 2019.

Fallon, J. A., Redig, P., Miller, T. A., Lanzone, M., & Katzner, T. (2017). Guidelines for evaluation and treatment of lead poisoning of wild raptors. *Wildlife Society Bulletin*, *41*(2)*,* 205–211.

Fenstad, A. A., Bustnes, J. O., Bingham, C. G., Öst, M., Jaatinen, K., Moe, B., Krøkje, Å. (2016a). DNA double-strand breaks in incubating female common eiders (*Somateria mollissima*): Comparison between a low and a high polluted area. *Environmental Research*, *151*, 297–303.

Fenstad, A. A., Bustnes, J. O., Lierhagen, S., Gabrielsen, K. M., Öst, M., Jaatinen, K., Krøkje, Å. (2017). Blood and feather concentrations of toxic elements in a Baltic and an Arctic seabird population. *Marine Pollution Bulletin*, *114*(2), 1152–1158.

Fenstad, A. A., Jenssen, B. M., Gabrielsen, K. M., Öst, M., Jaatinen, K., Bustnes, J. O., Krøkje. (2016b). Persistent organic pollutant levels and the importance of source proximity in Baltic and Svalbard breeding common eiders. *Environmental Toxicology and Chemistry*, *35*(6), 1526–1533.

Fenstad, A. A., Jenssen, B. M., Moe, B., Hanssen, S. A., Bingham, C., Herzke, D., Krøkje, Å. (2014). DNA double-strand breaks in relation to persistent organic pollutants in a fasting seabird. *Ecotoxicology and Environmental Safety*, *106*, 68–75.

Fernández, P., and Grimalt, J. O. (2003). On the Global Distribution of Persistent Organic Pollutants, *Envirornmental Analysis 57*(9), 514–521.

Franson, J. C. and Pain, D. J. (2011). Lead in birds. *In Environmental Contaminants in Biota: Interpreting Tissue Concentrations*. (pp. 563–593). CRC press, Boca Raton, 2nd edition.

Franson, J. C., Hollmen, T., Poppenga, R. H., Hario, M., and Kilpi, M. (2000a). Metals and trace elements in tissues of common eiders (*Somateria mollissima*) from the Finnish archipelago. *Ornis Fennica, 77*(2), 57–63.

Franson, J. C., Hollmen, T., Poppenga, R. H., Hario, M., Kilpi, M., and Smith, M. R. (2000b). Selected trace elements and organochlorines: Some findings in blood and eggs of nesting common eiders (*Somateria mollissima*) from Finland. *Environmental Toxicology and Chemistry, 19*(5), 1340–1347.

Fuchsman, P. C., Brown, L. E., Henning, M. H., Bock, M. J., and Magar, V. S. (2017). Toxicity reference values for methylmercury effects on avian reproduction: Critical review and analysis. *Environmental Toxicology and Chemistry, 36*(2), 294-319.

Galvan, I. (2014). Chronic exposure to low-dose radiation at Chernobyl favours adaptation to oxidative stress in birds. *Functional Ecology*, *28*, 1387–1403.

Gao, J., Liu, Q., Xu, Y., Gong, X., Zhang, R., Zhou, C., Hou, Y. (2015). PPARalpha induces cell apoptosis by destructing Bcl2. *Oncotarget*, *6*(42), 44635–44642.

Garbus Svend-Erik Ramskov (2016). Health, behaviour, egg failure and starvation-mortality of incubating common eiders (*Somateria mollissima*) at Christiansø, Central Baltic Sea. Master's thesis. (pp. 1-73). *Faculty of health and medical sciences, Univeristy of Copenhagen*, Copenhagen, Denmark.

Garbus, S., Lyngs, P., Peter, J., and Buchmann, K. (2018). Common Eider (*Somateria mollissima*) body condition and parasitic load during a mortality event in the Baltic Proper. *Avian Biology Research*, *11*(3), 167–172.

Gebbink, W. A., and Letcher, R. J. (2012). Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls. *Environmental Pollution*, *162*, 40–47.

Gilmore EH. (2015). Do contaminants in polar bears (*Ursus maritimus*) modulate the expression of selected genes and cause DNA strand breaks? Master's thesis. (pp. 1-53). *Department of Biosciences, University of Oslo.* Oslo, Norway.

Haarr, A., Hylland, K., Eckbo, N., Gabrielsen, G. W., Herzke, D., Bustnes, J. O., Borga, K. (2018). DNA Damage in Arctic Seabirds : Baseline , Sensitivity to a Genotoxic Stressor , and Association with Organohalogen Contaminants. *Environmental Toxicology and Chemistry, 37*(4), 1084–1091.

Hanssen, S. A., Folstad, I., & Erikstad, K. E. (2003). Reduced immunocompetence and cost of reproduction in common eiders. *Oecologia*, *136*(3), 457–464.

Hanssen, S. A., Hasselquist, D., Folstad, I., and Erikstad, K. E. (2005). Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1567), 1039–1046.

Hanssen, S. A., Sonne, C., Bustnes, J. O., Schnug, L., Bourgeon, S., Ballesteros, M., Ims, R. A. (2017). Anti-parasite treatment and blood biochemistry in raptor nestlings. *Canadian Journal of Zoology*, *95*(9), 685–693.

Harr, K. E. (2002). Clinical chemistry of companion avian species a review. *Veterinary Clinical Pathology*, *31*(3), 140–151.

Harry, M.O., Gary, H.H., (2011). Selenium in birds. In *Environmental Contaminants in Biota: Interpreting Tissue Concentrations*. (pp. 669-701). CRC press, Boca Raton, 2nd edition.

Hartwig, A., Pelzer, A., Asmuss, M., and Bürkle, A. (2003). Very low concentrations of arsenite suppress poly(ADP-ribosyl)ation in mammalian cells. *International Journal of Cancer*, *104*(1), 1–6.

Haukås, M., Berger, U., Hop, H., Gulliksen, B., and Gabrielsen, G. W. (2007). Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environmental Pollution*, *148*(1), 360–371.

HELCOM. (2010). Hazardous substances in the Baltic Sea – an integrated thematic assessment of hazardous substances in the Baltic Sea. *Balt. Sea Environ. ProcNo. 120B*.

HELCOM. (2017). Radioactive substances : Cesium-137 in fish and surface seawater Key Message. (pp. 1–21).

HELCOM. (2018). Inputs of hazardous substances to the Baltic Sea. *Balt. Sea Environ. ProcNo. PCL-6*.

Hoff, P. T., Van De Vijver, K., Dauwe, T., Covaci, A., Maervoet, J., Eens, M., De Coen, W. (2005). Evaluation of biochemical effects related to perfluorooctane sulfonic acid exposure in organohalogen-contaminated great tit (*Parus major*) and blue tit (*Parus caeruleus*) nestlings. *Chemosphere*, *61*(11), 1558–1569.

Hollmen, T., Franson, J. C., Poppenga, R. H., Hario, M., and Kilpi, M. (1998). Lead poisoning and trace elements in common eiders (*Somateria mollissima)* from Finland. *Wildlife Biology*, *4*(4), 193–203.

Hollmen, T., Franson, J., Hario, M., Sankari, S., Kilpi, M., and Lindstrom, K. (2001). Use of serum biochemistry to evaluate nutritional status and health of incubating common eiders (*Somateria mollissima)* in Finland. *Physiological and Biochemical Zoology*, *74*(3), 333–342.

Holmes, D., and Martin, K. (2009). A Bird's-Eye View of Aging: What's in it for Ornithologists? *The Auk*, *126*(1), 1–23.

Hong, S., Seong, J., Wang, T., Naile, J. E., Park, J., Kwon, B., Giesy, J. P. (2015). Bioaccumulation characteristics of perfluoroalkyl acids (PFAAs) in coastal organisms from the west coast of South Korea. *Chemosphere*, *129*, 157–163.

Hung, H., Katsoyiannis, A. A., Brorström-Lundén, E., Olafsdottir, K., Aas, W., Breivik, K.,Wilson, S. (2016). Temporal trends of Persistent Organic Pollutants (POPs) in arctic air: 20 years of monitoring under the Arctic Monitoring and Assessment Programme (AMAP). *Environmental Pollution, 217*, 52–61.

Jacobsen, M. L., Jaspers, V. L. B., Ciesielski, T. M., Jenssen, B. M., Løseth, M. E., Briels, N., Sonne, C. (2017). Blood clinical-chemical parameters and feeding history in growing Japanese quail (*Coturnix japonica*) chicks exposed to Tris(1,3-dichloro-2-propyl) phosphate and Dechlorane Plus in ovo. *Toxicological and Environmental Chemistry*, *99*(5–6), 938–952.

Jacqueline, C., Biro, P. A., Beckmann, C., Moller, A. P., Renaud, F., Sorci, G., Thomas, F. (2017). Cancer: A disease at the crossroads of trade-offs. *Evolutionary Applications*, *10*(3), 215–225.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., and Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, *7*(2), 60–72.

Jian-Li, H.E., Tao, P., Jie, X., Han-Hui, D., Dong-Dong, C., Zhen-Feng, Y., Chun-Lin, F., and Cun, L. (2015). Determination of 20 Perfluorinated Compounds in Animal Liver by HPLC- MS/MS. *Chinese Journal of Analytical Chemistry*, *43*(1), 40-48.

Kim, Y. E., Saeki, K., Tanabe, S., Tanaka, H., & Tatsukawa, R. (1997). Specific accumulation of mercury and selenium in seabirds. *Science*, *94*(3), 261–265.

Koivula, M. J., and Eeva, T. (2010). Metal-related oxidative stress in birds. *Environmental Pollution*. *158*(7), 2359–2370.

Korschgen, C. E. (1977). Breeding Stress of Female Eiders in Maine. *The Journal of Wildlife Management*, *41*(3), 360–373.

Krafft, M. P. and Riess, J. G. (2015). Per- and polyfluorinated substances (PFASs): environmental challenges. *Current Opinion in Colloid and Interface Science, 20*(3), 192-212.

Krøkje, Å., Bingham, C., Husmo Tuven, R., and Wing Gabrielsen, G. (2006). Chromosome aberrations and DNA strand breaks in glaucous gull (*Larus hyperboreus*) chicks fed environmentally contaminated gull eggs. *Journal of Toxicology and Environmental Health, Part A*, *69*, 159–174.

Kunacheva, C., Fujii, S., Tanaka, S., Seneviratne, S. T. M. L. D., Lien, N. P. H., Nozoe, M., Harada, H. (2012). Worldwide surveys of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in water environment in recent years. *Water Science and Technology, 66*(12), 2764–2771.

Larsson, K., Hajdu, S., Kilpi, M., Larsson, R., Leito, A., and Lyngs, P. (2014). Effects of an extensive Prymnesium polylepis bloom on breeding eiders in the Baltic Sea. *Journal of Sea, 88*, 21-28.

Laursen, K., Møller, A.P., (2014). Long-Term changes in Nutrients and Mussel stocks are Related to Numbers of Breeding Eiders (*Somateria mollissima)* at a large Baltic Colony. *PLOS One 9*(4)*,* 1-6.

Laursen, K., Møller, A.P., (2016). Your tools disappear when you stop eating: Phenotypic variation in gizzard mass of eiders. *Journal of Zoology, 299*, 213–220.

Laursen, K., Pape, A., and Öst, M. (2019a). Body condition of Eiders at Danish wintering grounds and at pre-breeding grounds in Åland. *Journal of Ornithology*, *160*(1), 239–248.

Laursen, K., Pape, A., Haugaard, L., Öst, M., and Vainio, J. (2019b). Allocation of body reserves during winter in eider (*Somateria mollissima*) as preparation for spring migration and reproduction. *Journal of Sea Research*, *144*, 49–56.

Lavoie, R. A., Jardine, T. D., Chumchal, M. M., Kidd, K. A., and Campbell, L. M. (2013). Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. *Environmental Science and Technology, 47*(23):13385–13394.

Lee, C. E., Remfert, J. L., Opgenorth, T., Lee, K. M., Stanford, E., Connolly, J. W., Tomke, S. (2017). Evolutionary responses to crude oil from the Deepwater Horizon oil spill by the copepod (*Eurytemora affinis)*. *Evolutionary Applications*, *10*(8), 813–828.

Lee, P. Y., Costumbrado, J., Hsu, C., and Kim, Y. H. (2012). Agarose Gel Electrophoresis for the Separation of DNA Fragments. *Journal of Visualized Experiments*, *62*, 1–5.

Lehman-McKeeman, L. D. (2013). Absorption, Distribution, and Excretion of Toxicants. In *Toxicology: The Basic Science of Poisons* (pp. 153–183).

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. *Nature*, *363*, 709-714.

Liu, C., Chang, V. W. C., Gin, K. Y. H., and Nguyen, V. T. (2014). Genotoxicity of perfluorinated chemicals (PFCs) to the green mussel (*Perna viridis*). *Science of the Total Environment*, *487*(1), 117–122.

Maeda, T., Eller, M. S., Hedayati, M., Grossman, L., and Gilchrest, B. A. (1999). Enhanced repair of benzo(a)pyrene-induced DNA damage in human cells treated with thymidine dinucleotides. *Mutation Research - DNA Repair*, *433*(2), 137–145.

Mallory, M. L., Braune, B. M., Wayland, M., Gilchrist, H. G., and Dickson, D. L. (2004). Contaminants in common eiders (*Somateria mollissima*) of the Canadian Arctic. *Environmental Reviews*, *12*(4), 197–218.

Maness, T. J., and Emslie, S. D. (2007). An Analysis of Possible Genotoxic Exposure in Adult and Juvenile Royal Terns in North Carolina, USA. *Waterbirds: The International Journal of Waterbird Biology*, *24*(3), 352-360.

Martin, J. W., Mabury, S. A.; Solomon, K. R.; Muir, D. C. (2003). Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). *Environmental Science and Technology, 22,* 196– 204.

Mason, R., Reinfelder, J., and Morel, F. (1995). Bioaccumulation of mercury and methylmercury. *Water, Air, and Soil Pollution, 80*, 915–921.

Matson, C. W., Franson, J. C., Hollmén, T., Kilpi, M., Hario, M., Flint, P. L., and Bickham, J. W. (2004). Evidence ofchromosomal damage in common eiders (*Somateria mollissima*) from the Baltic Sea. M*arine Pollution Bulletin, 49,* 1066–1071.

Matson, C. W., Lambert, M. M., McDonald, T. J., Autenrieth, R. L., Donnelly, K. C., Islamzadeh, A., Bickham, J. W. (2006). Evolutionary toxicology: Population-level effects of chronic contaminant exposure on the marsh frogs (*Rana ridibunda*) of Azerbaijan. *Environmental Health Perspectives*, *114*(4), 547–552.

Melvin, Maureen. (1987). *Electrophoresis, analytical chemistry by open learning*. (pp. 1-65). John Wiley and Sons ACOL, London, 1st edition.

Möller, A., Ahrens, L., Surm, R., Westerveld, J., Wielen, F. Van Der, Ebinghaus, R., and Voogt, P. De. (2010). Distribution and sources of polyfluoroalkyl substances (PFAS) in the River Rhine watershed. *Environmental Pollution*, *158*(10), 3243–3250.

Mueller, R., and Yingling, V. (2017). *History and Use of Per-and Polyfluoroalkyl Substances (PFAS)*. Interstate Technology Regulatory Council. www.itrcweb.org. Accessed on April 9, 2019.

Murvoll, K. M., Skaare, J. U., Jensen, H., and Jenssen, B. M. (2007). Associations between persistent organic pollutants and vitamin status in Brünnich's guillemot and common eider hatchlings. *Science of the Total Environment*, *381*(1–3), 134–145.

Nakayama, K., Iwata, H., Tao, L., Kannan, K., Imoto, M., Kim, E. Y., Tanabe, S. (2008). Potential effects of perfluorinated compounds in common cormorants from Lake Biwa, Japan: An implication from the hepatic gene expression profiles by microarray. *Environmental Toxicology and Chemistry*, *27*(11), 2378–2386.

Nobels, I., Dardenne, F., De Coen, W., Blust, R., 2010. Application of a multiple endpoint bacterial reporter assay to evaluate toxicological relevant endpoints of perfluorinated compounds with different functional groups and varying chain length. *Toxicolology in Vitro, 24*, 1768–1774.

Noer, H., 1991. Distributions and movements of eider (*Somateria mollissima*) populations wintering in Danish waters, analysed from ringing recoveries. *Danish Review of Game Biology, 14*, 1–32.

Noori, B. M. L. (2018). DNA double strand breaks and chemical elements in incubating female common eiders (*Somateria mollissima*) in Christiansø, Denmark. Master's Thesis. (pp. 1-59). *Department of Biology, Norwegian University of Science and Technology,* Trondheim, Norway.

Nordberg, G., Nogawa, K., and Nordberg, M. (2014). Toxicology of Metals: Overview, Definitions, Concepts, and Trends. In *Handbook on the Toxicology of Metals*, (pp. 1-11). Elsevier, Oxford, UK, 4th edition.

Nordén, M., Berger, U., and Engwall, M. (2016). Developmental toxicity of PFOS and PFOA in great cormorant (*Phalacrocorax carbo sinensis*), herring gull (*Larus argentatus*) and chicken (*Gallus gallus domesticus)*. *Environmental Science and Pollution Research*, *23*(11), 10855–10862.

Nowsheen, S., and Yang, E. S. (2013). The intersection Between Dna Damage Response and cell death pathways. *Experimental Oncology*, *34*(3), 243–254.

Østby, L., Gabrielsen, G. W., and Krøkje, Å. (2005). Cytochrome P4501A induction and DNA adduct formation in glaucous gulls (*Larus hyperboreus*), fed with environmentally contaminated gull eggs. *Ecotoxicology and Environmental Safety*, *62*(3), 363–375.

Pavlaki, M. D., Araújo, M. J., Cardoso, D. N., Silva, A. R. R., Cruz, A., Mendo, S., Loureiro, S. (2016). Ecotoxicity and genotoxicity of cadmium in different marine trophic levels. *Environmental Pollution*, *215*, 203–212.

Peck, L. E., Gilchrist, H. G., Mallory, C. D., Braune, B. M., and Mallory, M. L. (2016). Persistent organic pollutant and mercury concentrations in eggs of ground-nesting marine birds in the Canadian high Arctic. *Science of the Total Environment*, *556*, 80–88.

Perneger, T.V., 1998. What's wrong with Bonferroni adjustments. *BMJ, 316*, 1236–1238.

Pratte, I., Tomlik, M. D., Betsch, T. A., Braune, B. M., Milton, G. R., and Mallory, M. L. (2015). Trace elements in eggs of common eiders (*Somateria mollissima*) breeding in Nova Scotia, Canada. *Marine Pollution Bulletin*, *100*(1), 586–591.

Preston, R. J., and Hoffmann, G. R. (2013). Genetic Toxicology. In *Toxicology: The Basic Science of Poisons* (pp. 445-473)

R Core Team (v.3.5.3, 2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reid, N. M., Proestou, D. A., Clark, B. W., Warren, W. C., Colbourne, J. K., Shaw, J. R., Whitehead, A. (2016). The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. *Science*, *354*(6317), 1305–1308.

Robin, J.P., Cherel, Y., Girard, H., Geloen, A., Le Maho, Y. 1987. Uric acid and urea in relation to protein catabolism in long-term fasting geese. *Journal of Comparative Physiolog*y, *157,* 491–499.

Robin, J.P., Frain, M., Sardet, C., Groscolas, R., Le Maho, Y. 1988. Protein and lipid utilization during long-term fasting in emperor penguins. *Journal of Comparative Physiology, 254,* 61–68.

Rodvelt, R. H. (2018). Levels and Potential Genotoxic Effects of Organohalogenated Contaminants in Seabird Eggs from Svalbard. Master's Thesis. (pp. 1-77). *Department of Biology, Norwegian University of Science and Technology,* Trondheim, Norway.

Sahlin, S. (2017). PFAS in the Baltic Sea Region. Inventory of awareness, actions and strategies related to highly fluorinated substances, PFAS, including PFOS. *Policy Area Hazards, EUSBSR, Swedish Environmental Protection Agency*. (pp. 1-8).

Savinov, V. M., Gabrielsen, G. W., and Savinova, T. N. (2003). Cadmium, zinc, copper, arsenic, selenium and mercury in seabirds from the Barents Sea: Levels, inter-specific and geographical differences. *Science of the Total Environment*, *306*, 133–158.

Scheuhammer, A. (1987). The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: A review. *Environmental Pollution, 46*(4), 263–295.

Shaposhnikov, S. A., Salenko, V. B., Brunborg, G., Nygren, J., and Collins, A. R. (2008). Single-cell gel electrophoresis (the comet assay): Loops of fragments? *Electrophoresis*, *29*(14), 3005–3012.

Skerfving, S. and Bergdahl, I. (2014). Lead. *In Handbook on the Toxicology of Metals.* (pp. 911–967). Elsevier, Oxford, UK, 4th edition.

Sletten, S., Bourgeon, S., Bårdsen, B. J., Herzke, D., Criscuolo, F., Massemin, S., Bustnes, J. O. (2016). Organohalogenated contaminants in white-tailed eagle (*Haliaeetus albicilla*) nestlings: An assessment of relationships to immunoglobulin levels, telomeres and oxidative stress. *Science of the Total Environment*, *539*, 337–349.

Sonne, C., Bustnes, J.O., Herzke, D., Jaspers, V.L.B., Covaci, A., Halley, D.J., Minagawa, M., Moum, T., Eulaers, I., Eens, M., Ims, R.A., Hanssen, S.A., Erikstad, K.E., Johnsen, T., Schnug, L., Jensen, A.L. (2010). Relationships between organohalogen contaminants and blood plasma clinical-chemical parameters in chicks of three raptor species from Northern Norway. *Ecotoxicology and Environmental Safety, 73,* 7-17.

Sonne, C., Bustnes, J.O., Herzke, D., Jaspers, V.L.B., Covaci, A., Eulaers, I., Halley, D.J., Moum, T., Ballesteros, M., Eens, M., Ims, R.A., Hanssen, S.A., Erikstad, K.E., Johnsen, T.V., Rigét, F.F., Jensen, A.L. and Kjelgaard-Hansen, M. (2012). Blood plasma clinical-chemical parameters as biomarker endpoints for organohalogen contaminant exposure in Norwegian raptor nestlings. *Ecotoxicology and Environmental Safety, 80,* 76-83.

Sonne, C., Dietz, R., Kirkegaard, M., Letcher, R.J., Shahmiri, S., Andersen, S., Møller, P., Olsen, A.K. and Jensen, A.L. (2008). Effects of Organohalogen Pollutants on Haematological and Urine Clinical-Chemical Parameters in Greenland Sledge Dogs (*Canis familiaris*). *Ecotoxicology and Environmental Safety, 69,* 381-390.

Sonne, C., Rigét, F.F., Leat, E.H.K., Bourgeon, S., Borgå, K., Strøm, H., Hanssen, S.A., Gabrielsen, G.W., Petersen, Æ., Olafsdottir, K., Magnusdottir, E., Bustnes, J.O., Furness, R.W. and Kjelgaard-Hansen, M. (2013): Organohalogen contaminants and blood plasma clinical- chemical parameters in three colonies of North Atlantic Great skua (*Stercorarius skua*). *Ecotoxicology and Environmental Safety, 92,* 245-251.

Świergosz-Kowalewska, R., Bednarska, A., and Kafel, A. (2006). Glutathione levels and enzyme activity in the tissues of bank vole (*Clethrionomys glareolus)* chronically exposed to a mixture of metal contaminants. *Chemosphere*, *65*(6), 963–974.

Tartu, S., Gabrielsen, G. W., Blévin, P., Ellis, H., Bustnes, J. O., Herzke, D., and Chastel, O. (2014). Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in arctic breeding black-legged kittiwakes. *Environmental Science and Technology*, *48*(22), 13504–13510.

Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clement-Chastel, C., Chastel, O. (2013). To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. *Biology Letters*, *9*(4), 10-13.

Tengjaroenkul, B., Intamat, S., Boonmee, S., and Neeratanaphan, L. (2017). Chromosomal aberration assessment of silver rasbora fish (*Rasbora tornieri*) living near gold mine area with heavy metal contamination. *Human and Ecological Risk Assessment*, *23*(5), 1140–1152.

Theodorakis, C. W., D'Surney, S. J., and Shugart, L. R. (1994). Detection of Genotoxic Insult as DNA strand breaks in fish blood cells by agarose gel electrophoresis. *Environmental Toxicology and Chemistry*, *13*(7), 1023–1031.

Thermo Scientific (2016). Lambda DNA/Hindiii. https://assets.thermofisher.com/ TFS-Assets/LSG/manuals/MAN0012988_Lambda_DNA_HindIII_Marker_RTU_UG.pdf. Cited Date: [6 April 2019]

Tian, X., Seluanov, A., and Gorbunova, V. (2017). Molecular Mechanisms Determining Lifespan in Short- and Long-Lived Species. *Trends in Endocrinology and Metabolism*, *28*(10), 722–734.

UNEP, U. (2009). Report of the conference of the parties of the Stockholm Convention on persistent organic pollutants on the work of its fourth meeting. In *United Nations Environment Programme: Stockholm Convention on Persistent Organic Pollutants.* Geneva, page 112.

USEPA, 2002. Perfluoroalkyl sulfonates. *Proposed significant new rule*. Fed. Reg. 67 (236), 72854-72867.

Vecchiato, M., Argiriadis, E., Zambon, S., Barbante, C., Toscano, G., Gambaro, A., and Piazza, R. (2015). Persistent Organic Pollutants (POPs) in Antarctica: Occurrence in continental and coastal surface snow. *Microchemical Journal*, *119*, 75–82.

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

Vlasov, V. M. (1993). Fluoride ion as a nucleophile and a leaving group in aromatic nucleophilic substitution reactions. *Journal of Fluorine Chemistry*, *61*(3), 193–216.

Waisberg, M., Joseph, P., Hale, B., and Beyersmann, D. (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, *192*(2–3), 95–117.

Waltho, C. and Coulson, J. (2015). *The Common Eider.* (pp. 1-276). Bloomsbury Publishing, London, UK, 1st edition.

Wayland, M., Alisauskas, R. T., Kellett, D. K., and Mehl, K. R. (2008b). Trace element concentrations in blood of nesting king eiders in the Canadian Arctic. *Archives of Environmental Contamination and Toxicology*, *55*(4), 683–690.

Wayland, M., Alisauskas, R.T., Kellett, D., Traylor, J., Swoboda, C., Neugebauer, E., Mehl, K., (2007). Year-to-year correlations in blood metal levels among individuals of two species of North American sea ducks. *Environmental Pollution, 150*, 329–337.

Wayland, M., Drake, K.L., Alisauskas, R.T., Kellett, D.K., Traylor, J., Swoboda, C., Mehl, K., (2008a). Survival rates and blood metal concentrations in two species of free-ranging North American sea ducks. *Environmental Toxicology and Chemistry, 27*, 698–704.

Wayland, M., Garcia-Fernandez, A.J., Neugebauer, E., Gilchrist, H.G., (2001). Concentrations of cadmium, mercury and selenium in blood, liver and kidney of common eider ducks from the Canadian arctic. *Environmental Monitor Assessesment 71*, 255–267.

Wayland, M., Gilchrist, H. G., and Neugebauer, E. (2005). Concentrations of cadmium, mercury and selenium in common eider ducks in the Eastern Canadian Arctic: Influence of reproductive stage. *Science of the Total Environment*, 351, 323–332.

Wayland, M., Gilchrist, H., Marchant, T., Keating, J., and Smits, J. (2002). Immune function, stress response, and body condition in arctic-breeding common eiders in relation to cadmium, mercury, and selenium concentrations. *Environmental Research*, *90*(1), 47–60.

Wayland, M., Scheuhammer, A.M., (2011). Cadmium in birds. In *Environmental Contaminants in Biota: Interpreting Tissue Concentrations*. (pp. 645–669). CRC press, Boca Raton, 2nd edition.

Whitehead, A., Clark, B. W., Reid, N. M., Hahn, M. E., and Nacci, D. (2017). When evolution is the solution to pollution: Key principles, and lessons from rapid repeated adaptation of killifish (*Fundulus heteroclitus*) populations. *Evolutionary Applications*, *10*(8), 762–783.

Wielsøe, M., Long, M., Ghisari, M., and Bonefeld-Jørgensen, E. C. (2015). Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. *Chemosphere*, *129*, 239–245.

Wolfe, M. F., Schwarzbach, S., and Sulaiman, R. A. (1998). Effects of mercury on wildlife: A comprehensive review. *Environmental Toxicology and Chemistry, 17*(2), 146–160.

Zhang, W., Wang, W. X., and Zhang, L. (2016). Comparison of Bioavailability and Biotransformation of Inorganic and Organic Arsenic to Two Marine Fish. *Environmental Science and Technology*, *50*(5), 2413–2423.

Appendix

A. Methods and Materials

A.1. Agarose Gel electrophoresis

A.1.1. Chemicals and Mixtures

Table A.1. List of chemicals used in the agarose gel electrophoresis to complete the DNA-DSBs analysis including producer and product number.

Chemicals	**Producer**	**Product number**
Agarose	Sigma	A5093
Boric acid	Sigma	B7901
EDTA (ethylenediaminetetraacetic acid disodium salt dihydrate)	Biorad	E5134
proteinase K	Sigma	P2308
Sodium Chloride	Sigma	S3014
Trizma base (#T6066)	Sigma	T6066
1% LMPA (Low Melt Preparative Grade Agarose)	Biorad	162-0019
bromophenol blue	Fermentas	#R0611
ethidium bromide	Sigma	161-0433
lambda DNA Hind III digest (Sigma)	Fermentas	#SM0231
sodium dodecyl sulfate (SDS)	Biorad	161-0301

Table A.2. Mixtures, concentrations, and masses used in the agarose gel electrophoresis used to complete the DNA-DSBs analysis

Solution	Amount of chemicals
TE Buffer	10mM Trizma base 1 mM EDTA
Theodorakis lysis buffer	100 mM NaCl 10 mM Trizma buffer 10 mM EDTA SDS (0.5 %) (pH 7.0)
TBE Buffer	90 mM Trizma base 90 mM boric acid 2mM EDTA (pH 8.0)
Agarose Gel (0.6%)	0.6 g Agarose 100 mL TBE buffer (0.5x)
LMPA (1%)	20 mL TE Buffer 200 mLow melting agarose
Lambda DNA Ladder	100 µL Lambda HindIII digest 37 µL lambda DNA 24 µL DNA loading dye

A.1.2. Interpretation and Calculations of MML and DNA-FTM

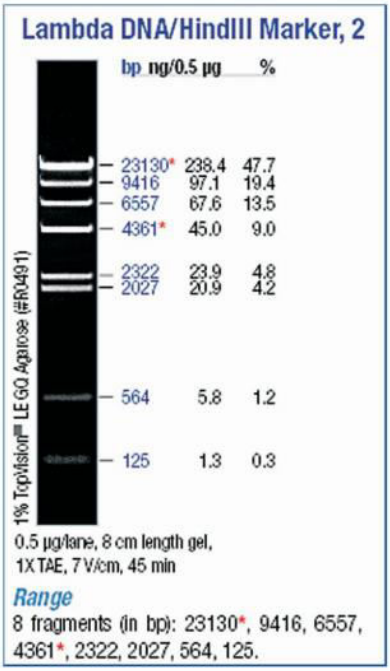


Figure A.3. Lambda DNA size fragments digested by HindIII. Size ladder is used as a reference to measure the MML of the unknown fragments from the biological samples. Image modified from Thermo Scientific (2016).

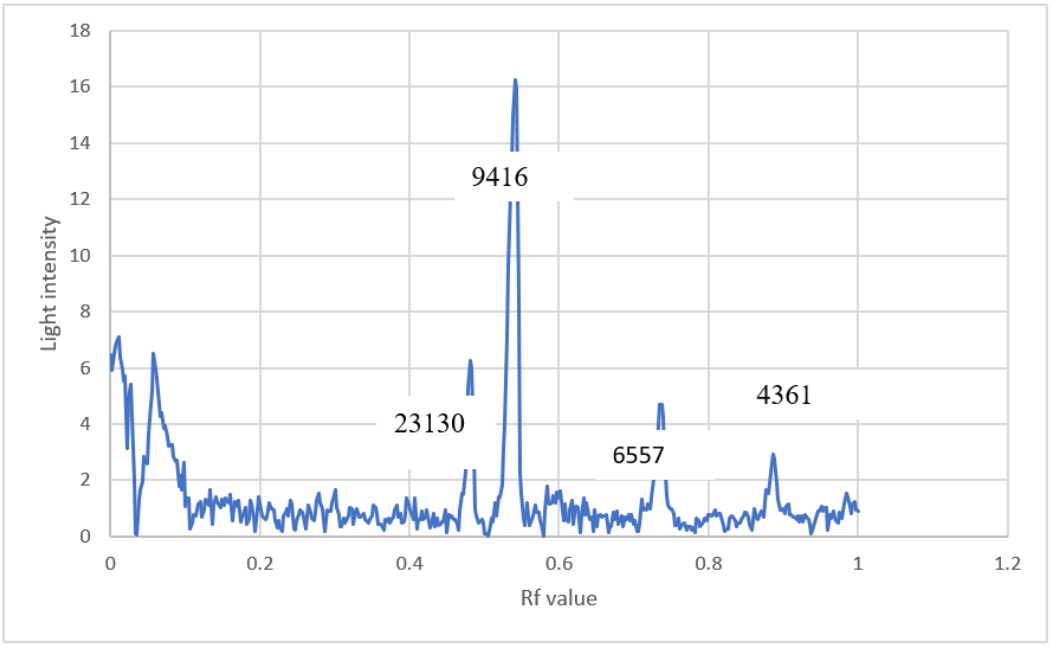


Figure A.4. Graph showing peaks, measured by Gel doc in light intensity, in relation to Rf value of the Lambda/HindiIII ladder. Each peak is labelled with its corresponding size marker in kbps.

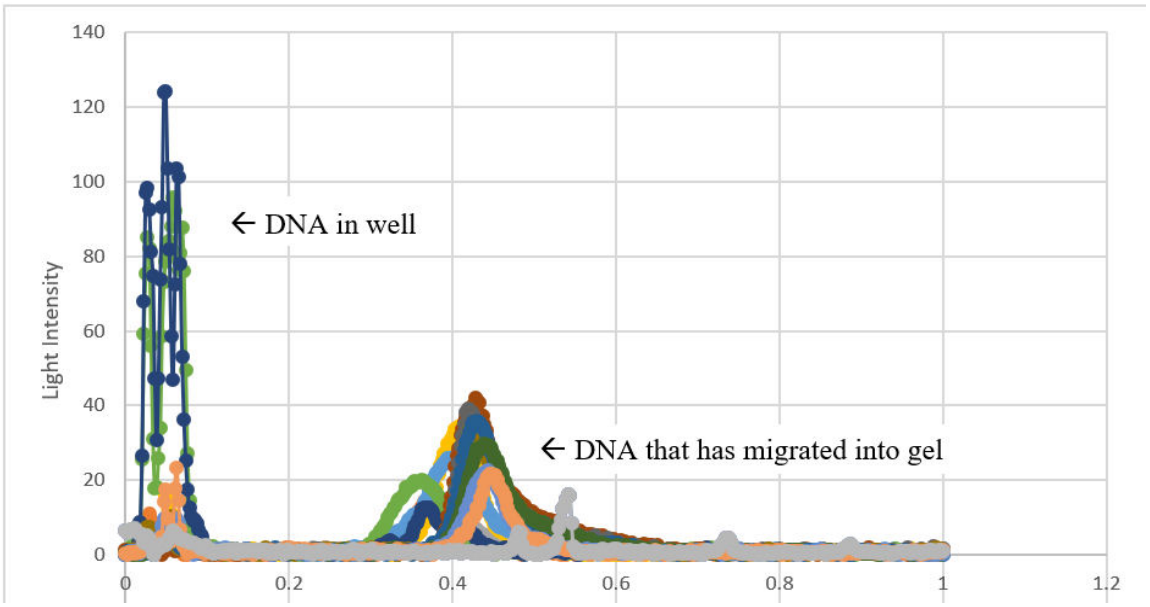


Figure A.5. Graph showing peaks, measured by Gel doc in light intensity, in relation to Rf value of the biological samples. Peak cluster are labelled with its corresponding position in the gel.

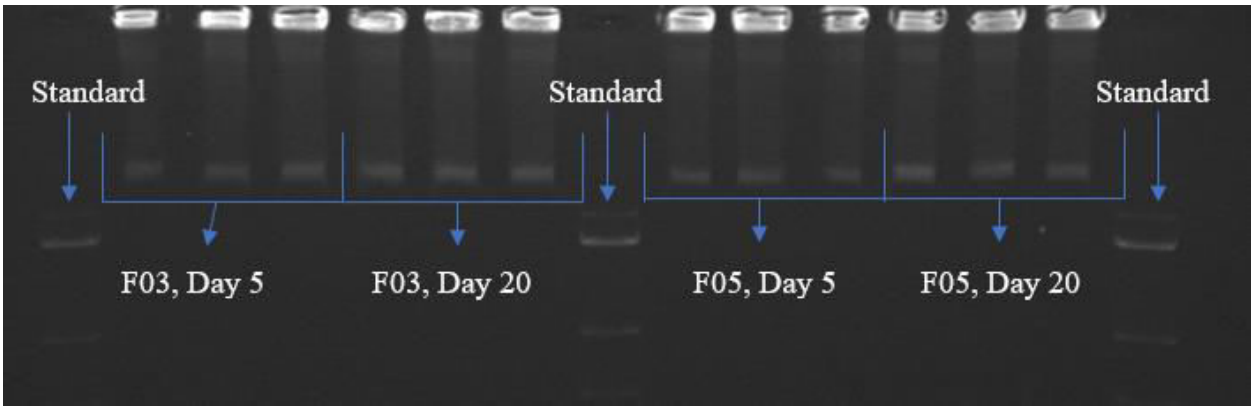


Figure A.6. Agarose gel set up. Triplicates of day 5 and day 20 for each individual were placed side by side for easier visual comparisons. Two identical gels were run simultaneously to create independence among samples. F03 and F05 are individuals, standard refers to the standard ladder used as a positive control and measurement of MML

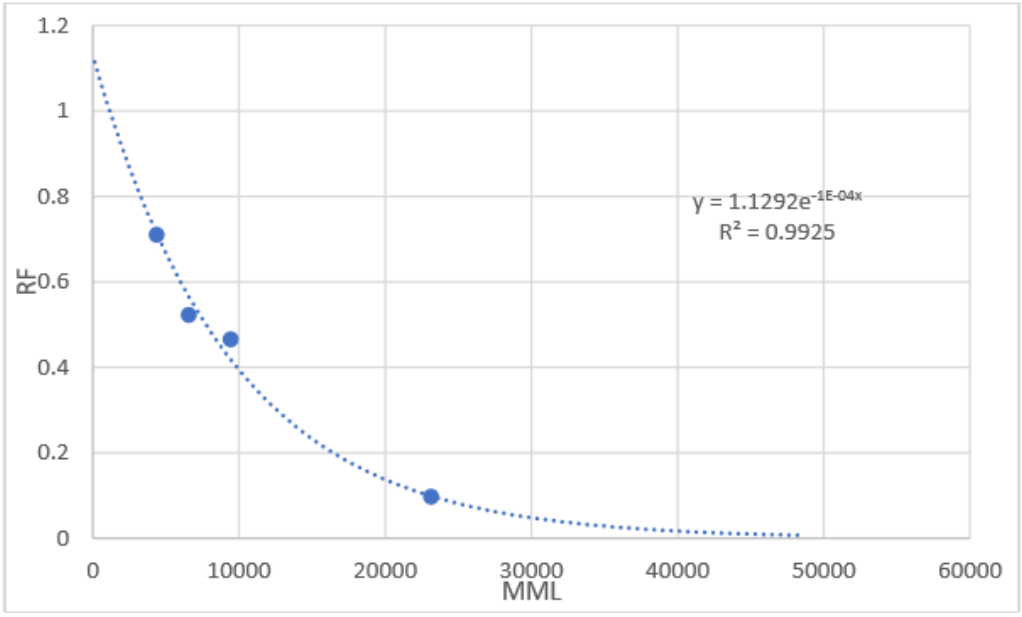


Figure A.7. Standard curve calculated from the Lambda DNA size markers with one extrapolated point at 400 Kbp. Using the standard curve equation MML was quantified from the rf value provided from the Gel Documentation machine.

A.2. HS-ICP-MS

Table A.3. Method limit of detection (MDL) for As, Cd, Hg, Pb, Ca, Co, Cu, Fe, K, Se, and Zn. MDL is calculated using three times the standard deviation of the blank or, if larger, the instruments detection limit. Elements' isotopes, resolution (LR, MR, HR = low, medium and high resolution respectively), and their MDL (μg/kg) are presented.

Compound	Isotope	Resolution	MDL
Toxic Elements			
As	75	HR	2.123
Cd	114	LR	0.1699
Hg	57	LR	0.1699
Pb	208	LR	0.1699
Essential Elements			
Ca	44	MR	169.9
Co	59	MR	0.3397
Cu	63	MR	2.5479
Fe	202	MR	1.699
K	39	MR	84.9
Se	78	MR	4.25
Zn	67	MR	3.397

A.3. GC-MS

Table A.4. PFASs analyzed in the present study with their full name, detection limit, and quantification limit.

Compound	**Full name**	**Detection limit** (ng/mL)	**Quantification Limit** (ng/mL)
PFBS	Perfluorobutane sulfonate	0.02	0.07
PFHxS	Perfluorohexanesulfonate	0.03	0.08
PFHpS	Perfluoroheptane sulfonate	0.04	0.11
PFOS	Perfluorooctane sulfonate	0.09	0.28
PFDS	Perfluorodecane sulfonate	0.12	0.37
PFOSA	Perfluorooctane sulfonamide	0.40	1.19
PFPeA	Perfluoropentanoic acid	0.06	0.19
PFHxA	Perfluorohexanoic acid	0.01	0.03
PFHpA	Perfluoroheptanoic acid	0.02	0.05
PFOA	Perfluorooctanoic acid	0.07	0.20
PFNA	Perfluorononanoic acid	0.09	0.27
PFDA	Perfluorodecanoic acid	0.03	0.09
PFUnA	Perfluoroundecanoic acid	0.05	0.15
PFDoA	Perfluorododecanoic acid	0.14	0.41
PFTrA	Perfluorotridecanoic acid	0.14	0.41
PFTeA	Perfluorotetradecanoic acid	0.14	0.41

A.4. Additional R Packages

Table A.5. Table showing the additional R packages used in the data analysis presented in this study. Description and version as described by the R Core Team (2018).

Package	**Description**	**Version**
Car	Companion to applied regression	3.0-2
carData	Companion to applied regression Data sets	3.0-2
dplyr	A grammar of data manipulation	0.8.0.1
effects	Displays for linear, generalized linear, and other models	4.1-0
ggplot2	Data visualizations using the grammar of graphics	3.1.0
ggpubr	'ggplot2' based publication ready plots	0.2
lme4	Linear mixed-effect models using 'Eigen and S4	1.1-2.1
lmerTest	Tests in linear mixed effects models	3.1-0
mdatools	Multivariate data analysis for chemometrics	0.9.1
MuMin	Multi-model interference	1.42.1
nlme	Linear and nonlinear mixed effects models	3.1-137
plsdepot	Partial least squares (PLS) data analysis methods	0.1.17
plyr	Tools for splitting, applying, and combining data	1.8.4
tidry	Tidy data with 'spread()' and 'gather90'	0.8.3

B. Results

B.1. Body Mass

Table B.1. Body mass (g) for day 5 and day 20, mass lost (g), average daily mass lost (g), and percent mass loss (%) of incubating female Eiders sampled in Christiansø, Denmark in the breeding season of 2018.

ID	Mass day 5	Mass day 20	Mass lost	daily mass lost	percent mass lost
F02	1960	1345	1941	32.37	0.313776
F03	1985	1365	1966	32.63	0.312343
F05	2210	1575	2190	31.75	0.28733
F06	2285	1575	2265	35.5	0.310722
F09	2125	1545	2107	32.22	0.272941
F14	2200	1445	2180	37.75	0.343182
F15	2380	1595	2362	43.61	0.329832
F16	1960	1355	1941	31.84	0.308673
F17	2110	1745	2090	18.25	0.172986
F19	1935	NA	NA	NA	NA
F21	2135	1645	2116	25.79	0.229508
F22	2120	1575	2102	30.28	0.257075
F26	2260	1625	2240	31.75	0.280973
F27	2280	1665	2260	30.75	0.269737
F28	2475	NA	NA	NA	NA
F29	1985	1525	1965	23	0.231738
F30	2410	1865	2390	27.25	0.226141
F31	2300	1675	2281	32.89	0.271739
F32	2190	1375	2170	40.75	0.372146
F33	2360	1695	2340	33.25	0.28178
F34	2220	NA	NA	NA	NA
F37	2275	1775	2256	26.32	0.21978
F38	1975	1225	1956	39.47	0.379747
F39	2280	1525	2260	37.75	0.33114
F40	2300	1565	2280	36.75	0.319565
F42	2135	1625	2115	25.5	0.238876

B.2. PFASs concentrations

Table B.2. PFASs blood concentrations for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018 for both day 5 and day 20 of incubation. Individual F39 has two repeats for day 5 and day 20 because this sample was run twice during analysis. All concentrations are given in ng/g^{-1} ww. Only PFASs above LOD in >60% of individuals are included.

ID	day	mass	PFHxS	PFHpS	PFOS	PFNA	PFDA	PFUnA
F02	5	1960	3.23	0.13	3.76	0.68	0.28	0.34
F02	20	1345	3.69	0.24	6.37	1.03	0.49	0.44
F03	5	1985	6.09	0.28	5.68	0.91	0.42	0.43
F03	20	1365	5.57	0.29	7.08	1.06	0.49	0.51
F05	5	2210	2.24	0.1	5.18	1.05	0.32	0.37
F05	20	1575	1.88	0.15	6.4	1.22	0.38	0.38
F06	5	2285	1.26	0.07	3.59	1.01	0.26	0.3
F06	20	1575	0.96	0.07	4.21	1.11	0.34	0.3
F09	5	2125	2.14	0.22	6.46	0.99	0.38	0.56
F09	20	1545	2.97	0.4	13	1.95	0.81	0.86
F14	5	2200	1.92	0.15	6.8	2.19	0.57	0.7
F14	20	1445	2.07	0.21	9.16	2.65	0.83	1.01
F15	5	2380	1.88	0.16	6.9	1.65	0.46	0.5
F15	20	1595	2.37	0.26	12.3	2.97	0.77	0.93
F16	5	1960	1.76	0.18	5.62	1.14	0.33	0.4
F16	20	1355	1.6	0.2	6.14	1.28	0.34	0.45
F17	5	2110	4.57	0.42	7.15	1.09	0.45	0.43
F17	20	1745	5.14	0.54	9.05	1.33	0.57	0.52
F21	5	2135	1.75	0.15	5.25	1.1	0.31	0.38
F21	20	1645	1.3	0.21	7.08	1.25	0.39	0.44
F22	5	2120	1.35	NA	3.4	0.65	0.21	0.24
F22	20	1575	1.43	0.14	4.96	0.94	0.27	0.34
F26	5	2260	2.48	NA	5.07	1.13	0.33	0.4
F26	20	1625	2.73	0.17	7.18	1.5	0.46	0.54
F27	5	2280	1.06	0.11	5.51	1	0.38	0.5
F27	20	1665	0.76	0.15	7.62	1.25	0.51	0.7
F28	5	2475	NA	NA	NA	NA	NA	NA
F29	5	1985	1.33	NA	3.85	0.89	0.24	0.27
F29	20	1525	NA	NA	NA	NA	NA	NA
F30	5	2410	1.01	NA	2.38	0.75	0.16	0.14
F30	20	1865	1.1	0.12	4.65	1.22	0.29	0.3

F31	5	2300	1.27	0.12	4.72	1.15	0.32	0.28
F31	20	1675	1.12	0.14	6.43	1.61	0.4	0.37
F32	5	2190	2.42	0.12	3.98	0.68	0.26	0.28
F32	20	1375	2.96	0.22	8.88	1.24	0.56	0.69
F33	5	2360	NA	NA	NA	NA	NA	NA
F33	20	1695	1.8	0.26	8.08	1.32	0.46	0.54
F34	5	2220	NA	NA	NA	NA	NA	NA
F37	5	2275	1.99	0.15	8.05	1.35	0.53	0.59
F37	20	1775	1.41	0.17	10.2	1.57	0.68	0.83
F38	5	1975	1.07	0.13	5.99	0.95	0.41	0.51
F38	20	1225	0.97	0.19	8.96	1.73	0.63	0.85
F39	5	2280	1.51	0.14	4.8	1.01	0.3	0.35
F39	5	2280	1.54	0.13	4.63	0.91	0.3	0.31
F39	20	1525	1.81	0.28	9.77	1.69	0.57	0.81
F39	20	1525	1.87	0.27	10.1	1.7	0.57	0.81
F40	5	2300	1.38	0.12	6.17	0.99	0.43	0.6
F40	20	1565	1.67	0.22	10.3	1.54	0.64	0.89
F42	5	2135	3.36	0.18	6.93	1.39	0.46	0.45
F42	20	1625	4.16	0.25	10.7	1.89	0.72	0.73

B.3. Chemical element concentrations

Table B.3. Chemical element blood concentrations of incubating female Eiders on Christiansø, Denmark in the breeding season of 2018 for day 5 and day 20 of incubation. All concentrations are given in µg/kg ww. Only chemical above LOD in >60% of individuals are included.

ID	day	As	Ca	Cd	Co	Cu	Hg	K	Pb	Se	Zn	Fe
F02	5	11.13	39541.73	0.58	0.78	306.63	439.77	1896158	182.79	2064	4825.6	501931.7
F02	5	12.73	39664	0.53	0.69	316.04	443.25	1806067	182.34	2025	4898.1	517601.4
F03	5	11.24	49872.68	0.48	0.79	301.68	297.88	1939393	69.79	2819	5703.59	500053.2
F05	5	11.53	72398.93	0.9	1.4	278.17	186.19	1960980	115.45	4640	5804.81	466288.5
F06	5	16.69	59160.83	0.61	1.06	291.74	120.44	1888648	96.3	3689	5968.25	466605.7
F09	5	13.66	44881.86	0.81	0.88	349.83	292.08	2009888	71.66	3055	5194.01	504734.3
F09	5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F14	5	15.2	58320.52	1.46	1.28	485.68	220.22	1996809	162.36	3745	5851.14	491011.3
F14	5	12.27	55391.57	1.32	1.35	477.5	196.09	1929287	148.31	3957	5644.22	475812.2
F15	5	12.25	53096.75	0.72	0.89	374.87	169.05	2071845	70.45	4149	5250.14	479931.2
F16	5	11.43	46936.56	0.68	0.84	415.99	114.84	1838034	164.91	2744	5217.05	468344.9
F17	5	17.2	65374.63	0.73	0.81	291.78	250.96	1854407	47.96	2857	5365.83	458756.8
F21	5	13.02	63856.71	0.55	1.07	338.66	362.04	1971898	45.16	4807	5400.73	483022.2
F22	5	12.98	54254.59	0.46	0.75	280.12	145.06	1725493	50.66	3045	4739.91	416348.8
F26	5	21.81	61182.37	2.37	2.66	487.5	165.1	2583159	268.96	2722	6438.9	736762.4
F27	5	8.55	72262.75	0.63	0.97	340.48	159.8	1928866	645.13	2271	5712.33	489308.4
F28	5	11.11	51840.65	0.47	0.85	372.29	121.86	1804582	1488.23	2284	4588.75	447501.1
F29	5	11.63	43063.44	0.95	1.21	453.01	228.89	1886036	204.85	2771	5042.45	460670.6
F30	5	10.13	92296.9	0.78	1.47	287.26	114.91	1731130	137.14	2316	6386.22	433061.1
F31	5	17.95	73780.56	0.83	1.42	258.73	81.57	1963554	98.93	2049	6122.29	464414
F32	5	10.8	50812.29	1.13	1.33	315.62	370.24	2104047	188.25	2327	4859.78	488872.1
F33	5	11.75	58314.82	0.6	1.13	282.68	218.67	1926088	149.85	3214	6159.88	467892.7
F34	5	7.26	52551.13	0.63	0.99	259.12	111.3	1920577	66.9	1687	5749.15	519823.6
F37	5	17.49	61567.15	0.58	1.19	277.55	149.18	2002901	75.41	2625	5355.58	522552
F38	5	8.83	49363.99	0.98	1.36	409.09	129.88	1908734	189.15	2765	5820.17	473036.3
F39	5	13.97	56275.17	0.86	0.74	415.53	169.95	2032011	64.6	3430	5287.49	521151.3

F40	5	12.13	105788.6	0.84	1.35	346.18	153.66	1929287	875.38	3445	6511.58	449310.6
F42	5	13.91	57875.52	0.62	1.31	308.97	234.26	1905845	107.37	2073	5853.01	475849.2
F02	20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F02	20	14.77	53107.27	0.99	1.21	348.99	388.36	1812730	455.61	1571	5118.49	434365.8
F03	20	8.99	38324.08	0.63	2.1	339.83	265.98	1709689	146.02	1860	5097.05	460295.9
F05	20	14.65	57272.24	1.29	0.92	362.62	150.25	1711959	176.78	3134	5157.67	424620.9
F06	20	15.16	50632.19	0.87	1.45	345.21	103.47	1750144	119.66	2917	5407.13	425728
F09	20	14.12	48918.08	1.31	1.25	390.01	295.96	1794930	213.3	2374	5483.92	453663.9
F09	20	13.19	47528.4	1.36	1.5	389.61	279.48	1810801	203.51	2485	5384.41	451745.4
F14	20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F14	20	14.88	52497.89	2.42	0.92	435.43	179.05	1725499	684.13	3277	5171.36	415844.4
F15	20	15.5	53497.79	1.11	1.02	401.91	145.89	1820147	125.05	3893	5659.34	431210.2
F16	20	11.47	37434.94	0.92	0.88	429.25	99.07	1743106	181.81	1824	4660.89	474340.3
F17	20	20.49	53238.13	0.76	0.66	301.67	204.35	1592110	67.95	1859	4231.81	403967
F21	20	13.9	54505.03	0.73	0.92	364.03	279.79	1653868	52.3	3365	4901.01	411791
F22	20	10.79	55685.78	0.61	1.73	337.68	155.27	1613614	144.53	2441	4943	399317
F26	20	9.43	36874.74	0.55	0.65	161.34	67.5	1175555	30.08	1413	2984.48	286192.2
F27	20	6.73	50688.62	0.92	1.34	367.73	146.72	1712505	651.6	1767	5159.91	424717.1
F28	20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F29	20	12.54	52815.15	1.65	1.27	541.73	213.32	1809339	176.66	2113	4833.64	432896.3
F30	20	8.57	57576.39	1.37	2.17	404.23	111.1	1702126	237.89	1882	5746.89	415573
F31	20	17.45	54553.85	1.23	1.51	338.8	65.05	1750345	147.16	1845	5641.82	426401.6
F32	20	11.93	55713.97	1.86	0.9	345.01	321.91	1850180	186.22	1861	4667.21	444089.3
F34	20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F33	20	7.34	49159.29	1.05	1.87	342.23	224.3	1778408	275.02	2945	6001.71	462964.9
F37	20	16.12	54237.19	0.69	0.88	315.96	107.77	1606720	52.66	1580	4895.52	416014.1
F38	20	16.12	47366.22	2.13	1.68	452.96	121.35	1820195	264.83	2168	5256.22	451639.4
F39	20	16.6	51690.78	1.9	1.05	419.16	146.39	1785411	156.49	2587	5508.76	442315.7
F40	20	12.77	57949.65	1.31	0.92	374.64	108.89	1544105	1050.08	2983	5594.03	390149.3
F42	20	18.5	53318.68	0.97	1.66	376.39	218.71	1786553	352.81	1453	5357.32	455746.8

B.4. BCCPs

B.4.1. BCCP concentrations

Table B.4. Blood plasma chemical-concentrations parameters (BCCPs) (units specified) for day 5 and day 20 for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018. Paired Wilcoxon test was performed to account for non-normality of data. Compounds that were not detected in >60% of individuals were excluded from analysis.

	day 5 of incubation			**day 20 of incubation**			**paired Wilcoxon test**	
BCCP	**Mean ± SD**	**median**	**range**	**Mean ± SD**	**median**	**range**	**V**	**p**
Liver Enzymes								
Alanine aminotransferase (ALAT; U/L)	11.39±3.27	11.0	7-17	5.48±0.99	6.0	4-7	253	<0.001
Aspartate aminotransferase (ASAT; U/L)	19.96±6.83	20.0	10-37	13.35±2.89	13.0	10-18	220	0.002
Alkaline phosphatase (ALKP; U/L)	168.0±144.65	137	25-554	42.09±21.71	36.0	23-117	275	<0.001
Gamma glutamyl transferase (GGT; U/L)	6.17±2.85	6.0	1-11	5.87±1.01	6.0	2-7	167	0.004
Electrolytes/Minerals								<0.001
Iron (Fe; mmol/L)	39.69±25.57	31.0	19-116	13.52±2.56	13.0	9-20	276	<0.001
Calcium (Ca; mmol/L)	3.33±0.69	3.2	2.6-5.3	2.57±0.10	2.6	2.3-2.7	253	<0.001
Sodium (Na; mmol/L)	159.04±3.23	159.0	153-166	156.78±4.01	157.0	146-163	157	0.0146
Chloride (Cl; mmol/L)	115.48±3.72	116.0	108-164	112.35±3.77	113.0	104-119	199	0.004
Phosphate (PHOS; mmol/L)	1.33±0.33	1.3	0.9-2.2	0.89±0.22	0.9	0.6-1.4	209	<0.001
Protein/Carbohydrate groups								
Albumin (ALB; g/L)	15.74±189	16.0	13-19	12.69±1.18	13.0	10-15	210	<0.001
Total Protein (TP; g/L)	46.60±6.05	45.0	38-60	41.91±5.56	41.0	30-53	228	0.006
Fructosamine (FRU; U/L)	132.91±16.74	135.0	101-164	142.04±9.81	143.0	119-154	59.5	0.018
Metabolizing enzymes								
Lipase (U/L)	7.22±0.85	7.0	6-9	6.26±0.54	6.0	6-8	190.5	<0.001
lactate dehydrogenase (LDH; U/L)	221.56±111.68	193.0	100-586	164.43±54.65	155.0	102-334	208.5	0.03
Amylase (AMY; U/L)	1384.39±289.08	1403.0	884-1943	655.09±193.83	635.0	407-1338	276	<0.001

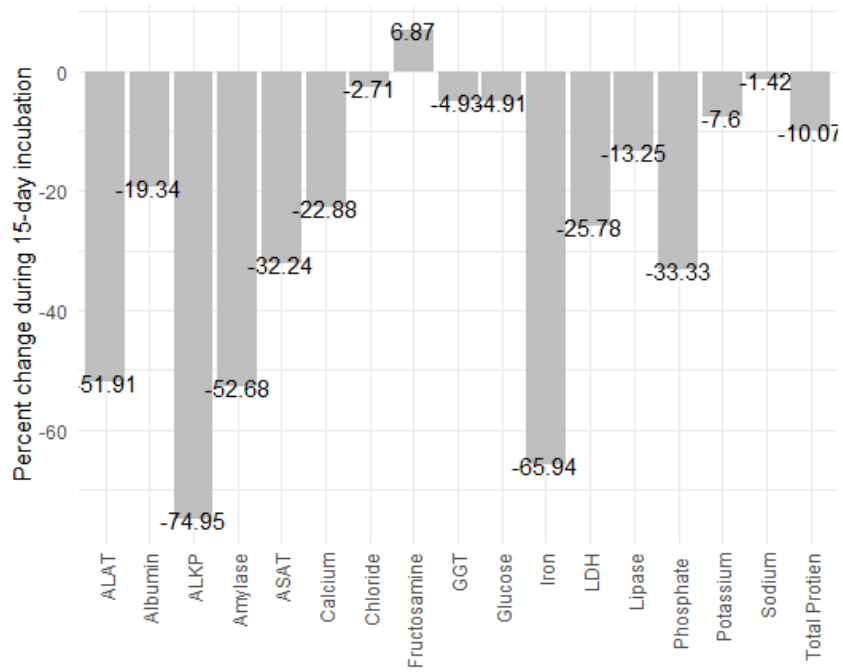


Figure B.1. Bar graph of percent change of BCCPs during the 15-day incubation period for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018.

Table B.5. Significant correlations between BCCPs and body mass (g) for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018. Data was log-transformed to create normality. If data was still not normal a spearman correlation test was performed.

Parameter	p-value	r_s	Correlation test	Parameter	p-value	r_s	Correlation test
Phosphate	<0.001	0.73	Spearman	Albumin	<0.001	0.77	Pearson
Alanine aminotransferase	<0.001	0.74	Pearson	Calcium	<0.001	0.74	Pearson
Alkanine phosphatase	<0.001	0.53	Spearman	Amylase	<0.001	0.87	Pearson
Total Protein	<0.001	0.47	Pearson	Iron	<0.001	0.86	Spearman
Aspartate aminotransferase	<0.001	0.52	Spearman	Lipase	<0.001	0.51	Pearson

B.5. Correlation Analyses

B.5.1. Relationship between toxic elements and essential elements

Table B.6. Correlations between toxic elements and essential elements for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018. All data was log-transformed to create normality. Significant correlations between chemical elements and essential elements when individual F27 on day 5 and F26 on day 20 has been removed are also presented. The asterisks (*,**,**) denote a p-value of <0.05, <0.01 and <0.001 respectively.

Essential element	**As**	**As w/o ID 27.1, 26.2**	**Cd**	**Cd w/o ID 27.1, 26.2**	**Hg**	**Hg w/o ID 27.1, 26.2**	**Pb**	**Pb w/o ID 27.1, 26.2**
Ca	0.29*							
Co			0.45**	0.39**			0.41**	0.36*
Cu			0.68***	0.65***			0.51**	0.46**
Fe				-0.34*	0.33*	0.30*		
K					0.30*			
Se								
Zn								

B.5.2. Relationship between PFASs and BCCPs

Table B.7. Significant correlations between Blood plasma chemical-concentrations parameters (BCCPs) and PFASs and Hg for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018. All PFASs and BCCP data was log-transformed to create normality. If data was still not normal a spearman correlation test was performed (FRU, Fe, ALKP). Asterisks denote significance ($p<0.05$*, $p<0.001$**, $p<0.0001$***)

BCCPs	PFOS	PFNA	PFDA	PFHxS	PFHpS	PFUnA	ΣPFASs
Liver/digestive Enzymes							
Alanine aminotransferase (ALAT)	-0.36*						
Aspartate aminotransferase (ASAT)	-0.30*						
Alkaline phosphatase (ALKP)	-0.39**		-0.37**		-0.32*	-0.32*	-0.35*
Amylase (AMY)	-0.56***	-0.39**	-0.49***		-0.39*	-0.453**	-0.47***
Electrolytes/Minerals							
Iron (Fe)	-0.54***	-0.49**	-0.48**		-0.45**	-0.44**	-0.46**
Calcium (Ca)	-0.43**	-0.37*	-0.40**			-0.40**	-0.41**
Chloride (Cl)	-0.40**		-0.31*			-0.30*	-0.30*
Phosphate (PHOS)	-0.32*		-0.32*		-0.36*		-0.31*
Protein/Carbohydrate groups							
Albumin (ALB)	-0.31*					-0.30*	
Fructosamine (FRU)	0.42**		0.42**		0.32*	0.34*	0.41**
Metabolic products							
Cholesterol (CHOL)	0.62***	0.45**	0.61***	0.35*	0.55***	0.60***	0.62***
Bile	0.56***	0.48***	0.46**		0.58***	0.50***	0.52***

B.5.3. Relationship between PFASs, chemical elements and DNA-DSBs

Table B.9. Significant correlations between chemical elements, ΣPFASs and; DNA-FTM, MML, and body mass for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018. All PFASs and chemical element data was log-transformed to create normality. If data was still not normal a spearman correlation test was performed. Asterisks denote significance (p<0.05*, p<0.001**, p<0.0001***)

				w/o ID 27.1, 26.2		
Compound	**Body mass**	**DNA-FTM**	**MML**	**Body mass**	**DNA-FTM**	**MML**
As						0.32*
Ca	0.42**			0.39*		
Cd				-0.35*	0.29*	
Co						
Cu	-0.30*				0.31*	
Fe	0.54***	-0.38*		0.52**	-0.37**	
Hg						
K	0.63***	-0.35*		0.60***	-0.38**	
Pb			-0.45**			-0.44**
Se	0.35*			0.39**		
Zn	0.48**			0.47**		
ΣPFASs	-0.54**			-0.54**		

B.6. Principal Component Analysis

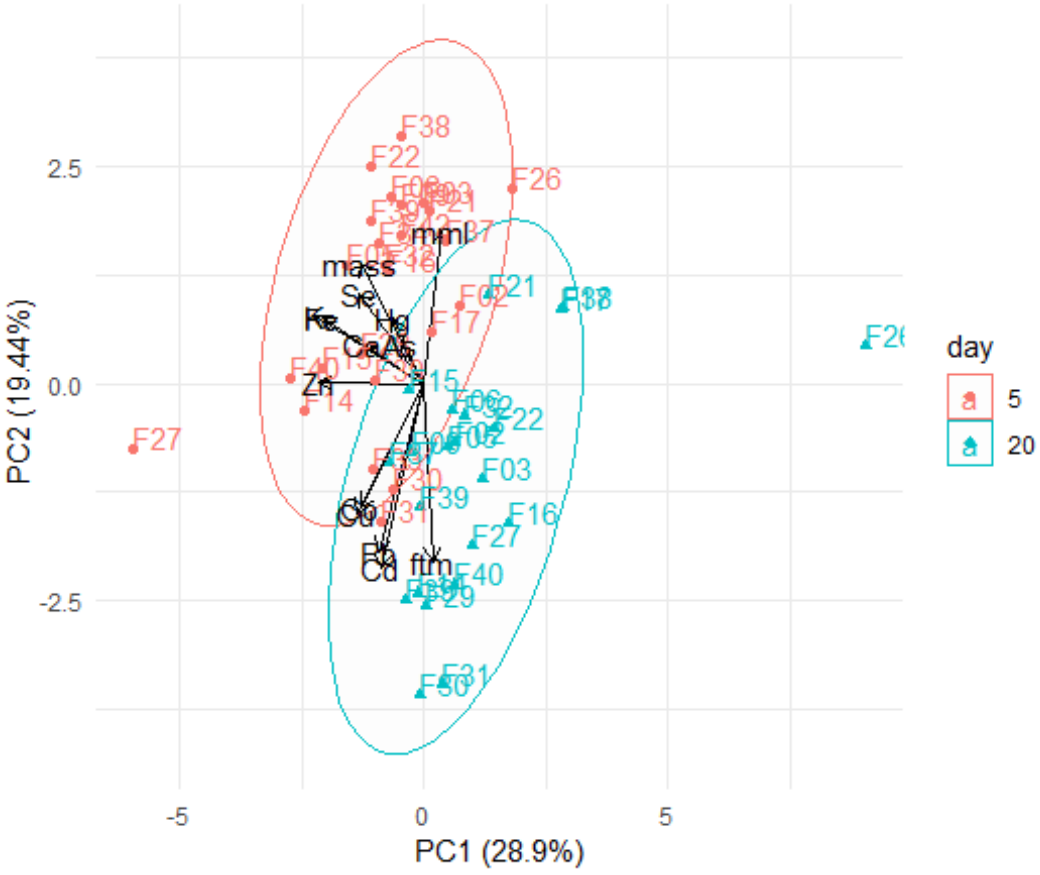


Figure B.2. Principal component analysis score plot overlaid with biplot of PFASs, chemical elements, body mass, DNA-FTM, and MML all measured for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018 (n=23). Day 5 of incubation is represented by orange circles and day 20 by green triangles. The loading plot shows the clustering between day 5 and day 20 measurements. The biplot shows the relationship between the above listed variables. All PFASs, DNA-FTM, MML and chemical elements have been log transformed to account for non-normality.

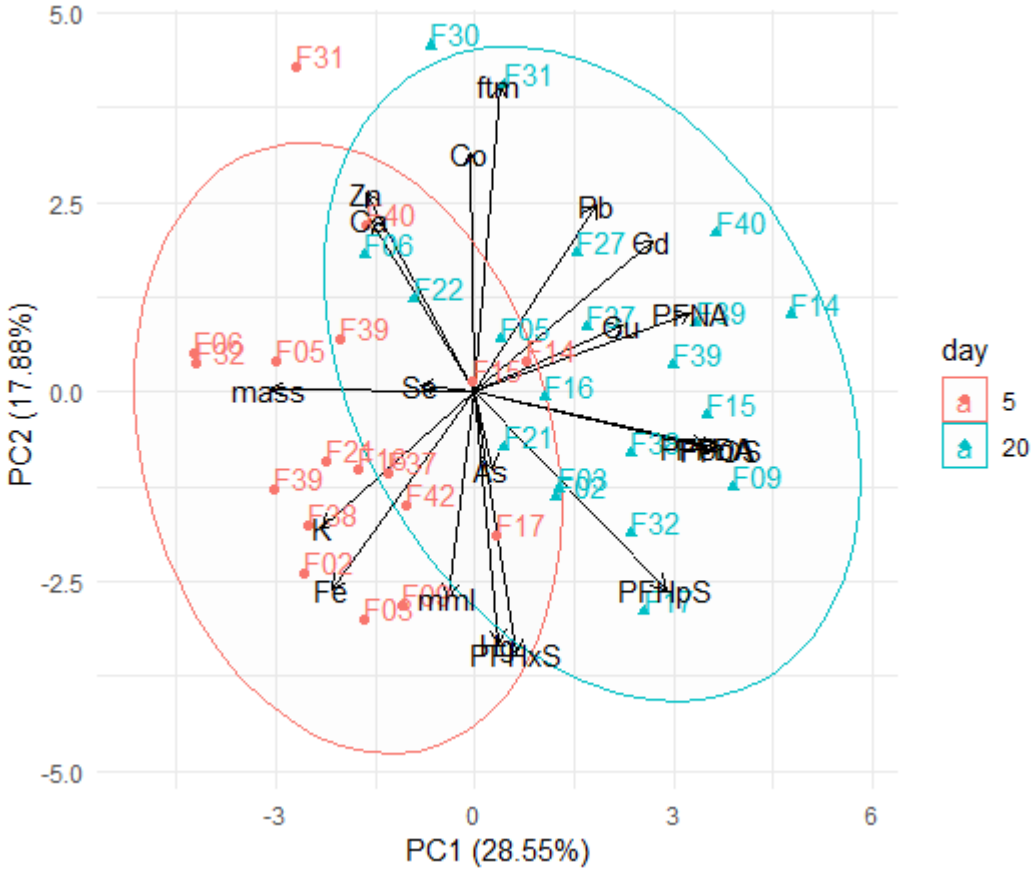


Figure B.3. Principal component analysis score plot overlaid with biplot of PFASs, chemical elements, body mass, DNA-FTM, and MML all measured for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018 (n=23). Individuals F26 and F27 have been removed, based off results from Figure B.2. Day 5 of incubation is represented by orange circles and day 20 by green triangles. The loading plot shows the clustering between day 5 and day 20 measurements. The biplot shows the relationship between the above listed variables. All PFASs, DNA-FTM, MML and chemical elements have been log transformed to account for non-normality.

B.7. Linear models

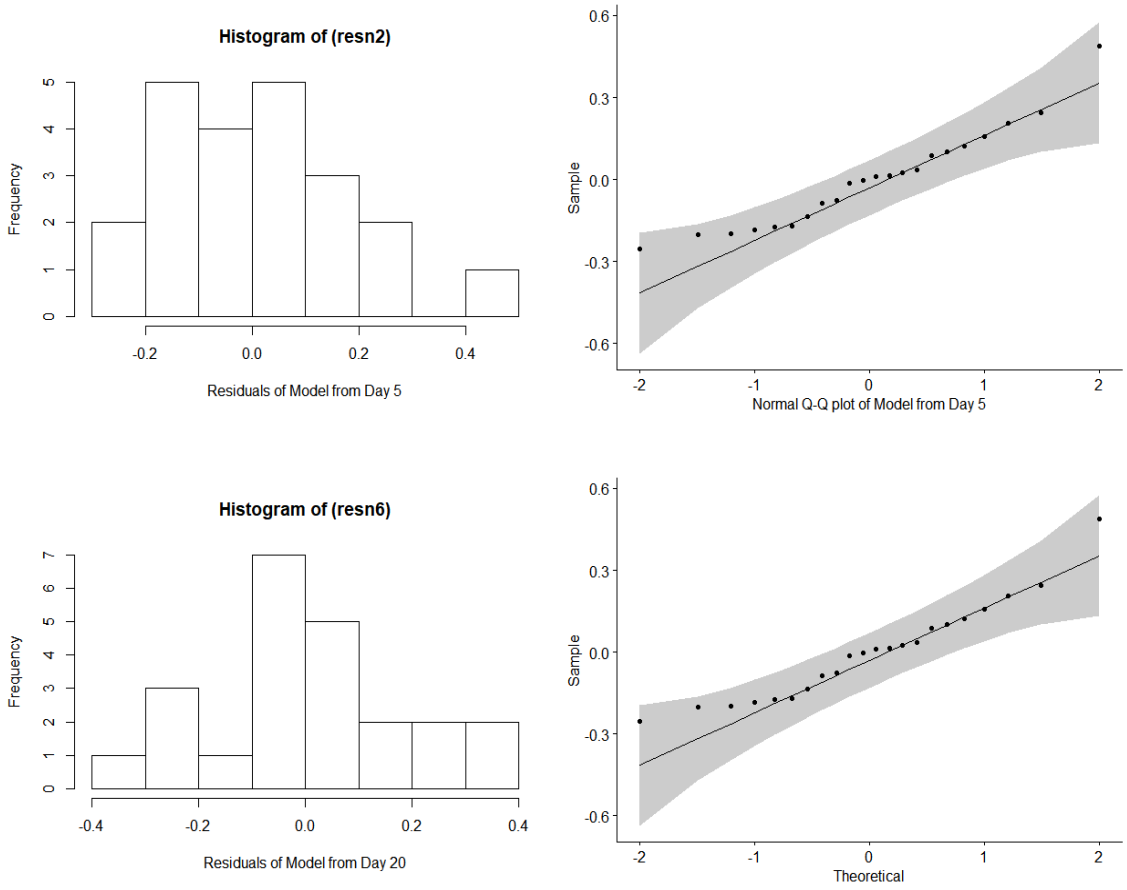


Figure B.4. Histograms and quantile-quantile plots from linear models from day 5 (top) and day 20 (bottom) referred to in Table 3.7 for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018. Plots illustrate the models fit, residual normality, and the individuals weighting the models more heavily. DNA-FTM, Hg, and PFASs were both log-transformed.

B.8. DNA-FTM and MML raw data

Table B.10. DNA-fraction migrated into gel (DNA FTM, %) and median molecular length (MML, kbps) for incubating female Eiders on Christiansø, Denmark in the breeding season of 2018 on day 5 and day 25 of incubation. Standard deviation and coefficient of variation are presented for every tri-replicate ran in each gel.

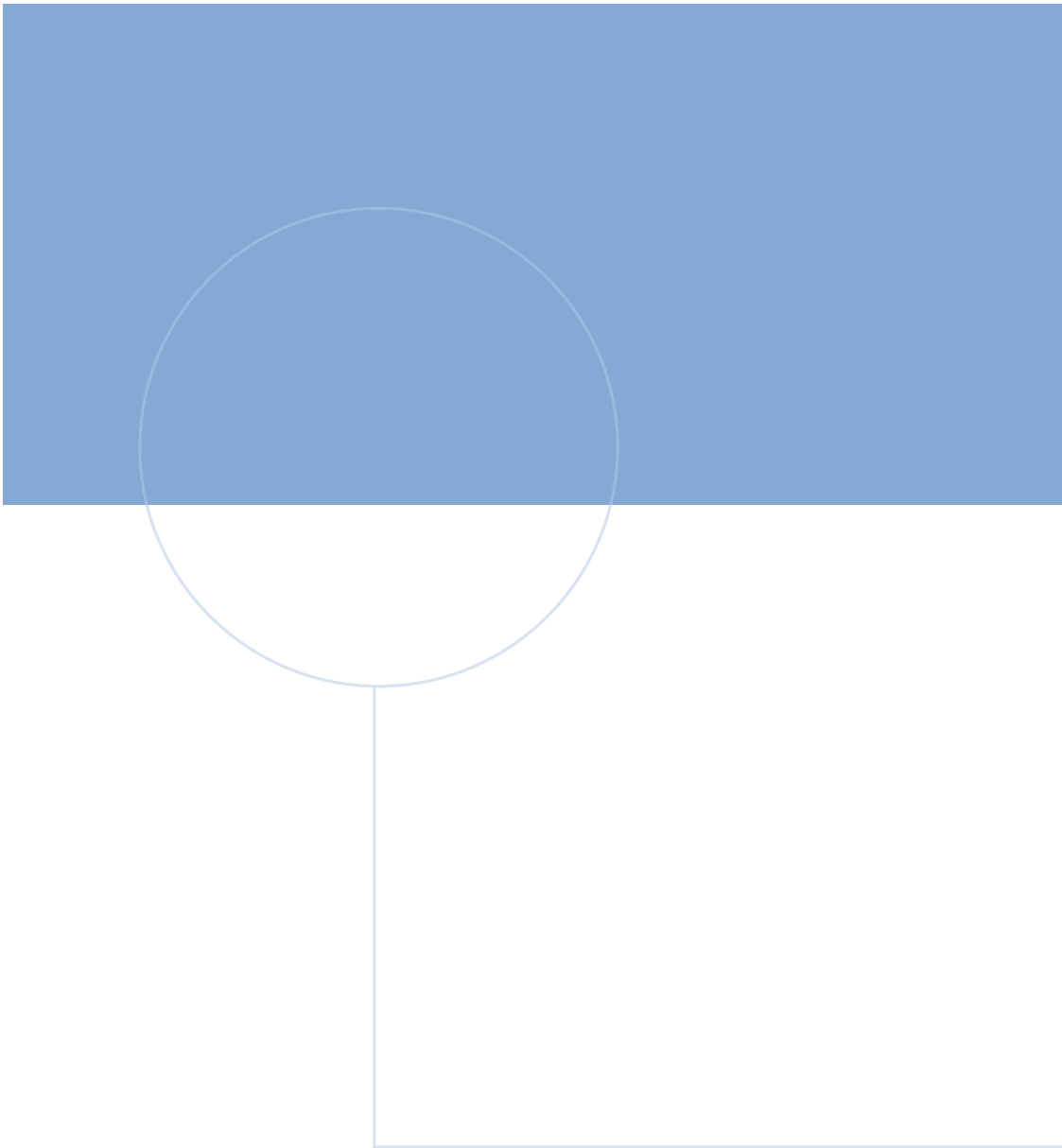
			MML (kbps)			DNA FTM (%)		
ID	**Day**	**Gel**	**MML (kbps)**	**SD MML**	**CV MML**	**DNA FTM (%)**	**SD DNA FTM (%)**	**CV DNA FTM (%)**
F02	5	1	365.57	24.65	6.74	12.49	1.17	9.40
F02	5	2	284.76	25.21	8.85	13.78	1.56	11.29
F02	20	1	491.94	8.63	1.76	11.48	0.77	6.70
F02	20	2	448.34	48.21	10.75	14.57	2.79	19.14
F03	5	9	311.64	20.45	6.56	17.04	2.31	13.55
F03	5	10	370.34	10.31	2.79	14.82	1.97	13.32
F03	5	13	472.60	17.92	3.79	11.94	2.12	17.72
F03	5	14	400.09	6.51	1.63	11.34	1.04	9.21
F03	20	9	328.92	21.99	6.68	19.22	2.72	14.16
F03	20	10	320.43	13.09	4.08	16.98	2.13	12.56
F03	20	13	430.82	29.35	6.81	11.03	1.87	16.93
F03	20	14	386.36	23.05	5.97	11.54	0.70	6.10
F05	5	9	431.01	24.20	5.62	14.50	1.48	10.21
F05	5	10	353.02	9.72	2.75	13.34	0.87	6.56
F05	5	13	411.82	21.04	5.11	11.79	2.11	17.85
F05	5	14	320.18	18.85	5.89	10.82	1.01	9.38
F05	20	9	436.10	11.91	2.73	18.22	2.16	11.86
F05	20	10	307.04	3.13	1.02	18.78	1.42	7.56
F05	20	13	385.66	14.33	3.72	9.90	2.32	23.46
F05	20	14	287.54	7.00	2.44	11.52	1.84	15.99
F06	5	11	440.39	9.38	2.13	14.88	1.78	11.95
F06	5	12	505.80	8.40	1.66	15.20	2.23	14.69
F06	5	15	555.86	18.89	3.40	12.04	1.92	15.93
F06	5	16	460.90	7.93	1.72	12.05	1.14	9.44
F06	20	11	379.70	13.58	3.58	16.33	1.90	11.66
F06	20	12	450.54	7.20	1.60	17.66	2.16	12.25
F06	20	15	483.24	17.44	3.61	11.14	1.54	13.85

ID	Day	Gel	MML (kbps)	SD MML	CV MML	DNA FTM (%)	SD DNA FTM (%)	CV DNA FTM (%)
F06	20	16	404.84	10.64	2.63	13.87	1.32	9.51
F09	5	11	422.18	6.10	1.44	12.60	1.04	8.22
F09	5	12	479.01	11.62	2.43	13.51	2.12	15.72
F09	5	15	429.29	17.10	3.98	8.97	0.95	10.57
F09	5	16	404.84	19.62	4.85	9.65	0.50	5.21
F09	20	11	401.37	5.20	1.30	14.48	1.53	10.58
F09	20	12	433.26	8.40	1.94	15.80	1.84	11.67
F09	20	15	387.41	35.06	9.05	8.99	1.24	13.80
F09	20	16	398.50	8.24	2.07	10.79	2.24	20.76
F14	5	17	303.26	17.67	5.83	15.58	1.48	9.52
F14	5	18	372.14	12.59	3.38	15.40	1.59	10.30
F14	5	21	375.01	17.25	4.60	14.69	1.92	13.05
F14	5	22	456.01	10.36	2.27	15.20	0.97	6.40
F14	20	17	316.58	15.98	5.05	18.28	1.94	10.64
F14	20	18	346.50	14.12	4.07	17.89	1.79	9.99
F14	20	21	381.57	13.55	3.55	13.97	1.75	12.53
F14	20	22	459.31	12.99	2.83	14.90	1.31	8.80
F15	5	17	396.74	31.26	7.88	12.68	1.45	11.43
F15	5	18	361.42	5.95	1.65	10.91	1.30	11.94
F15	5	21	336.21	39.46	11.74	11.33	1.81	16.01
F15	5	22	439.85	14.56	3.31	14.04	1.12	7.95
F15	20	17	457.70	14.25	3.11	12.45	0.96	7.73
F15	20	18	378.14	13.62	3.60	12.14	1.06	8.75
F15	20	21	253.37	19.92	7.86	12.38	1.07	8.63
F15	20	22	423.86	15.57	3.67	15.30	1.18	7.71
F16	5	19	294.67	14.98	5.08	13.90	1.83	13.20
F16	5	20	289.92	43.09	14.86	11.78	1.69	14.35
F16	5	23	388.53	17.15	4.42	15.83	3.69	23.32
F16	5	24	431.87	16.12	3.73	11.12	0.94	8.45
F16	20	19	307.45	11.12	3.62	13.08	2.02	15.42
F16	20	20	335.55	18.09	5.39	10.70	1.12	10.45
F16	20	23	345.62	16.42	4.75	21.61	2.04	9.45
F16	20	24	370.21	15.75	4.26	18.05	1.78	9.87
F17	5	19	316.97	13.84	4.37	11.55	1.61	13.91

ID	Day	Gel	MML (kbps)	SD MML	CV MML	DNA FTM (%)	SD DNA FTM (%)	CV DNA FTM (%)
F17	5	20	387.40	15.03	3.88	10.78	0.95	8.82
F17	5	23	397.34	10.82	2.72	12.77	1.55	12.16
F17	5	24	398.88	11.77	2.95	11.68	1.32	11.30
F17	20	19	298.96	20.75	6.94	11.53	1.77	15.33
F17	20	20	415.95	18.52	4.45	13.01	1.40	10.78
F17	20	23	376.43	9.19	2.44	14.33	2.61	18.23
F17	20	24	353.24	11.99	3.39	12.49	1.28	10.24
F21	5	25	488.63	31.19	6.38	22.40	2.99	13.36
F21	5	26	429.37	20.71	4.82	18.04	2.13	11.82
F21	5	29	425.25	14.82	3.49	12.99	1.25	9.63
F21	5	30	424.14	15.43	3.64	11.81	1.80	15.27
F21	20	25	457.93	18.06	3.94	18.72	2.92	15.57
F21	20	26	498.10	16.94	3.40	16.66	1.49	8.93
F21	20	29	421.20	22.62	5.37	12.47	1.03	8.23
F21	20	30	434.81	9.49	2.18	12.53	1.91	15.23
F22	5	25	434.75	27.88	6.41	18.54	3.05	16.46
F22	5	26	463.01	13.06	2.82	15.97	1.87	11.68
F22	5	29	383.77	18.14	4.73	13.08	1.20	9.16
F22	5	30	442.42	21.05	4.76	10.56	0.83	7.87
F22	20	25	405.03	27.77	6.86	16.42	0.88	5.33
F22	20	26	494.86	20.08	4.06	16.07	1.92	11.97
F22	20	29	327.50	19.87	6.07	14.35	2.02	14.05
F22	20	30	366.97	22.04	6.01	10.85	0.82	7.58
F26	5	27	461.65	38.68	8.38	16.57	0.99	5.98
F26	5	28	538.77	24.39	4.53	14.13	1.60	11.36
F26	5	31	494.07	10.79	2.18	14.88	1.84	12.36
F26	5	32	387.79	7.31	1.89	18.74	2.47	13.18
F26	20	27	456.23	32.66	7.16	18.82	1.17	6.19
F26	20	28	454.62	14.80	3.26	15.26	1.93	12.64
F26	20	31	499.45	29.35	5.88	16.85	2.50	14.86
F26	20	32	426.34	23.53	5.52	19.89	2.63	13.21
F27	5	27	400.78	23.58	5.88	15.44	1.39	9.02
F27	5	28	498.60	22.39	4.49	13.43	0.94	7.01
F27	5	31	384.94	12.79	3.32	15.87	1.55	9.74

ID	Day	Gel	MML (kbps)	SD MML	CV MML	DNA FTM (%)	SD DNA FTM (%)	CV DNA FTM (%)
F27	5	32	350.35	16.65	4.75	15.60	2.13	13.62
F27	20	27	476.43	38.51	8.08	22.24	2.70	12.16
F27	20	28	519.52	21.00	4.04	16.28	1.34	8.25
F27	20	31	411.06	14.35	3.49	14.58	1.92	13.16
F27	20	32	322.72	10.44	3.23	13.57	0.65	4.76
F28	5	3	351.81	21.97	6.25	14.44	1.55	10.71
F28	5	4	338.25	15.21	4.50	11.27	1.49	13.23
F29	5	33	384.78	19.07	4.96	23.93	3.87	16.18
F29	5	34	307.96	10.59	3.44	17.61	1.73	9.83
F29	5	37	301.60	11.20	3.71	24.47	3.92	16.02
F29	20	33	389.76	16.07	4.12	19.21	2.05	10.66
F29	20	34	368.16	14.09	3.83	16.17	2.67	16.51
F29	20	37	301.05	14.01	4.65	25.56	3.66	14.31
F30	5	33	372.46	9.34	2.51	29.55	4.25	14.39
F30	5	34	378.08	25.28	6.69	21.86	3.22	14.72
F30	5	37	273.81	10.25	3.74	20.57	3.77	18.32
F30	20	33	377.95	12.02	3.18	26.82	4.04	15.07
F30	20	34	392.90	14.58	3.71	35.71	6.53	18.30
F30	20	37	271.89	15.75	5.79	28.12	4.05	14.41
F31	5	38	254.47	21.74	8.55	31.58	2.10	6.65
F31	5	39	221.93	8.36	3.76	20.65	2.23	10.80
F31	20	38	247.65	12.59	5.08	27.87	4.76	17.09
F31	20	39	177.99	5.17	2.90	25.21	3.41	13.53
F32	5	3	405.06	7.84	1.94	11.54	1.54	13.34
F32	5	4	455.98	10.75	2.36	11.06	1.98	17.94
F32	20	3	405.96	17.12	4.22	11.88	0.45	3.75
F32	20	4	473.46	9.85	2.08	10.93	1.04	9.52
F33	5	38	320.77	15.57	4.85	22.48	4.74	21.09
F33	5	39	232.44	20.21	8.70	21.37	2.61	12.20
F33	20	38	296.82	15.05	5.07	26.01	3.04	11.70
F33	20	39	213.34	12.74	5.97	27.34	3.39	12.38
F34	5	3	387.12	5.71	1.47	12.52	1.96	15.66
F34	5	4	394.22	30.06	7.62	12.15	1.66	13.70
F37	5	40	442.22	10.35	2.34	12.68	0.59	4.66

ID	Day	Gel	MML (kbps)	SD MML	CV MML	DNA FTM (%)	SD DNA FTM (%)	CV DNA FTM (%)
F37	5	41	449.45	11.23	2.50	13.60	1.59	11.68
F37	20	40	409.71	9.41	2.30	13.67	1.22	8.95
F37	20	41	379.20	8.60	2.27	14.29	2.51	17.55
F38	5	40	557.89	21.75	3.90	9.47	0.81	8.60
F38	5	41	494.92	11.34	2.29	10.36	1.15	11.07
F38	20	40	526.78	34.93	6.63	10.55	1.74	16.46
F38	20	41	463.95	13.17	2.84	12.01	1.21	10.08
F39	5	42	449.29	16.93	3.77	12.33	1.04	8.43
F39	5	43	471.67	11.47	2.43	10.75	2.46	22.86
F39	5	46	494.54	240.57	0.00	29.26	3.86	13.19
F39	20	42	412.41	12.49	3.03	18.25	3.11	17.04
F39	20	43	451.56	15.21	3.37	13.31	1.38	10.38
F39	20	46	379.02	17.29	4.56	19.76	3.17	16.02
F40	5	42	405.28	11.70	2.89	11.89	1.48	12.43
F40	5	43	462.36	356.12	11.04	9.68	1.02	10.55
F40	5	46	383.40	12.90	3.37	17.46	2.01	11.53
F40	20	42	347.82	20.57	5.91	14.29	2.21	15.46
F40	20	43	347.97	398.06	12.65	11.88	1.49	12.56
F40	20	46	433.45	18.03	4.16	20.41	1.74	8.55
F42	5	1	544.53	21.26	3.90	12.47	1.55	12.43
F42	5	2	466.73	9.04	1.94	12.57	1.42	11.29
F42	20	1	533.60	7.05	1.32	11.44	0.92	8.05
F42	20	2	421.26	11.98	2.84	12.23	1.48	12.11



NTNU
Norwegian University of
Science and Technology