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DNA double strand breaks and chemical elements in incubating female common eiders (*Somateria mollissima*) in Christiansø, Denmark.

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Abstract

Genotoxic agents are ubiquitous in the Baltic Sea and may cause effects not only at a molecular level but at an individual and population level as well. Fasting during reproduction may lead to a state of oxidative stress and enhance the genotoxicity of non-essential elements due to low levels of essential elements and nutrients. Therefore genotoxic agents pose a threat to fasting species. The present study aimed to assess DNA double strand breaks (DNA DSBs) in relation to body mass and non-essential elements in blood of female common eiders (*Somateria mollissima*) in the Southern Baltic Sea (Christiansø, Denmark) at the beginning (day 5) and end (day 25) of incubation. Furthermore, the present study aimed to investigate the relationships between non-essential and essential elements in whole blood of female common eiders (*Somateria mollissima*) on day 5 and day 25 of incubation. This was a unique study because each incubating eider was sampled twice and therefore acted as its own control. The present study analyzed DNA DSBs using gel electrophoresis to quantify DNA-fraction, of total DNA, that migrated into the gel (DNA-FTM).

During incubation DNA-FTM increased significantly throughout incubation (0.4 - 70 %). Body mass decreased significantly (17 - 44 %) throughout incubation. Significantly increasing levels of Cd were associated with decreasing levels of Ca and Zn and increasing levels of Cu, which may demonstrate an increase in absorption of Cd from day 5 to day 25 of incubation. While significantly increasing levels of Pb were significantly correlated with decreasing levels of Ca, which may indicate Pb was released from medullary bone during incubation. As and Hg were not found to significantly increase. Hg was found to be positively and significantly correlated with Se, suggesting a protective effect of Se on Hg.

DNA-FTM was found to be negatively and significantly correlated to body mass and positively correlated to Hg (not significantly). Given the high levels of DNA DSBs in the current study compared to previous studies in Baltic Sea eiders, there may be other factors at play, apart from non-essential elements, causing DNA DSBs. However, the high levels of DNA DSBs and body mass loss may reflect the overall health of this endangered population, which is exposed to multiple stressors.

Abbreviations

ADME	Absorption, distribution, metabolism and excretion
AICc	Aikaike's information criteria for small sample sizes
AICc wt	AICc weight
ΔAICc	Difference in AIC between models
ALAD	Aminolevulinate dehydratase
ANOVA	Analysis of variance
As	Arsenic
ATM	ATM serine/threonine kinase
ATR	ATR serine/threonine kinase
BRCA1	Breast cancer 1, DNA repair associated
Ca	Calcium
°C	Celcius
Cd	Cadmium
Co	Cobalt
Coef.value	coefficient value
Cr	Chromium
Cu	Copper
CV	Coefficient of variation
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
DNA-FTM	DNA fraction that migrated
DSB	Double strand break
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron
g	Gram
GI	Gastrointestinal tract
GSH	Glutathione
GPx	Glutathione peroxidase

HBCDD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
Hg	Mercury
HgSe	Molar ratio between mercury and selenium
HR	Homologous recombination
HR-ICP-MS	High resolution inductively coupled mass spectrometry
HSD	Honest significant difference
IDL	Instrument detection limit
IQR	Interquartile range
K	Parameters
kbp	Kilobasepairs
LMPA	Low melting point agarose
LOD	Limit of detection
MDL	Method detection limit
MeHg	Methylmercury
mg	Milligrams
mg/l	Milligrams per liter
mtDNA	Mitochondrial DNA
ml	Milliliter
mm	Millimeter
mM	Millimolar
MML	Median molecular length
MT	Metallothionein
NaCl	Sodium chloride
NHEJ	Non-homologous end joining
nm	Nanometer
NTNU	Norwegian University of Science and Technology
p-value	Probability of rejecting null hypothesis
PAH	Polycyclic aromatic hydrocarbon
Pb	Lead

PBDA	Polybrominated diphenyl ether
PCA	Principal component analysis
PC1	Principal component one
PC2	Principal component two
PCB	Polychlorinated biphenyl
PFOS	Perfluorooctanesulfonic acid
POPs	Persistent organic pollutants
p53	Tumor protein p53
QQ	quantile-quantile
R^2c	Conditional R-squared
R^2m	Marginal R-squared
rf	Relative front
ROS	Reactive oxygen species
rpm	Revolutions per minute
S phase	Synthesis phase
SDS	Sodium dodecyl sulfate
Std.error	Standard error
TAC	Total antioxidant capacity
TBT	Tributyltin
tGSH	Total GSH
t-value	t-statistic from student's t-test
Vcm^{-1}	Volts per centimeter
V(D)J	Variable diversity and joining genes
ww	Wet weight
WWII	World War two
Zn	Zinc

Contents

1 Introduction

1.1 Contaminants in the Baltic Sea

The Baltic Sea is considered to be one of the most polluted seas in the world (HELCOM, 2010). As a semi-closed, relatively shallow marine basin, the Baltic Sea has a long water residence time of around 30 years enabling it to accumulate large amounts of pollutants. With a population exceeding 85 million people in nine countries boarding the Baltic Sea, this brackish-water environment receives contaminants from multiple hot spots and through atmospheric deposition (HELCOM, 2010).

Main sources of contaminants into the Baltic Sea include runoff from industrial sources, waste water treatment plants, municipalities and farmland. Accidental oil spills, hazardous substances released from ships and local hot spots (waste dumping sites of industrial chemicals and World War II (WWII) munitions) also contribute to pollution in the Baltic Sea. In addition, atmospheric deposition is important in the transport of heavy metals and persistent organic pollutants (POPs) that originate from combustion processes in countries around the Baltic Sea (HELCOM, 2010; Leipe et al., 2013). The Baltic Sea has a long history of contaminant exposure. After a peak in most pollutant levels between the 1980s and 1990s some successful attempts have been made to mitigate these levels. However, based on evaluations in sediments, seawater and biota, 137 out of 144 areas assessed in the Baltic Sea were classified as being disturbed by hazardous substances (HELCOM, 2010).

1.1.1 Toxic elements

Due to their nature, toxic elements such as heavy metals, are ubiquitous in the Baltic Sea. Metals are non-biodegradable and readily bioaccumulate and biomagnify in Baltic Sea marine food webs (Polak-Juszczak, 2009, 2012). In addition, heavy metals are read-

ily redistributed in the environment through natural biological and geological cycling and have long residence times in soils (Uścinowicz, 2011). Therefore, biota face the risk of long term exposure to these toxic substances. Despite this, heavy metal levels in sediments and from atmospheric deposition have generally declined since the 1990's in the Baltic Sea (HELCOM, 2010). Sediments function as an ultimate sink for contaminants as they are physically undisturbed and may therefore reflect changes in the marine environment (Uścinowicz, 2011). Environmental monitoring of elements in sediments may help shed light on exposure to benthic and benthic feeding organisms (HELCOM, 2010). Sediment analyses generally show a peak of toxic elements including heavy metals around 20-30 years ago followed by a decline (Leipe et al., 2013; Zalewska et al., 2015). In addition, the total the atmospheric deposition of heavy metals to the Baltic Sea has decreased by from 1990 to 2015 by 63% for cadmium (Cd), 34% for mercury (Hg), and 80% for lead (Pb) (Bartnicki et al., 2017). However, recent sediment studies show that certain elements (arsenic (As), Cd, Hg and Pb) are still of concern and higher than background levels in certain areas of the Baltic Sea (HELCOM, 2010; Leipe et al., 2013; Zalewska et al., 2015). In certain species, levels of Hg and Cd have shown an increasing temporal trend in some areas of the Baltic Sea (HELCOM, 2010). In blue mussels (*Mytilus edulis*), concentrations of Hg and Cd were shown to be above threshold values associated with natural background levels in all areas of the Baltic Sea (HELCOM, 2010). As has been of concern in the Baltic Sea because of chemical weapons and warfare agents dumped after WWII. Sediment analyses in the Bornholm Basin, show elevated As concentrations around the dumping sites (Emelyanov et al., 2010). In addition, benthic organisms, like the blue mussel, may accumulate high levels of As compared to pelagic organisms (Neff, 1997). Therefore, the risk of exposure to benthic and benthic feeding species is high.

1.1.2 Persistent organic pollutants

POPs are resistant to degradation and may be transported long distances, making them ubiquitous in the environment. As most POPs are highly lipophilic substances, they may bioaccumulate and biomagnify in food chains and therefore pose a threat to marine environments (Henny et al., 2003; Nfon et al., 2008). POPs show a similar trend as heavy metals in the Baltic Sea with a general decline since the 1990's and after their regulation through the Stockholm convention, however, because of their persistence certain POPs have been a concern in biota in the Baltic Sea (UNEP, 2009). Polychlorinated biphenyls (PCBs) and Dichlorodiphenyltrichloroethane/Dichlorodiphenyldichloroethylene

(DDT/DDE), and Polybrominated diphenyl ethers (PBDEs) are still found to exceed threshold levels in sediments, seawater and biota in almost all sub-basins of the Baltic Sea (HELCOM, 2010; Skov, 2011). Of the emerging contaminants, both Hexabromocyclododemaye (HBCDD) and Perfluorooctane sulfonate (PFOS), show increasing trends in guillemot eggs and have been at the same level in herrings for decades (HELCOM, 2010). In addition, tributyltin (TBT) has been shown to plague Baltic Sea benthic organisms (HELCOM, 2010). Avian species, especially marine top predators, are at risk for toxic effects from POPs because of their position in the marine food web (Fisk et al., 2001).

1.2 Non-essential element toxicity in seabirds

Seabirds are considered suitable bioindicators because of their sensitivity to heavy metal pollution (Furness and Camphuysen, 1997; Burger et al., 2008). One of the main ways non-essential elements elicit their toxic effects in seabirds is through the onset of oxidative stress (Ercal et al., 2001; Koivula and Eeva, 2010). This occurs when there is an imbalance between antioxidants and reactive oxygen species (ROS) favoring ROS (Ercal et al., 2001). These species are produced normally from oxidative respiration but are detoxified by antioxidants (Sies, 1997; Finkel and Holbrook, 2000). However, antioxidants have a limited capacity and an excess production of ROS may not always be accounted for (Sies, 1997). ROS are highly reactive species and an excess may lead to oxidation of biomolecules (Stohs and Bagchi, 1995; Halliwell and Gutteridge, 2015). Redox inactive non-essential elements like As, Cd, Hg and Pb deplete the cell's major antioxidants (especially thiol containing antioxidants) leading to an imbalance between the levels of ROS and antioxidants. (Ercal et al., 2001; Valko et al., 2006).

1.2.1 Essential and non-essential element interactions

Another key feature in non-essential elements' mechanisms of action is their ability to mimic essential elements (specifically essential metals) and disturb metal homeostasis (Scheuhammer, 1987; Goyer, 1997). This may either enhance or diminish their absorption, distribution, biotransformation and elimination (ADME) and therefore their toxicity (Tokar et al., 2015). For example, absorption of non-essential elements may be enhanced through dietary deficiencies while their distribution is enhanced by their ability to mimic essential elements (Foulkes, 2000). However, non-essential element's elimination may increase when they are bound to certain proteins (Elder et al., 2014).

It is important to note that non-essential elements' ADME may vary greatly depending on age and gender (Franson et al., 2000a; Robinson et al., 2012). Deficiencies in certain essential elements and their interactions with non-essential elements may greatly increase or decrease non-essential elements' toxic effects (Goyer, 1997). Some essential elements may also be in excess in the environment due to anthropogenic pollution, however, this is beyond the realm of the present study.

1.2.2 Arsenic

Both inorganic and organic arsenic may exist in the marine food web. Organic arsenic, especially as arsenobetaine, may be present in relatively high concentrations in marine food webs because of its potential to biomagnify (Neff, 1997; Zhang et al., 2016). However, organic As is less toxic than other forms of As. Inorganic As (As^{3+}) is the most toxic form of As and is well absorbed in the GI tract at 80 to 90% (Fowler et al., 2014). Once absorbed As is distributed throughout the body and may accumulate in the liver and kidneys. Here As may be methylated, becoming toxic to these organs (Scheuhammer, 1987; Fowler et al., 2014).

1.2.3 Cadmium

Bioavailable Cd mostly exists in the Cd^{2+} form in the environment and exposure may occur both through drinking water and ingesting prey (Scheuhammer, 1987; Furness, 1996). Cadmium absorption through the GI tract occurs at a relatively low rate (5-10%) (Tokar et al., 2015). However, dietary deficiencies in calcium (Ca), copper (Cu), iron (Fe) and zinc (Zn) may enhance the absorption of Cd through molecular mimicry in avian species (Scheuhammer, 1987, 1996; Goyer, 1997; Wayland and Scheuhammer, 2011). Once absorbed into the body Cd binds to albumin and other higher molecular weight proteins but is taken up rapidly by tissues and stored mainly in the liver and kidneys where it may have a long biological half-life (Frazier, 1979; Garcá-Fernández et al., 1996). In the liver, Cd may mimic Zn and Cu and bind to metallothionein (MT), a cysteine rich transporter protein, when organisms are deficient in Cu and Zn (Scheuhammer, 1987; Tokar et al., 2015). In fact, studies have shown that an increase in dietary Zn may decrease the toxicity of Cd (Jacobs et al., 1983; Imed et al., 2008; Nordberg et al., 2014). Once bound to MT cadmium is considered relatively stable and non-toxic (Nordberg et al., 2014).

1.2.4 Lead

For seabirds, high lead concentrations may occur from lead ammunition either from ingestion or from hunting (Helander et al., 2009). In addition, seabirds may be exposed through ingestion of prey (Furness, 1996; Franson and Pain, 2011). Most non-essential elements may vary to a certain degree in avian species depending on gender, breeding condition, age and diet (Franson and Pain, 2011). This is however, especially true for Pb and levels may vary greatly depending on these factors (Franson et al., 2000a,b). Dietary deficiencies in Ca, Fe and Zn may greatly increase the absorption of Pb through molecular mimicry (Abadin et al., 2007; Skerfving and Bergdahl, 2014). Once absorbed approximately 5% of Pb remains in blood and 95% of it is distributed to soft tissues like the kidney and liver and later to bones (Ethier et al., 2007; Skerfving and Bergdahl, 2014). In blood Pb is known to inhibit the activity of delta-aminolevulinic acid dehydratase (ALAD) by replacing Zn and leading to anemia (Franson et al., 2000b; Ethier et al., 2007). In addition, Pb may mimic Ca thereby replacing Ca in bone (Goyer, 1997; Ethier et al., 2007). Pb therefore accumulates in bone with age and may have a half long life between 20-30 years compared to other tissues with a Pb half life between 30 and 40 days. Consequently, Pb released from bone may contribute up to 50% of Pb found in blood (Skerfving and Bergdahl, 2014). In breeding birds the medullary bone acts as a source of Ca for developing eggs, therefore Pb may be mobilized during egg development (Franson and Pain, 2011; Williams et al.). Due to both Pb and Cd's mimicry of Ca, they are seen in high concentrations of species like blue mussels (*Mytilus edulis*) (Phillips, 1976; Eisler, 2009). This contributes to exposure of Pb to mussel eating seabirds.

1.2.5 Mercury

Methylmercury (MeHg) is of concern in marine food webs because of its chemical stability, lipophilicity and ability to biomagnify (Mason et al., 1995; Lavoie et al., 2013). In marine birds MeHg has been shown to make up >95% of total Hg in blood (Wayland et al., 2001; Fournier et al., 2002). MeHg, is well absorbed through the GI tract and about 95% and is distributed to all tissues in 30 hours where 10% goes to the brain and 5% remains in the blood with a half life of approximately 60 days in avian species (Wolfe et al., 1998). Hg is known to bind to proteins and therefore has been shown to accumulate in lean tissues like the liver, kidneys and muscle in birds (Kenow et al., 2007; Seewagen et al., 2016). By binding to sulfhydryl groups on antioxidants like glutathione (GSH) and on proteins, Hg depletes the cell's major sulfhydryl reserves. These antiox-

idants are then not available to scavenge ROS produced by normal cellular respiration or by other stressors, like pollutants, leading to a state of oxidative stress (Ercal et al., 2001; Koivula and Eeva, 2010). However, the toxic effects of Hg may be combated with selenium (Se) whereby Se binds directly to Hg to form an insoluble complex or acts as a co-factor in glutathione peroxidase (GPx) and reduces free radicals with GSH (Kenow et al., 2008). Evidence shows that a molar excess of Se to Hg (more than 1) in blood indicates a protection of Se from Hg toxicity in tissue (Scheuhammer, 1987; Kim et al., 1996).

1.3 Genotoxicity and DNA double strand breaks

Genetic toxicology attempts to assess the effects of chemical and physical agents on genetic material (DNA (deoxyribonucleic acid)) and genetic processes (Preston and Hoffman, 2015). Such effects are initiated by a spectrum of DNA damage. For example, damage to DNA bases may lead to apyrdmidnic and apurinic sites, pyrimidine dimers and free radical formation (Mehta and Haber, 2014). DNA damage also includes intra- and interstrand cross links, DNA-protein cross links and both single and double strand breaks (DSBs) (Lindahl, 1993). Of these, DSBs are considered to be one of the most cytotoxic forms of damage because the phosphate backbones of the two complementary strands are broken simultaneously and therefore the continuity of the DNA template is disrupted (Mehta and Haber, 2014) (see Fig. 1.1). Without an intact template it is difficult to synthesize new complementary strands (Jackson, 2002).

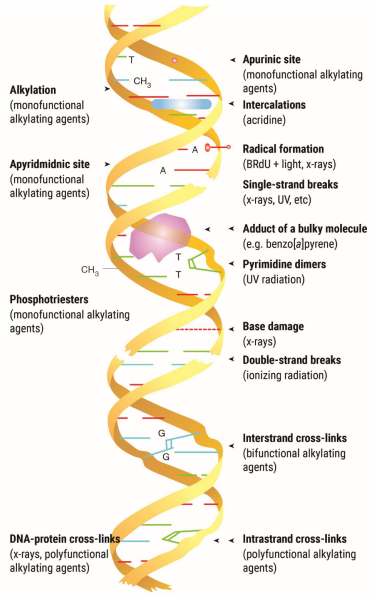


Figure 1.1: The spectrum of DNA damage induced by both physical and chemical agents. Modified from Preston and Hoffman (2015).

There are a number of endogenous and exogenous causes of DSBs. Exogenous causes of DSBs include radiation and certain chemicals. Endogenous causes of DSBs are found, for example, during DSB induced recombination in meiosis, during mechan-

ical stress on chromosomes and from oxidative induced damage (Lieber, 2010; Kryston et al., 2011; Mehta and Haber, 2014). DSBs may also occur during DNA replication when unrepaired lesions are encountered leading to a fork collapse (Hoeijmakers, 2001). If left unrepaired these DSBs may lead to mutations, chromosomal aberrations, tumorigenesis, carcinogenesis, and/or apoptosis (Pfeiffer, 1998; van Gent et al., 2001; Vilenchik and Knudson, 2003; Bignold, 2009). Furthermore, accumulation of DNA damages may lead to neurodegenerative diseases and accelerated aging (Friedberg et al., 2005). Given the complexity and severity of DSBs, cells have developed repair systems that are highly conserved across pro/eukaryotic evolutionary borders.

The cell has a variety of sensing and repair systems responding to DNA DSBs. First, cell cycle check points are crucial for sensing DNA DSBs so that these damages are not passed down to daughter cells and so that chromosomal aberrations are not created. Two main checkpoints include the G_1-S and G_2-M cell cycle checkpoints and they allow a dividing cell to slow down and repair DNA DSBs (Khanna and Jackson, 2001). In addition, protein kinases are activated by DNA DSBs and recruit various downstream substrates that are involved in DSB repair (Jazayeri et al., 2006; Caestecker and Van de Walle, 2013; Daley and Sung, 2014). If DNA damages are numerous and severe, repair systems may not be able handle them and the cell may go into apoptosis (Davis and Chen, 2013).

1.3.1 Repair of DNA double strand breaks

There are then two main pathways of DSB repair: Non-homologous end joining (NHEJ) and homologous recombination (HR) (Fig. 1.2).

In HR repair, nucleotide sequences are exchanged between two similar molecules of DNA (Rothkamm et al., 2003). The DNA section where the DNA DSB occurred is resectioned and the resulting single strands invade a homologous double stranded template ultimately leading to dissolution and repair (Lans et al., 2012) (Fig. 1.2). This type of repair is considered more efficient and less error prone than NHEJ repair (Takata et al., 1998; Rothkamm et al., 2003; Daley and Sung, 2014).

NHEJ occurs when two ends of DSBs are simply ligated together (Davis and Chen, 2013) (Fig. 1.2). Such repair is error-prone because the two broken ends of the DSB are simply spliced together and this may result in small deletions leading to mutations. (Davis and Chen, 2013). In fact, this pathway of repair is so prone to deletions and mutations that developing B and T-lymphocytes use NHEJ (called V(D)J recombination for these cells) as a way to promote antibody diversity (Soulas-Sprauel et al., 2007).

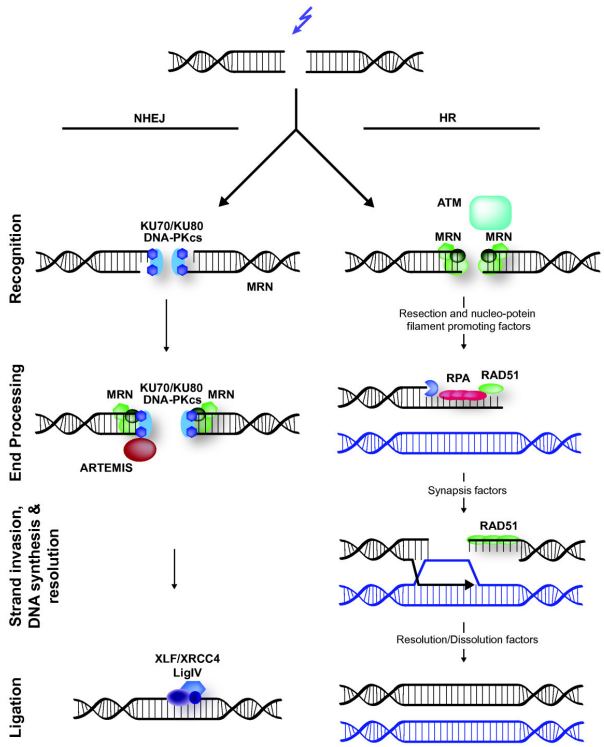


Figure 1.2: The two main repair pathways of DNA DSBs. The non-homologous end joining (NHEJ) repair pathway is represented on the left and the homologous recombination (HR) repair pathway on the right. Both involve the stages of recognition, end processing and ligation, while only HR involves strand invasion, DNA synthesis and resolution. Modified from Lans et al. (2012).

1.3.2 Non-essential elements and DNA double strand breaks

An important environmental source of DNA damage and DSBs is exposure to non-essential elements (Mehta and Haber, 2014), which generally exert their toxic effects through the onset of oxidative stress (Ercal et al., 2001) as mentioned above. Free radicals, like the hydroxyl radical (OH-) are then free to oxidize nucleobases or the sugar phosphate backbones of DNA (Valavanidis et al., 2006). When too many damages are accumulated, DNA repair mechanisms fail leading to DNA DSBs (Friedberg et al., 2005). When free radicals attack DNA simultaneously, two neighboring single strand breaks may be created (i.e. DNA DSBs) (Tchounwou et al., 2012). In addition, persistent damage to DNA may lead to replication errors, upregulation of signal transduction pathways, arrest or induction of transcription and genomic instability, events that are linked to carcinogenesis (Marnett, 2000; Cooke et al., 2003).

Exposure to non-essential elements *in vitro* has also shown inhibition of DNA repair mechanisms (Davidson et al., 2014; Morales et al., 2016). As has been shown to methylate or "silence" DNA repair proteins (Mass and Wang, 1997; Klein et al., 2007). Other non-essential elements, such as Hg and Cd, may also inhibit repair proteins (Waisberg et al., 2003; Davidson et al., 2014). In addition, some heavy metals in their ionic form may be very reactive, forming DNA adducts (Friedberg et al., 2005). Pb and Cd may replace Zn on zinc finger proteins involved in DNA repair and chromosome stability (Witkiewicz-Kucharczyk and Bal, 2006). Taken together, inhibited repair mechanisms and oxidative stress, may lead to the formation of DNA DSBs.

Many *in vitro* studies exist on the effects of non-essential elements on DNA damage (Costa et al., 1991; Klein et al., 2007; Morales et al., 2016). However, in the environment these non-essential elements may exist in mixtures and may have synergistic, additive or antagonistic effects (Tchounwou et al., 2012). Metals are ubiquitous in the environment, especially in the Baltic Sea and therefore pose a constant genotoxic threat in many species.

1.3.3 Population level effects

Although the initial damage caused by chemical pollutants is at the molecular level, there are emergent effects which appear later at population levels (Bickham et al., 2000). Genotoxicants may induce heritable changes that are passed down through generations (Bickham, 2011). Chronic exposure to environmental contaminants results in a stress to individuals that may lead to the selection of certain alleles associated with survival and successful reproduction (Bickham et al., 2000; Bickham, 2011) thus decreasing genetic diversity in a population (van Straalen and Timmermans, 2002). In turn this may lead to population bottlenecks, changes in migration patterns, and reduced fitness in inbred populations (Brown et al., 2009). These events may lead to a collapse of populations (Bickham, 2011). Studies in laboratories *in vivo* of fish and flies have shown the eco-genotoxicological effects of Hg and Cd in the form of changes in allelic frequencies or reduced survivorship (Shirley and Sibly, 1999; Tatara et al., 1999).

However, contaminants occur in mixtures in the environmental and it is therefore challenging to predict how they will elicit eco-genotoxicological effects. Avian species in the environment may be good bioindicators of eco-genotoxicological effects because of their diversity, presence across the world and the availability of their historical data (Bonisoli-Alquati, 2014)

1.4 The common eider *(Somateria mollissima)*

The common eider (*Somateria mollissima*, hereafter called eider), a long-lived species, is structurally the largest and heaviest bird in the northern hemisphere (Waltho and Coulson, 2015). As partially migratory birds, eiders breed around the mid- and high-latitude coasts in the northern hemisphere where populations breeding in the Danish Baltic Sea generally winter in the Dutch Wadden Sea (Skov, 2011; Waltho and Coulson, 2015). The most important food source for eiders in the Baltic and Wadden Seas is the blue mussel (*Mytilus edulis*). As low trophic feeders, eiders are therefore exposed to a relatively low level of pollutants in comparison with top predatory birds (Dahl et al., 2003; Bustnes et al., 2010). Eiders have a low reproductive rate and a long life expectancy where adult survival rates are historically high (Waltho and Coulson, 2015). Population growth rates are therefore sensitive to changes in adult survival (Coulson, 2010).

1.4.1 Population declines

Starting in the late 1990s eider populations have seen drastic decreases, especially in Europe where they are now considered endangered (Birdlife International, 2016). Europe contains approximately 60% of the global population of eiders (*Somateria mollissima*) so declines in this region are of global significance (Wetlands International, 2012). Within Europe, the Baltic and Wadden Seas represent the largest flyway population of eiders (Birdlife International, 2016). Between 1990 and 2000 the total Baltic/ Wadden Sea flyway population decreased by 36% followed by a 48% decline in the number of breeding eiders between 2000 and 2009 (Desholm et al., 2003; Ekroos et al., 2012). Paralleling this drastic population decrease, was a shift in the population sex ratio from a female bias to an increasingly male bias (Lehikoinen et al., 2008). In addition, breeding colonies have experienced mass mortality events. For example, in Christiansø, Denmark mass morality events in 2007 and 2015 left 125 and 110 eiders dead, respectively (Garbus, 2016).

The reasons for such a sharp, geographically broad decline are multiple, interconnected and poorly understood (Christensen, 2008). Some of the hypotheses are increased predation (Christensen, 2008), disease like avian cholera (Pedersen et al., 2003), parasites, oil pollution (Thieltges et al., 2006), environmental contaminants (Desholm et al., 2003; Sonne et al., 2012; HELCOM, 2013c) and changes in the quality and quantity of food stocks (Camphuysen et al., 2002; Laursen and Møller, 2014). Others attribute the population decline and skewed sex ratio to increased mortality of breeding female

eiders (Lehikoinen et al., 2008).

1.4.2 The stress of breeding

Breeding in eiders is considered to be an energetically costly event as eiders are largely considered capital breeders (Parker and Holm, 1990; Waltho and Coulson, 2015). Only females participate in incubation, at which time they fast and rely on endogenous energy stores built up mostly from their respective wintering grounds, to provide the nutrients and energy for not only producing and incubating a clutch of eggs but also for self preservation (Milne, 1976; Korschgen, 1977; Parker and Holm, 1990). In preparation for breeding, it is therefore vital that females build up sufficient body reserves. However, recent evidence suggests females may in fact feed some at local breeding grounds during incubation. Jaatinen et al. (2016) showed that heavier females relied mostly on stored reserves at the beginning of the breeding season to produce their eggs while lighter females relied more on local feeding. This, however, could be related to mussel stock quality at the wintering grounds. Possibly due to climate change, warmer winters result in poorer mussel quality and consequently eiders may have to rely more on local feeding (Hobson et al., 2015; Jaatinen et al., 2016). Therefore, in the face of climate change eiders may increasingly adopt an income breeding strategy.

Figure 1.3: Incubating female common eider (*Somateria mollissima*) in Christiansø, Denmark. Photo: Brenley Noori.

Females still loose a significant amount of weight during incubation loosing between 20-45% of their pre-laying body mass (Korschgen, 1977; Parker and Holm, 1990; Fenstad et al., 2014). Incubation lasts approximately 26 days and females lay on average between 3 to 6 eggs (Waltho and Coulson, 2015). Cherel et al. (1988) suggested three phases of energy expenditure in fasting in avian species. The first phase is an adaptation phase, in which protein catabolism decreases and lipid mobilization increases. The second phase is marked primarily by lipid catabolism, where incubating birds spend most of their time. The third phase is characterized by an increase in protein catabolism, which is marked by decreases in albumin and total protein levels. Eiders have been shown to enter phase three towards the end of incubation (Parker and Holm, 1990; Hollmén et al., 1998). Furthermore, Parker and Holm (1990) found that of a total body

mass loss of 30.6%, 81.4% was lipids and 36.8% was protein in Svalbard eiders. These percentages may change depending on the initial health of individual eiders (Parker and Holm, 1990). Such drastic changes in body mass may mobilize non-essential elements stored in tissues that are bound to proteins and possibly adipose tissue into the blood in the circulatory system (Wayland et al., 2001, 2005; Provencher et al., 2016). In addition, lipophilic pollutants like some POPs stored adipose tissue may be released in the blood circulation during incubation and fasting (Bustnes et al., 2012; Fenstad et al., 2014). These pollutants may be distributed to vulnerable tissues where they may exert a toxic, adverse effect, creating a state of oxidative stress and even damaging DNA (Fenstad et al., 2014, 2016a).

In addition, at the end of incubation females have depleted their energy reserves and are therefore in poor body condition showing signs of severely suppressed immune systems and oxidative stress due to incubation and fasting (Hanssen et al., 2003, 2005). A general assumption in the evolution of life histories is that an increased portion of energy or resources in one function means redirecting energy or resources from another function (Stearns, 1992). In the realm of reproduction, when an organism reproduces, less energy is available for self-maintenance (Alonso-Alvarez et al., 2004). Studies have shown that a high reproductive effort increases basal and field metabolic rates and because metabolism is greater more ROS is produced (Alonso-Alvarez et al., 2004). Free radicals are then able to interact with biomolecules like DNA (Ercal et al., 2001).

At the end of incubation eiders may also have very low levels of essential elements which may facilitate the absorption, distribution and toxicity of non-essential elements (Scheuhammer, 1987). Nutritional deficiencies alone may also be toxic (Sonne et al., 2012). A combination of nutritional deficiencies, a state of oxidative stress and exposure to environmental contaminants may contribute to genotoxic effects. There is evidence that a combination of severe mass loss from starvation, POPs and Hg may elicit negative effects in the form of DNA damage in eiders (Bustnes et al., 2010; Fenstad et al., 2014, 2016a). Taken together these toxic effects may help shed light on some of the factors causing a population decline in the eider population in the Baltic Sea.

1.5 Aim

The aim of the present study was to study the frequency of DNA DSBs throughout incubation in female eiders and to investigate the potential effects of body mass loss and non-essential elements on DNA DSBs. In addition, the aim was to investigate lev-

els of non-essential elements in relation to essential elements throughout incubation. It is hypothesized that DNA DSBs will increase from the beginning to the end of incubation and that DNA DSBs will be correlated with an increase in concentration of non-essential elements and a decrease in body mass. It is also hypothesized that non-essential elements will increase throughout incubation and be related to decreasing essential elements.

2 | Materials and Methods

2.1 Field sampling

2.1.1 Location

Field sampling was conducted on Christiansø, Gudhjem, Denmark in the Southern Baltic Sea (55°19'N 15°11'E) (Fig. 2.1) during the 2017 breeding season. There was a total of approximately 1680 breeding females in 2017. New nests were searched for every day at the beginning of the breeding season (March 31st to April 13th). New nests were marked with an identification tag and their GPS position was recorded. In addition, nesting eiders were previously ringed with identification numbers during prior breeding seasons. Blood sampling took place between April 8th to the 13th 2017 followed by a second sampling from May 1st to May 7th 2017.

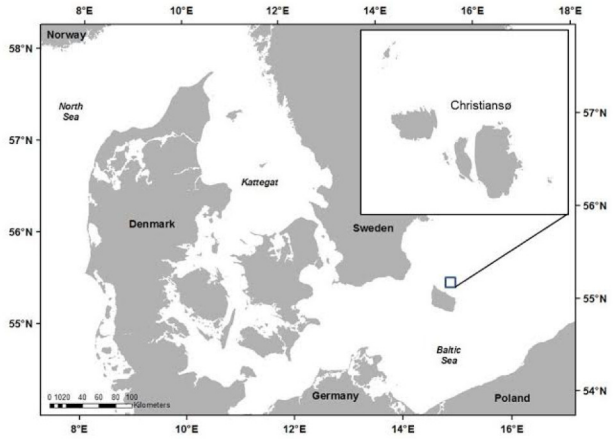


Figure 2.1: Study area in the Central Baltic Sea. The common eider (*Somateria mollissima*) colony was located on the island of Christiansø (55°19'N 15°11'E). Modified from: Garbus (2016).

2.1.2 Blood sampling

Blood samples were obtained from incubating female eiders (n=28) at the beginning (day 5) and end (day 25) of incubation. Day zero of incubation was defined as the day the last egg was laid. The females were caught on the nest by using a pole with a black cloth attached at the end. Body mass was recorded using a Pesola spring balance (3000g). Whole blood samples (8-10 ml) were collected from the brachial vein using a heparinized syringe. A sample of 1 ml, for DNA analyses was transferred to an eppendorf tube, and snap frozen (-196°C) in a nitrogen dry shipper tank within 30 minutes of sampling. Samples were transported to the Norwegian University of Science and Technology (NTNU) at the end of the field season and stored at -80°C until later analysis. The rest of the blood was stored at -20°C for later chemical analyses.

2.2 Detection of DNA double strand breaks

The analyses of DNA DSBs in the blood cells of female eiders was performed by agarose gel electrophoresis at the Department of Biology, NTNU.

The chemicals used were as follows: TE buffer, TBE buffer, Lysis buffer, 1% LMPA (low melting point agarose), 0.6% agarose, lambda DNA ladder, ethidium bromide, and proteinaseK (see Appendix A.1 for more details).

2.2.1 Principles of applied gel electrophoresis

Quantitative analyses of DNA DSBs are made possible through gel electrophoresis as described by (Theodorakis et al., 1994). Whole blood cells are embedded in LMPA as to protect DNA from procedural damage and shearing forces characteristic of conventional methods of pipetting samples into gel wells. Because LMPA remains fluid at 37°C, enzymatic digestions are made possible within the plug without the gel solidifying (Sambrook and Russell, 2012). The resulting plugs are then added to a digestion buffer where cells are lysed, nucleases are digested, and DNA associated proteins are removed. What remains is tightly wound, supercoiled DNA. Under gel electrophoresis, DNA DSBs cause these supercoils to relax, releasing fragments of DNA into the gel (Khan and Ali, 2017; Collins et al., 2008). Under neutral electrophoretic conditions (pH 7), DNA DSBs cause these supercoils to relax, releasing fragments of undistributed, duplex structured DNA into the gel (Khan and Ali, 2017; Collins et al., 2008; Theodorakis et al., 1994).

During gel electrophoresis the negatively charged sugar phosphate backbone of DNA is attracted to the anode of the gel electrophoresis chamber causing DNA fragments to migrate when an electric field is applied (Yılmaz et al., 2012). The agarose gel acts as a sieve, enabling smaller DNA molecules to pass more easily through the pores of the gel (Lee et al., 2012). Where larger fragments are able to migrate further with a lower gel percentage. Shorter fragments move faster and migrate further than longer fragments. DNA is therefore separated by size (Lee et al., 2012).

The resulting gel is then stained with ethidium bromide, an intercalating dye, containing a tricyclic planar group that intercalates between stacked bases of DNA . When UV radiation is applied, Ethidium bromide re-emits energy at 590 nm as a red-orange color, making the DNA visible (Sambrook and Russell, 2012).

2.2.2 Preparation of plugs

Agarose plugs for gel electrophoresis were prepared according to the procedures described by Theodorakis et al. (1994) and Krøkje et al. (2006) with modifications. Briefly, whole blood (10 µl) was added to 500 µl of TE buffer at 37°C. An equal amount (500 µl) of pre-melted 1% LMPA was added at 37°C and briefly centrifuged up to a speed of 8 000 rpm. From this mixture 50 plugs were cast in plug moulds and cooled at 4°C for 1 hour. The plugs were transferred to lysis buffer (100mM NaCl, 10mM Tris, 10mM EDTA, 0.5% SDS) with freshly added proteinase K (~1mg/ml) and incubated for 16 hours at 55°C. After incubation, plugs were cooled for 4 hours at 4°C.

2.2.3 Applied gel electrophoresis

Plugs were loaded into the wells of a 0.6% agarose gel in TBE buffer (90mM tris base, 90mM boric acid, 2mM EDTA, pH 8) and sealed in place with 1% LMPA. Whole linearized Lambda DNA and Lamda-DNA Hind III digest fragments were used as molecular size markers (Appendix A.2). The molecular size markers were heated for 5 minutes at 65° C and then placed on ice for 5 minutes before being loaded into the gels.

Electrophoresis was run at 2.3Vcm^{-1} in 0.5×TBE buffer (Appendix A.1) for 14 hours at room temperature. The gel was then stained for 120 minutes in a solution of ethidium bromide mixed in TBE buffer (~0.1 mg/l). The gels were then rinsed in tap water to remove excess ethidium bromide. Two gels with an identical setup were run simultaneously. Each gel contained 2 individuals with samples from day 5 and day 25 of incubation in triplicate with 3 molecular size markers.

2.2.4 Quantification and semi-quantification of DNA double strand breaks

The gel was photographed under UV light using a BioRad Gel Doc 2000 system and gel image data was obtained. For each triplicate of a sample, 3 staining intensity curves (Relative front vs. intensity) were used to calculate DNA-FTM and MML. The relative front represents values between 0-1 and indicate the movement of the bands from top to bottom of the gel. Intensity represents the intensity of fluorescence of the bands. The fraction of DNA that migrated in the gel (DNA-FTM) compared to the total amount of DNA loaded was calculated by:

$$DNA\text{-}FTM = \frac{DNA\ in\ gel}{DNA\ in\ gel + DNA\ in\ well} \times 100 \tag{2.1}$$

Where DNA that migrated in the gel and DNA left in the well were calculated based on the areas under the intensity curves generated by the gel image (See Appendix A.3). DNA-FTM is an indication of DNA DSB frequency (Fenstad et al., 2014).

Median molecular length (MML) of the DNA that migrated in the gel was determined by the median of the area under the intensity curve. The corresponding rf value (relative front) was compared to a standard curve (rf vs. kilo-base pairs (kbp)) generated by a Hindiii digest of λ-phage DNA of known molecular length. Molecular lengths were represented in kbp.

2.3 Chemical analysis of elements

Whole blood samples were analyzed for concentrations of elements at the Department of Chemistry, NTNU using High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS).

2.3.1 Preparation of samples and acid digestion

Before beginning the ICP-MS, between 500-1000 mg of whole blood was transferred to acid washed 15 ml Teflon tubes designed for UltraClave and 2 ml of Scanpure nitric acid 50% (HNO_3) was added to each vial. Samples were then digested for 2 hours in an UltraClave (Milestone), a high pressure microwave system reaching up to temperatures

of 240°C and a pressure of 160 bar. The samples were then diluted with Milli-Q water to a volume of 24-27 ml and transferred to 15 ml vials for HR-ICP-MS analysis.

2.3.2 HR-ICP-MS and quantification

HR-ICP-MS was carried out using a Thermo Finnigen model Element 2 instrument. To ensure the quality of the analysis, three reference material samples (Seronorm trace elements whole blood L-2, lot 1206264, REF 210105) were analyzed with the samples. Three blanks were also added to monitor contamination during each analysis. The reference material was within the approved range for all analyzed elements (See Table A.4 in Appendix A.4).

The lower limit of detection (LOD) was set to the highest value of either the calculated instrument detection limit (IDL) or three times the standard deviation of the blanks (Table A.3 in Appendix A.4). Calculations of IDL were made by analyzing solutions containing decreasing concentrations each element. The concentration resulting in a relative standard deviation of 25% (n=3 scans) was chosen as the IDL with baseline corrections. Element concentrations are presented in µg/kg wet weight (ww).

2.4 Statistical analyses

All statistical analyses were done in the program R (R Core Team (2015)). Additional packages are presented in Appendix A.5.

2.4.1 Treatment of the samples

Elements chosen for further analyses were As, Ca, Cd, cobalt (Co), Cu, Fe, Hg, potassium (K), Pb, Se, and Zn and were chosen based on their genotoxic effects and/or their relation to genotoxic elements. Chromium (Cr) and nickel (Ni) were also investigated, however more than 50% of individuals had concentrations below the LOD and were therefore excluded from further statistical analyses. All other elements had concentrations greater than the calculated LODs for all individuals. The molar ratio between Hg and Se (HgSe) was also calculated.

For applied gel electrophoresis, multiple gels were run for the samples, therefore gels with the least noise were chosen to analyze for DNA DSBs. If gels were considered equally as good an average was taken between the gels.

Additionally, a day 5 sample for one individual was missing for element analysis, so this individual was excluded from further statistical analyses including elements.

2.4.2 Analysis of variance

One way mixed effect ANOVAs (analysis of variance) were created with each variable as the dependent variable, the day of measurement (day 5 and day 25) as the independent variable and individual identity as the random factor. This allowed for detection of significant differences in each variable from day 5 to day 25 of incubation. The ANOVA models were supplemented with Tukey's honest significant difference (HSD) test as a post hoc test. Assumptions of the ANOVA models were checked by evaluating the residuals of the models through quantile-quantile (QQ) plots and Shapiro-Wilk's test for normality. If assumptions were not met, independent variables were log transformed. Hence As, Cd, Se and Zn were log transformed. However, they are presented in box plots and graphs without log transformation. If assumptions were still not met, as with K, Kruskal-Wallis's one-way analysis of variance test was used.

2.4.3 Correlation analyses

For correlation analyses among elements and body mass both Pearson's product moment correlation and Spearman's rank correlation were used. Variables were tested for normality using Shapiro-Wilk's test for normality. If the variables were found to not follow normality they were ln-transformed, as with As, Cd and Zn and if normality was still not found correlation analysis was done with Spearman's rank correlation, as with Ca, K and Pb. Bonferroni's correction for multiple variables was not used to avoid producing type two errors (Perneger, 1998; Moran, 2003)

2.4.4 Multivariate statistics

DNA-FTM instead of MML was chosen for multivariate statistics as both are a measure of DNA DSBs. DNA-FTM had lower mean coefficient of variation (CV) and is less influenced by individual interpretation compared to MML.

Principal component analysis

Principal component analysis (PCA) was carried out in order to visualize correlations among variables. Variables included DNA-FTM, body mass, As, Ca, Cd, Co, Cu, Fe, Hg, K, Pb, Se and Zn. The molar ratio between Hg and Se (HgSe) was added in as a supplementary variable and therefore did not contribute to the PCA. All variables were centered and scaled to unit variance prior to PCA.

Linear mixed effect modeling

Linear mixed effect models were used to investigate how non-essential elements and body mass related to DNA-FTM. Each eider's individual ID (identification) number was treated as a random effect. Only non-essential elements and not essential elements were chosen to include in the model because of their potential genotoxic effects at low concentrations (Davidson et al., 2014) and to avoid issues of multicollinearity and over fitting of the models (Zuur et al., 2009). In addition, although HgSe showed a positive relationship to DNA-FTM in the PCA, this variable was not included in the linear mixed effect models to avoid issues of colinearity with Hg. Furthermore, it is important to note that HgSe is merely a supplementary variable in the PCA and therefore does not contribute to the PCA. Cd and Pb were found to be correlated with body mass so an interaction term was included with body mass and Cd and body mass and Pb (body

mass:Cd and body mass:Pb). Some independent variables were present at much higher concentrations compared to others so they were scaled to be within 0 and 3 integers of each other. Body mass was presented in kg, As, Hg and Pb in mg/kg and Cd in µg/kg.

The full model therefore included DNA-FTM as the dependent variable and body mass, As, Cd, Hg, Pb, body mass:Cd and body mass:Pb as the independent/ explanatory variables. From this model the final models were chosen using backward selection with Akaike information criterion for small sample sizes (AICc). AICc takes into account the goodness of fit (R^2) of the models and the model complexity (number of parameters). The best model has a ΔAIC of 0 and all other models with a ΔAIC within a range of 0-2 are considered to substantially explain the model and should also be taken into account. Also, the lower the ΔAIC, the better the fit of the model (Burnham and Anderson, 2004).

R^2 for the top models were determined using R^2 conditional (R^2c) and R^2 marginal (R^2m). R^2c represents the variance explained by fixed factors only while R^2m explains the variance explained by both fixed and random factors. These R^2 values are considered to better fit linear mixed effect models compared to the traditional R^2 for linear regression (Nakagawa and Schielzeth, 2013).

To ensure that assumptions of the models were met, residuals were checked for normality using QQ plots and Shapiro-Wilk's test for normality. In addition, the dependent variable, DNA-FTM was checked for normality using the same procedures.

3 Results

3.1 Body mass

Body mass (BM) was significantly lower at the end of incubation (day 25) relative to the beginning (day 5) in the incubating female eiders (Tukey HSD p<0.001) as illustrated in Fig. 3.1. The mean body mass ± the standard deviation on day 5 was 2335.4 ± 165.4 g, and 1621.4 ± 154.1 g on day 25. This resulted in a mean percent decrease of approximately 31.2%. Body mass is presented in Table 3.1 and individual body masses are presented in Appendix B.1.

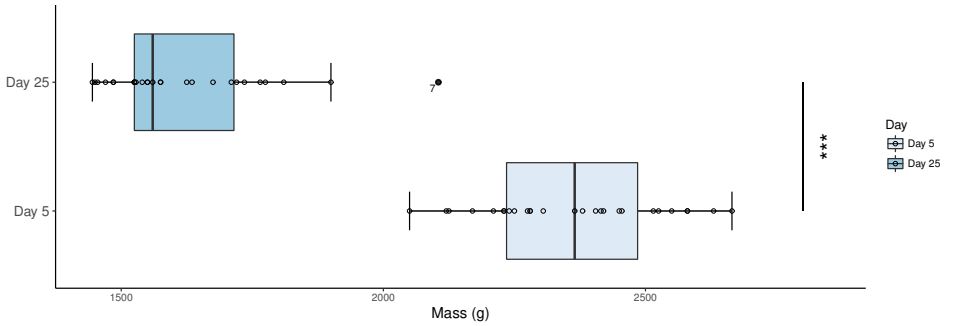


Figure 3.1: Box plot showing the body mass (g) from the same individual common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 (n=28) of incubation. The plot shows a median (thick vertical line) with the interquartile range (IQR, box), maximum and minimum values and potential outliers which were labelled with the individual's identification number (ID). The asterisks to the right of the boxes (***) denotes a p-value <0.001 from Tukey's HSD test and a significant decrease in body mass.

Table 3.1: Table showing body masses (g) of female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation (n=28). The table presents the mean, standard deviation (SD), median and range for body mass and the % difference ± SD. The asterisks (***) indicates a p-value < 0.001 (Tukey's HSD test).

	Mean ± SD	Median	Range	Δ Diff. ± SD
Day 5 (n=28)	2355.4 ± 165.4	2335	2050.0-2665.0	734.0 ± 183.3
Day 25 (n=28)	1621.4 ± 154.1	1567.5	1445.0-2105.0	
% Difference	-31.0 ± 8.6***	24.0%	16.6%-43.6%	

3.2 Elements

3.2.1 Non-essential elements

Non-essential elements that were significantly higher at the end of incubation compared to the start were Cd and Pb with a mean increase of 60% and 71% respectively (Tukey's HSD test p< 0.001 and 0.01 respectively) (See Fig.3.2 and Table 3.2). The mean concentration of As increased slightly by 16% from day 5 to day 25 of incubation, however, this difference was not significant (Tukey's HSD test). Hg increased slightly by 0.01% but this difference was not significant (Fig. 3.2 and Table 3.2). Noteworthy is one individual (individual 26) with Pb concentrations 9 and 7 times higher than the mean Pb concentrations on day 5 and day 25 respectively. This individual had Pb concentrations of 354.93 µg/kg ww on day 5 and 366.09 µg/kg ww on day 25 of incubation respectively (Fig.3.2 and Table 3.2). When this individual was taken out the difference between day 5 and day 25 was still significant (Tukey's test p<0.01) The highest to lowest concentrations of non-essential elements were as follows: Hg > Pb > As > Cd. Hg, the non-essential element with the highest concentration had mean concentrations of 175.40 µg/kg ww and 179.59 µg/kg ww and maximum concentrations of 295.74 µg/kg ww and 318.12 µg/kg ww on day 5 and day 25 respectively. See Appendix B.2 for raw data.

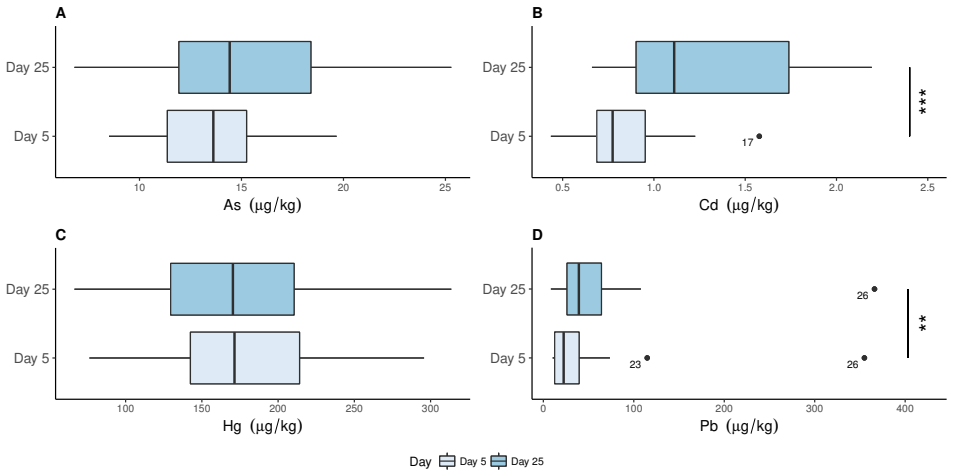


Figure 3.2: Box plots showing following non-essential elements: A) arsenic (As), B) cadmium (Cd), C) mercury (Hg) and D)lead (Pb), quantified in µg/kg ww in whole blood from the same individual common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 (n=28) and day 25 (n=27) of incubation. The plots show a median (thick vertical line) with the interquartile range (IQR, box), maximum and minimum values and potential outliers which were labelled with the individual's identification number (ID).The asterisks, *,**,**, represent a p-value of <0.05, <0.01 and <0.001 (Tukey's HSD test) respectively, denoting a significant difference in that element from day 5 to day 25 of incubation in the same individuals.

Table 3.2: Concentrations of non-essential elements (µg/kg ww) in whole blood from the same individual female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 (n=27) and day 25 (n=28) of incubation. The mean ± standard deviation (SD), median and range of non-essential elements and percent difference ± standard deviation (% Diff. ± SD) are presented. The asterisks (*,**,**) represent a p-value of <0.05, <0.01 and <0.001 (Tukey's HSD test) respectively, denoting a significant difference in that element from day 5 to day 25 of incubation in the same individuals.

	Day 5 of incubation (n=27)			Day 25 of incubation (n=28)			
	Mean ± SD	Median	Range	Mean±SD	Median	Range	% Diff. ± SD
As	13.62 ± 3.84	13.62	8.51-19.67	15.53 ± 4.68	14.45	6.81-25.29	15.6 ± 35.4
Cd***	0.82 ± 0.30	0.77	0.44-1.58	1.27 ± 0.46	1.09	0.66-2.19	60.3 ± 43.9
Hg	175.40 ± 63.72	171.40	76.24-295.74	179.59 ± 62.89	173.67	66.42-318.12	0.01 ± 15.0
Pb**	41.65 ± 66.29	22.47	10.290-354.93	55.65 ± 65.60	39.34	8.34-366.09	71.1 ± 86.4
Without ID. 26							
Pb**	29.60 ± 24.72	21.44	10.29-114.84	44.15 ± 25.00	39.27	8.34-107.88	73.4 ± 86.4%

3.2.2 Essential elements

Essential elements that were significantly lower at the end of incubation were Ca, Fe, Se, and Zn with a mean percent decrease of 41%, 6%, 20%, 24% (Tukey's HSD test $p<0.001$, 0.01, 0.001 and 0.001 respectively) (see Table 3.3 and Fig. 3.3). Cu was significantly higher at the end of incubation with a 9% mean percent increase (Tukey's HSD test $p<0.05$). Mean concentrations of Co were slightly higher at day 25, however, this difference was not significant (Tukey's HSD test $p<0.05$). Mean K concentrations were slightly lower on day 25 and this difference was statistically significant (see Fig. 3.2 and Table 3.3). See Appendix B.2 for raw data.

Table 3.3: Concentrations of essential elements (µmol/kg) in the same individual female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 (n=27) and day 25 (n=28) of incubation. The table presents the mean ± standard deviation (SD), median and range of essential elements. A mean percent difference ± standard deviation (% Diff. ± SD) is also presented. The asterisks (*,**,**) represent a p-value of <0.05, <0.01 and <0.001 (Tukey's HSD test) respectively, denoting a significant difference in that element from day 5 to day 25 of incubation in the same individuals.

	Day 5 of incubation (n=27)			Day 25 of incubation (n=28)			
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	% Diff. ± SD
Ca***	95697.45 ± 36703.89	85629.69	49496.52- 220548.56	50488.43 ±4575.59	51465.86	38932.69- 59655.92	-41.0 ± 17.7
Co	1.44 ± 0.55	1.40	0.56-2.47	1.55 ± 0.63	1.57	0.60-2.83	-3.5 ± 46.5
Cu*	336.66 ± 88.06	334.08	196.30-459.69	356.73 ± 49.28	350.91	270.01-443.30	8.5 ± 17.1
Fe**	440494.18 ± 95770.63	434255.49	328143.69-520644.49	407241.54 ± 46580.87	404013.68	335338.72- 488426.46	-6.3 ± 11.7
K	2282404.11 ± 553741.21	2197450.21	1587858.45- 3156427.00	2156514.39 ± 457569.31	2061199.98	1650041.94-3352737.16	-4.1 ± 20.7
Se***	3868.68 ± 1379.23	3945.43	1718.38-6447.68	2991.64 ± 982.25	2975.49	1649.52- 6011.90	-20.0 ± 14.9
Zn***	7019.85 ± 1914.75	6598.15	5076.84-10526.32	5151.38 ± 812.76	5120.75	3419.70-6841.50	-24.0 ± 16.6

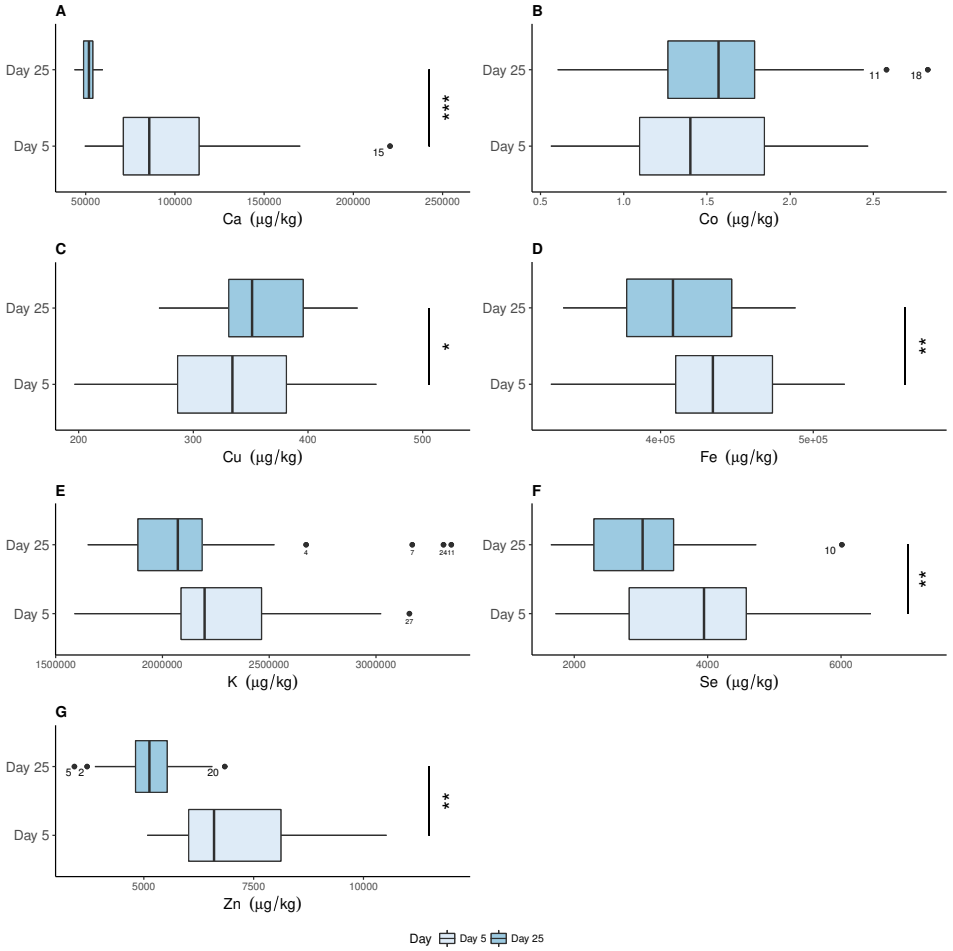


Figure 3.3: Box plots showing the following essential elements: A) calcium (Ca), B) cobalt (Co), C) copper (Cu), D) iron (Fe), E) potassium (K), F) selenium (Se) and G) zinc (Zn) quantified in µg/kg ww in whole blood from the same individual common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 (n=27) and day 25 (n=28) of incubation. Elements are presented in µg/kg ww. The plots show a median (thick vertical line) with the interquartile range (IQR, box), maximum and minimum values and potential outliers which were labelled with the individual's identification number (ID). The asterisks (*,**,**) represent a p-value of <0.05, <0.01 and <0.001 (Tukey's HSD test) respectively, denoting a significant difference in that element from day 5 to day 25 of incubation in the same individuals.

3.3 Relationships between elements and body mass

3.3.1 Non-essential elements and body mass

Cd and body mass were significantly correlated with a negative relationship (r_s= -0.55, p<0.001). Pb and body mass were negatively and significantly correlated (r_s=-0.38, p<0.01). As and Hg were not significantly correlated to mass. When individual 26 with high concentrations of Pb was removed the correlation decreased slightly but remained significant (p= 0.0042 with individual 26 and p=0.013, r_s=-0.34 without individual 26). Plots in Fig. 3.4 show these relationships and that Cd and Pb generally increased in whole blood of the incubating eiders as mass decreased from day 5 to day 25. See Appendix B.3 for p-values.

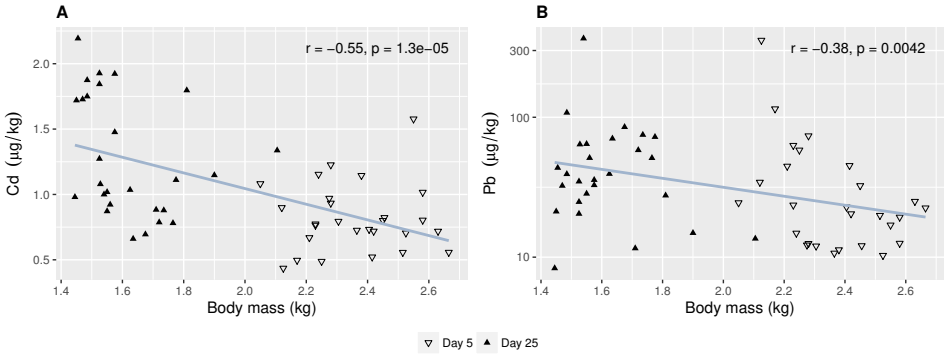


Figure 3.4: Plots showing the significant correlations between body mass (kg) and A) cadmium (Cd) and B) lead (Pb) presented in µg/kg ww. Open triangles represent incubating female common eiders (*Somateria mollissima*) on day 5 and closed triangles represent the same individuals on day 25 (n=27) of incubation. Spearman correlation coefficients (r) and p-values (p) are displayed on each graph. Linear regression lines are displayed for presentation purposes only. Note the y-axis on plot B is presented on a log scale.

3.3.2 Essential elements and body mass

The essential element Ca, was positively and significantly correlated with body mass (r_s=0.79 p<0.001). Zn was positively and significantly correlated to body mass (r_s= 0.63 p<0.001). Fe and K were positively and significantly correlated with body mass (r_s= 0.36 p<0.01 and r_s= 0.34 p<0.05 respectively). The other essential elements, Co, Cu, K and Se were not significantly correlated to body mass. Plots in Fig. 3.5 show these relationships and that Ca, Cd, Fe, Se and Zn generally decreased in whole blood of the incubating eiders as mass decreased from day 5 to day 25. See Appendix B.3 for p-values.

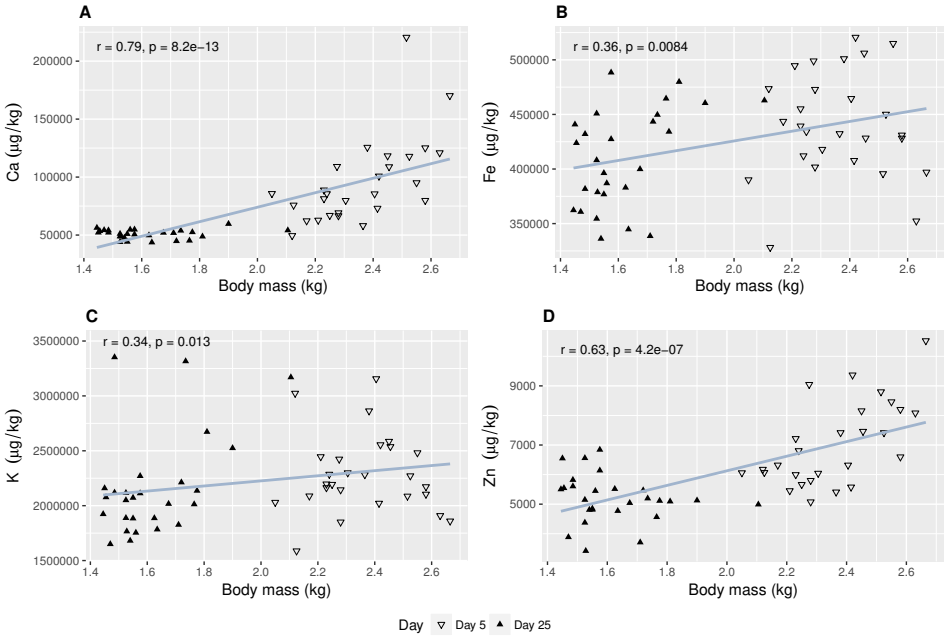


Figure 3.5: Plot showing the significant correlations between body mass (kg) and A) calcium (Ca), B) iron(Fe), C) potassium (K), and D) zinc (Zn) presented in µg/kg ww. Open triangles represent incubating female common eiders (*Somateria mollissima*) on day 5 and closed triangles represent the same individuals on day 25 of incubation (n=27). Spearman correlation coefficients (r) and p-values (p) are displayed on each graph. Linear regression lines are displayed for presentation purposes only.

3.4 Relationships between non-essential and essential elements

Many correlations existed between non-essential and essential elements. Only correlations considered especially toxicologically relevant are focused on in the present study. For a summary of all significant correlations see Appendix B.3.

Cd-Ca were negatively and significantly correlated (r_s=-0.37 p<0.01). Hg and Se were positively and significantly correlated (r=0.37, p<0.01). In addition, the molar ratio between Hg and Se (HgSe) increased significantly from a mean of 0.019 (0.9:49(µmol/kg)) to 0.023 (0.9:37.9) (µmol/kg)) from day 5 to day 25 (Tukey's HSD test p<0.01) (see Fig. 3.7 and Table 3.4. All HgSe values for each individual are presented in Appendix B.2).

For correlations between Pb and essential elements, Pb-Ca, Pb-K and Pb-Zn were negatively and significantly correlated (r_s= -0.47, -0.36 and -0.42, p< 0.001, 0.01 and 0.01 respectively). Pb-Ca and Pb-Zn were negatively and significantly correlated (r_s= -0.47, -0.42 and p<0.001, 0.01, respectively).

Noteworthy, was the negative relationship between Pb and Fe with a p-value of 0.068 (r_s= -0.25). However, when individual 26, with high Pb concentrations, was taken out the p-value was no longer nearly significant at (r_s= -0.16 p= 0.26). Correlations between Pb-Ca and Pb-Zn remained at similar significance levels with individual 26 removed (r_s= -0.47 and -0.42, p<0.001 and p<0.01 respectively). See Appendix B.3 for correlation summaries.

Table 3.4: The molar ratio between mercury (Hg) and selenium (Se) (HgSe) quantified in whole blood of the same individual incubating female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 (n=27) and day 25 (n=28) of incubation. Both the calculated molar ratio and the molar ratio in Hg (µmol/kg) : Se (µmol/kg). The mean ± the standard deviation (SD), median and range and a % difference (% Diff.) is presented. The asterisks denotes a p-value < 0.01 (Tukey's HSD test).

	Mean ± SD	Median	Range
Day 5			
HgSe (molar ratio)	0.018 ± 0.0069	0.019	0.0094 - 0.030
Hg (µmol/kg) : Se (µmol/kg)	0.87:49.00 ± 0.32:17.47	0.85:49.97	0.38:21.76 - 1.47:81.66
Day 25			
HgSe (molar ratio)	0.023 ± 0.0081	0.024	0.010 - 0.039
Hg (µmol/kg) : Se (µmol/kg)	0.85:37.89 ± 0.30:12.44	0.84:37.68	0.32:30.89 - 1.56:76.14
% Diff. ± SD	26.2% ± 13.3		

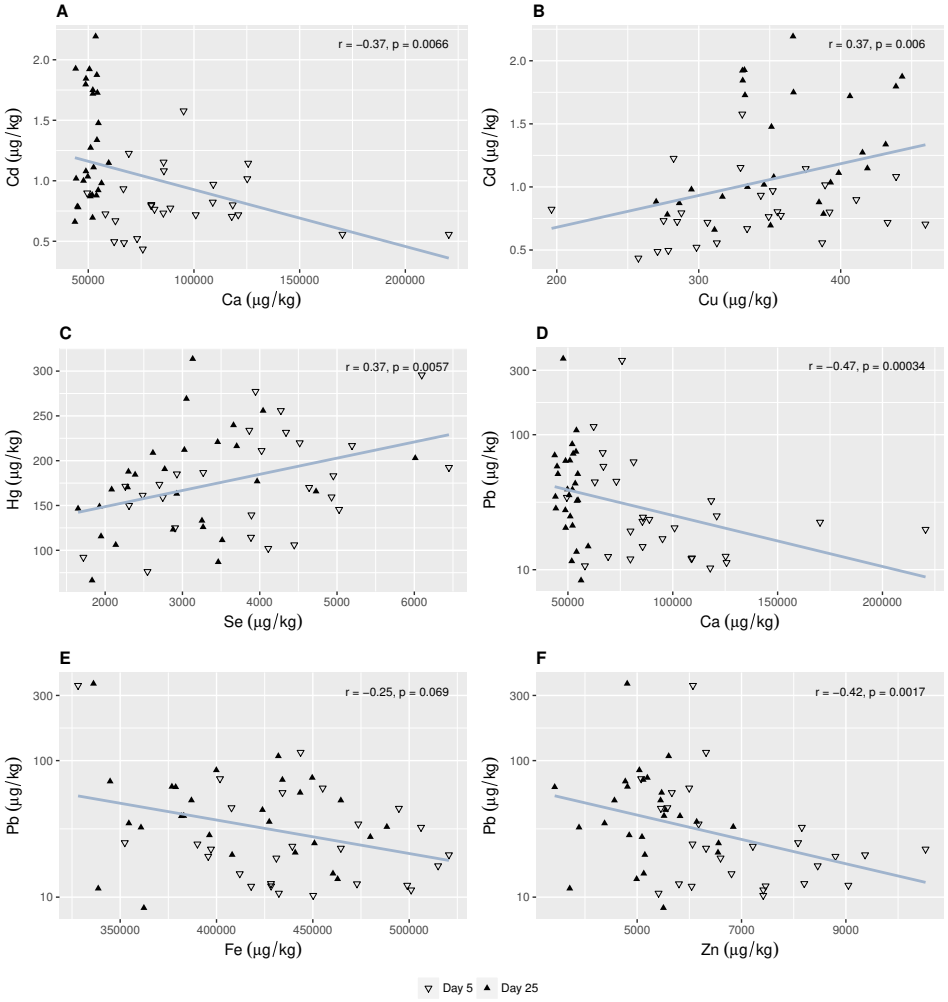


Figure 3.6: Plots showing the significant correlations between non-essential and essential elements (µg/kg ww) between the following elements: A) calcium (Ca) and cadmium (Cd), B) selenium (Se) and mercury (Hg), C) Ca and lead (Pb), D) iron (Fe) and Pb, E) zinc (Zn) and Pb. Open triangles represent incubating female common eiders (*Somateria mollissima*) on day 5 and closed triangles represent the same individuals on day 25 (n=27) of incubation. Pearson (B, C) and Spearman (A, D-F) correlation coefficients (r) and p-values (p) are displayed on each graph. Linear regression lines are displayed for presentation purposes only. Note the y-axis on plots D-F are presented on a log scales.

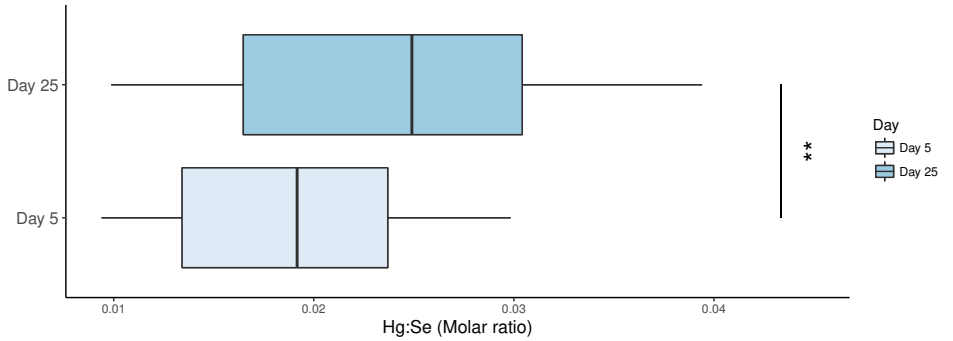


Figure 3.7: Box plot showing the molar ratio between Hg (Mercury) and Se (selenium) (HgSe) from the same individual female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 (n=27) and day 25 (n=28) of incubation. The plot shows a median (thick vertical line) with the interquartile range (IQR, box), maximum and minimum values. There was a significant decrease in mass from day 5 to day 25 of incubation. The asterisks to the right of the boxes (**) denotes a p-value<0.01 from Tukey's HSD test

3.5 DNA double strand breaks

DNA-FTM was significantly higher at the end of incubation (day 25) relative to the beginning of incubation (day 5) (Tukey HSD p<0.001). The mean DNA-FTM on day 5 was 63.4% and the mean on day 25 was 79.1%. The range of DNA-FTM was between 44.8 - 85.5% on day 5 and 68.1-91.9% on day 25. The mean increase in DNA-FTM for each individual was 27.4% (see Fig. 3.8 and Table 3.5). The standard deviation between individuals for DNA-FTM on day 5 and 25 was 10.6 and 7.9 respectively. The mean CV for all lanes ran for each individual on day 5 was 12.1% and 8.3% on day 25.

MML was significantly lower on day 25 relative to day 5 (Tukey HSD p<0.001). The mean MML on day 5 was 311.6 kbp and 239.4 kbp on day 25. The mean decrease in MML for each individual was 21.4% (see Fig. 3.9 and Table 3.5). The standard deviation between individuals for MML on day 5 and day 25 was 46.5 and 48.6 respectively, with a CV of 14.9% and 20.3%. The mean CV for all lanes ran for each individual was 4.6% and 5.4%.

The CV between gels that were run more than once were between 0.5-18% for DNA-FTM and 0.7%-32% for MML. See Appendix B.5 for raw data and Appendix B.6 for CVs and standard deviations between gels, triplicates and parallels.

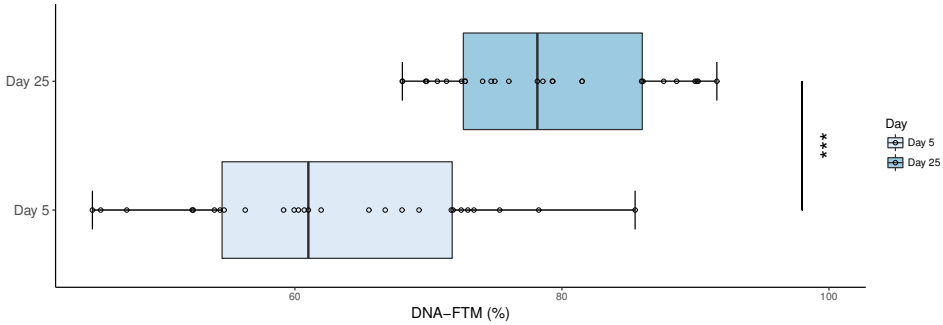


Figure 3.8: Box plot showing DNA-fraction of total DNA that migrated into the gel (DNA-FTM (%)) measured in whole blood of the same individual common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation (n=28). The plot shows a median (thick vertical line) with the interquartile range (IQR, box), maximum and minimum values. There was a significant increase in DNA-FTM (%) from day 5 to day 25 of incubation. The asterisks to the right of the boxes (***) denotes a p-value<0.001 from Tukey's HSD test.

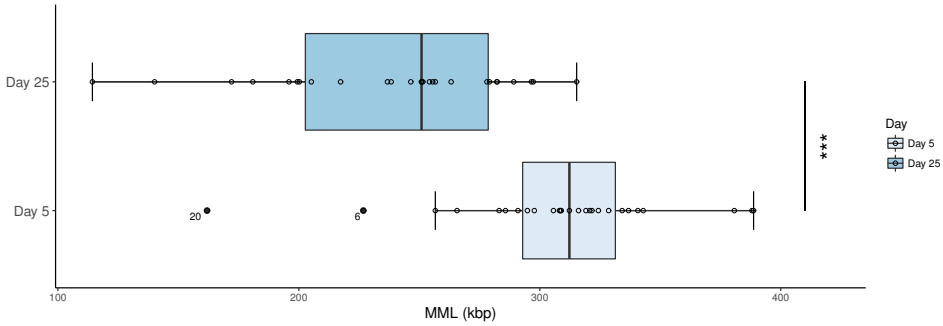


Figure 3.9: Box plot showing Median molecular length (MML), in kilobase pairs (kbp) measured in whole blood from the same individual common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation (n=28). The plot shows a median (thick vertical line) with the interquartile range (IQR, box), maximum and minimum values and potential outliers which were labelled with the individual's identification number (ID). There was a significant decrease in MML from day 5 to day 25 of incubation. The asterisks to the right of the boxes (***) denotes a p-value<0.001 from Tukey's HSD test

Table 3.5: DNA-fraction of total DNA that migrated into the gel (DNA-FTM (%)) and median molecular length (MML) in whole blood from the same individual common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation (n=28). The table presents the mean, standard deviation (SD), median and range. A % difference ± SD was also calculated.

	Day 5 of incubation (n=28)			Day 25 of incubation (n=28)			
	Mean ± SD	Median	Range	Mean	Median	Range	% difference±SD
DNA-FTM (%)***	63.4 ± 10.6	61.5	44.8-85.5	79.1 ± 7.9	78.4	68.1-91.9	27.4 ± 18.1
MML***	311.6 ± 46.5	310.6	161.9-388.7	239.4 ± 48.6	250.9	144.3-315.3	-21.4 ± 16.8

3.6 Relationships DNA DSBs, BM and non-essential elements

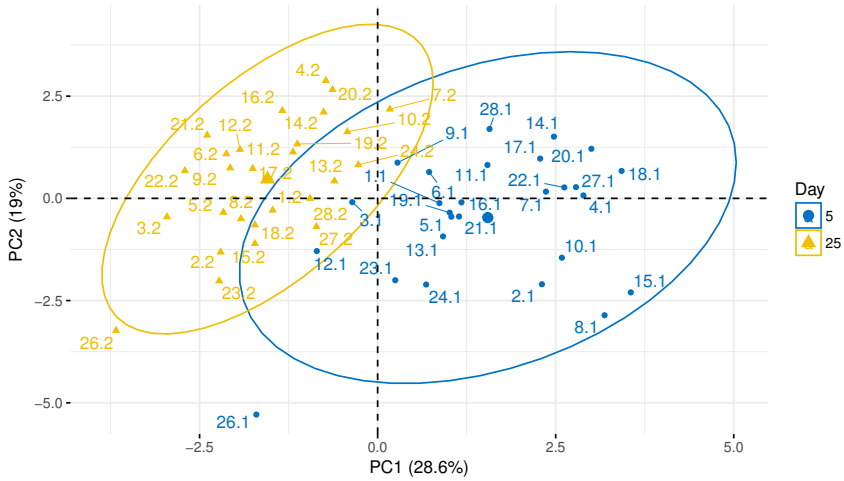
Individual 26 with high Pb concentrations was kept in the data set for further statistical analyses as these high concentrations may be as a result of natural variation and therefore these high Pb concentrations were considered not to be true outliers.

3.6.1 PCA

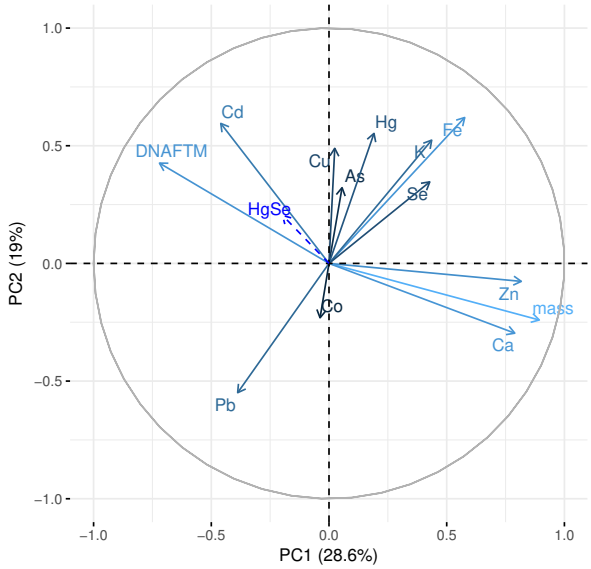
The Principal component analysis was performed for initial visualization of possible relationships between elements, body mass and DNA-FTM. The PCA (Fig. 3.10) includes the essential elements, Ca, Co, Cu, Fe, K, Se and Zn, non-essential elements, As, Cd, Hg and Pb, body mass and DNA-FTM. The molar ratio between Hg and Se was added as a supplementary variable and did therefore not contribute to the plot. The PCA resulted in two principal components accounting for 47.6% of the variation. The score plot (Fig. 3.10a) shows a clear difference between the same individuals on day 5 (blue) and day 25 (yellow).

In the loading plot (Fig. 3.10b) it is clear that the essential elements Fe, K and Se are clustered together along with the non-essential elements As and Hg and the essential element Cu, which are clustered slightly closer to PC1. This indicated a positive correlation between these variables. As shown earlier through correlation analysis, Se and Hg were positively and significantly correlated. Opposite this cluster was Pb. This indicates a negative relationship between the essential elements Fe, Se and Pb which was confirmed to be significant through correlation analysis (see above section 3.4).

In addition, it is clear that Ca and Zn are grouped close to body mass along PC1 indicating positive correlations. Opposite this cluster is a cluster containing DNA-FTM, Cd, and the molar ratio between Hg and Se indicating a positive correlation among



(a) Principal component analysis score plot



(b) Principal component analysis loading plot

Figure 3.10: Principal component analysis score plot (a) and loading plot (b) of DNA-FTM, mass, As, Ca, Cd, Co, Cu, Fe, Hg, K, Pb, Se, and Zn measured in whole blood female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation (n=27). PC1=28.6%, PC2=19%. The score plot (a) shows the relationships between the individuals sampled (labelled with their identification numbers). Each individual is represented twice both on day 5 (blue closed circles) and day 25 (yellow triangles) of incubation. The loading plot (b) shows the relationship between the variables (represented in blue). The blue dashed line represents the molar ratio between Hg and Se (HgSe) and was added as a supplementary variable.

these variables and negative correlations to Ca, Zn and body mass. Correlations between body mass and Ca, Cd and Zn were confirmed to be significant through correlation analysis above. Co is positioned close to the origin and as such did not correlate significantly with any other elements or body mass.

3.6.2 Linear mixed effect models

As mentioned above (Section 2.4), in the full mixed effect model body mass and all non-essential elements measured (As, Cd, Hg, Pb) were included as independent variables (fixed effects). DNA-FTM was included as the dependent variable and the each bird's identification number as the random effect. In addition, based on the PCA and correlation analyses, Pb and Cd were both shown to have negative, significant correlations to body mass and were therefore included independently and as interaction terms with body mass in the linear mixed effect model.

All models are shown in Table 3.6. When comparing the models using ΔAICc, three models had ΔAICc values between 0-2 and were therefore considered to explain the data equally as well. The top model explaining the DNA-FTM with a ΔAICc score of 0 was body mass and Hg followed by the second best model which only included body mass, with a ΔAICc score of 0.98. The third best model included body mass, Hg and body mass in interaction with Cd (body mass : Cd) with a ΔAICc score of 1.30.

Table 3.6: Model selection table of linear mixed effect models including all the models predicting blood levels of DNA-FTM (%) in relation to body mass (mass) (kg) and non-essential elements (As, Hg and Pb in mg/kg and Cd in µg/kg) in female eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation (n=27). Individual was treated as a random factor. Mass here is body mass. Models were ranked based on Akaike's Information Criterion (AICc). K is the number of parameters in the model. ΔAICc shows the difference between each model in relation to the best model (model 1). AICc weight (AICc wt) indicates the probability that the model is best for the observed data.

Models	Model ID	K	AICc	ΔAICc	AICcWT
DNA-FTM~ Mass + Hg	1	5	380.76	0.00	0.42
DNA-FTM~ Mass	2	4	381.74	0.98	0.26
DNA-FTM~ Mass + Hg + Mass:Cd	3	6	382.07	1.30	0.22
DNA-FTM~ Mass + Hg + Mass:Cd + Mass:Pb	4	7	384.64	3.88	0.06
DNA-FTM~ Mass + Hg + Pb + Mass:Cd + Mass:Pb	5	8	386.50	5.74	0.02
DNA-FTM~ Mass + Cd + Hg + Pb + Mass:Cd + Mass:Pb	6	9	388.71	7.95	0.01
DNA-FTM~ Mass + As + Cd + Hg + Pb + Mass:Cd + Mass:Pb	7	10	391.30	10.54	0.00

The top three models summaries are presented in Table 3.7. Body mass was the only significant variable explaining DNA-FTM in all three models ($p<0.001$). However,

Hg was nearly significant in both models one and three (t=1.87, p= 0.074 and t=1.91, p=0.069 respectively). The interaction term between body mass and Cd was not significant in the third model. The negative coefficient value of body mass in all three models shows that as body mass decreases DNA-FTM increases. While the positive coefficient value of Hg indicates increasing DNA-FTM values with increasing Hg concentrations.

The R^2m (marginal R^2) shows the proportion variability explained by the models (the goodness of fit) with fixed effects only (non-essential elements and body mass). While the R^2c shows the proportion of variability explained by both fixed effects and random effects. The goodness of fit of all the models increased when accounting for both the fixed effects and the random effects (individual variation).

Table 3.7: The top three model summaries based on AICc predicting DNA-FTM (%) based on body mass (kg) and non-essential elements (Cd: µg/kg and Hg: mg/kg) in female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation (n=27). Individual was treated as a random factor. The summary table includes the coefficients or response variables and coefficient values (coeff. value), the standard error (std.error), t-values and the significance levels (p-values). The value of the intercept represents the y-axis intercept and the sum of the coefficient values represents the slope. The significance level was set to p= 0.05. The asterisks (***) represents a significance level of p<0.001. P-values with a significance level of <0.1 are underlined. R^2m and R^2c represent the marginal and conditional R squares, respectively.

DNA-FTM							
Model ID	Coefficients	Coeff. value	Std.error	t-value	p-value	R^2m	R^2c
1	Intercept	105.27	5.88	17.91	0.000***	0.54	0.78
	Mass	−21.21	2.02	−10.54	0.000***		
	Hg	43.70	23.41	1.87	0.074		
2	Intercept	112.89	4.21	26.84	0.000***	0.50	0.79
	Mass	−21.19	1.98	−10.69	0.000		
3	Intercept	100.89	6.93	14.55	0.000***	0.55	0.75
	Mass	−20.98	2.11	−9.95	0.000***		
	Hg	43.24	22.69	1.91	0.069		
	Mass:Cd	2.01	1.77	1.13	0.268		

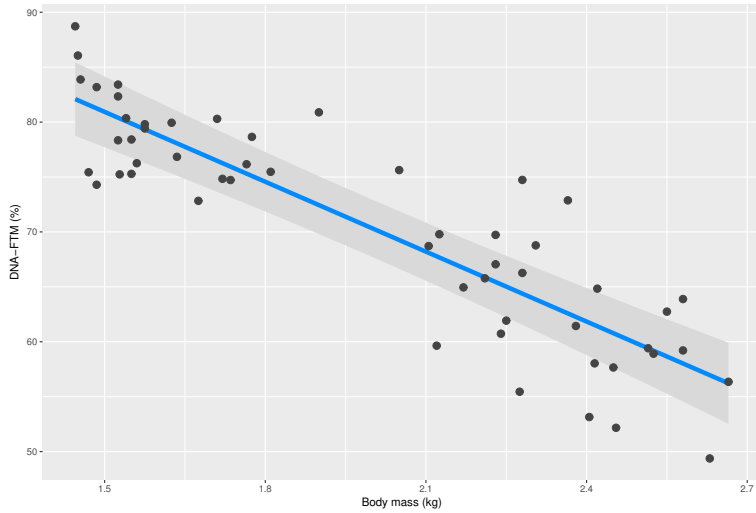
Fig. 3.11 shows part residual plots of the top model from the linear mixed effect models (see Table 3.6 and Table 3.7). The plots show the effect of each variable (Hg or body mass) after the other variable and the random effect (individual) had been controlled for. Fig. 3.11a shows a clear increase in DNA-FTM with decreasing body mass. Also noteworthy is the clustering of individuals on the plot. One cluster contains individuals with a DNA-FTM of more than 70% and a body mass generally less than 1.8 kg. The second cluster contains individuals with generally a DNA-FTM less than 70% and a body mass of more than 2.1kg. Fig. 3.11b shows an increasing DNA-FTM percentage

with increasing concentrations of Hg after the fixed effect mass and the random effect individual has been controlled for. Notice how the data points in Fig. 3.11b are more spread out than the data points in Fig. 3.11a. This is reflected in their p-values (Table 3.7) where mass has a significant p-value and Hg has a nearly significant p-value.

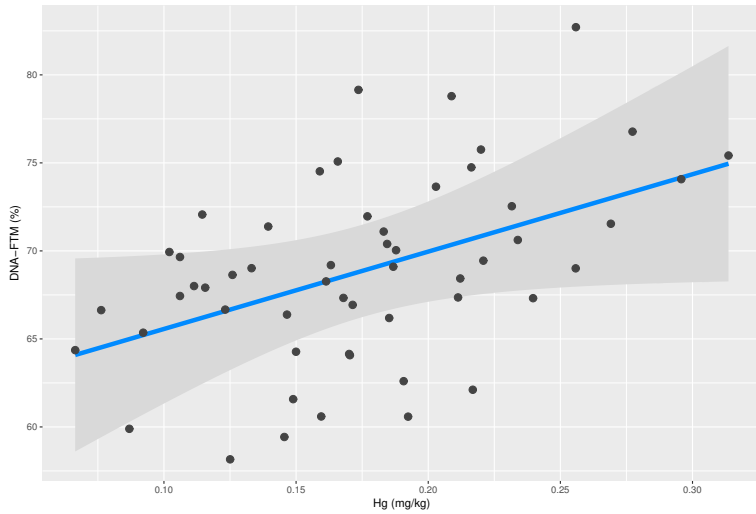
Hg and body mass were included in the best models despite Hg concentrations not changing throughout incubation. It was therefore decided to further investigate the relationship between Hg and body mass to see how the change in body mass affected the change in Hg levels. Upon testing this relationship the two were found to be nearly significantly correlated (p= 0.054 r=-0.38) (Fig. 3.12). The greater the change in body mass, the greater the increase in Hg. However, this relationship was not significant and did not apply to all individuals, as some individuals lower Hg levels on day 25 compared to day 5 of incubation, therefore an interaction term in the linear mixed effect model was not included.

Fig. 3.13 shows the conditional plots of the residuals of the first model (Table 3.6 and Table 3.7) with Hg and body mass explaining DNA-FTM (%). The plot (Fig. 3.13) is a partial residual plot showing how body mass may affect Hg when the random effect (individual) has been controlled for. 3.13 is split up into cross sections between the 10th, 50th and 90th percentiles of the model (*Somateria mollissima*). The graphs show that for some individuals at lower body masses, Hg increases and DNA-FTM (%) decreases.

Fig. 3.14 also shows conditional plots of the residuals of the third best model (Table 3.6 and Table 3.7) with Hg, body mass and the interaction between body mass and Cd as variables explaining DNA-FTM. Fig. 3.14 shows how body mass affects the relationship between DNA-FTM and Cd after the other fixed effects and the random effect in the model was controlled for. The plots in Fig. 3.14 are also split up into cross sections between the 10th, 50th and 90th percentiles of the model. As body mass decreases, Cd concentrations increase, and DNA-FTM increases. Note, in the first panel individuals (gray points) are generally clustered around low Cd concentrations, however, as body mass decreases, more and more are clustered around higher Cd concentrations.



(a)



(b)

Figure 3.11: Partial residual plots from the top model with body mass (kg) and Mercury (Hg) (mg/kg) explaining DNA-FTM (%) measured in female common eiders (*Somateria mollissima*) on day 5 and day 25 of incubation. Plots show the effect of each variable after the other fixed effect and the random effect have been controlled for. Plot a) shows DNA-FTM (%) plotted against body mass (kg). Plot b) shows DNA-FTM (%) plotted against Hg (µg/kg ww). The dark gray dots are partial residuals, the blue line is a linear regression through these points and the light grey area around the regression line represents a 95% confidence interval.

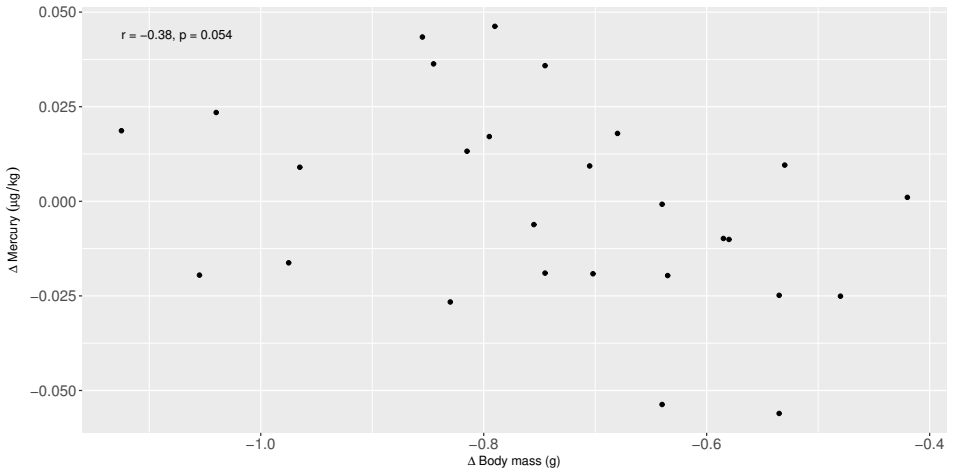


Figure 3.12: Plot showing the relationship between the difference (Δ) in body mass (kg) and mercury (Hg) (µg/kg ww) in whole blood of female common eiders (*Somateria mollissima*) in Christiansø, Denmark from day 5 to day 25 of incubation. The difference was calculated by subtracting day 25 values by day 5 for each individual. Each point represents one individual (n=27). Pearson's correlation coefficient (r) and the p-value is displayed on the graph.

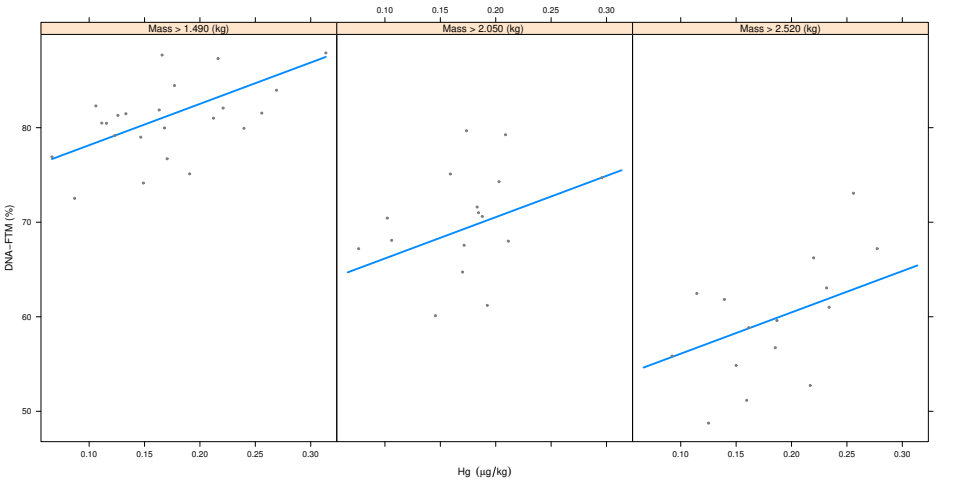


Figure 3.13: Partial residuals plots from the third best model explaining DNA-FTM (%) measured in female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation. The plots illustrate how body mass (mass (kg)) affects the relationship between DNA-FTM (%) and mercury (Hg µg/kg ww) after other fixed effects and the random effect have been controlled for. Plots are cross sectioned into 10th (body mass (mass) > 1.490 kg), 50th (mass > 2.050 kg) and 90th (mass > 2.520 kg) percentiles of the model. The dark gray points are partial residuals, the blue line represents the linear regression line.

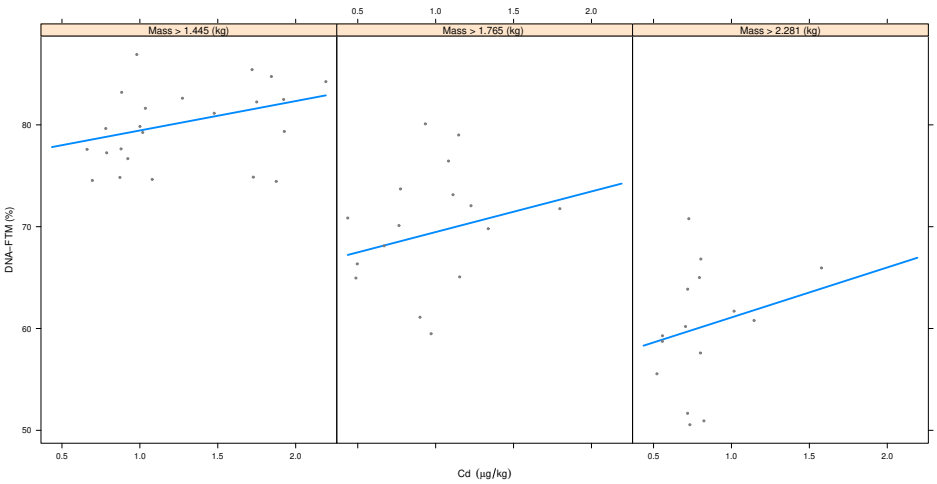


Figure 3.14: Partial residuals plots from the third best model explaining DNA-FTM (%) measured in female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation. The plots illustrate how body mass (mass (kg)) affects the relationship between DNA-FTM (%) and Cadmium (Cd µg/kg ww) after other fixed effects and the random effect have been controlled for. Plots are cross sectioned into 10th (body mass (mass) > 1.445 kg), 50th (mass > 1.765 kg) and 90th (mass > 2.281 kg) percentiles of the model. The dark gray points are partial residuals, the blue line represents the linear regression line.

4 Discussion

The present study investigated how body mass and levels of non-essential and essential elements changed in relation to each other from day 5 to day 25 of incubation in a female Baltic Sea eider population. In addition, the present study investigated how non-essential elements and body mass affected DNA DSBs (measured as DNA-FTM) on day 5 and day 25 of incubation. This was a unique study because each incubating eider was sampled twice and therefore acted as its own control.

4.1 Body mass

One of the main goals of assessing body mass in female eiders at day 5 and day 25 of incubation was to see how body mass changed over the incubation period and to give insight into the health of the Christiansø, Denmark eider population. Due to the poor health of the Baltic / Wadden Sea flyway population (Desholm et al., 2003; Christensen, 2008; HELCOM, 2013a), body mass was expected to greatly decrease in the present study. The current findings confirm these hypotheses as the body mass drastically decreased (by 17 - 44 %) from the beginning to the end of incubation. This finding is in agreement with another study on Danish eiders reporting a body mass loss ranging between 31 - 46% in 1996 (Bolduc and Guillemette, 2003). However, Bolduc and Guillemette (2003) measured initial body mass on day 0 of incubation, before eggs had been laid while the present study measured at day 5, after eggs had been laid. So the body mass loss reported in the present study may be considered greater. In addition, the health of the Baltic Sea eider population has decreased since 1996 (Desholm et al., 2003; Ekroos et al., 2012). In the Northern Baltic Sea Fenstad et al. (2016b) reported a mean body mass (1474g ± 101 g) 9% lower than the eiders in the present study (1621 ± 154 g) at the end of incubation. However, body mass at the beginning of incubation

was not reported by Fenstad et al. (2016b), making it difficult to compare to the present study.

Reports of body mass loss in incubating eiders from Norway show a mean body mass loss ranging between 18 - 23% (Bustnes et al., 2012). While reports in Svalbard show a mean body mass loss between 19 - 21% (Fenstad et al., 2014). Incubating eiders in Svalbard have been known to be in worse conditions at the end of incubation due to harsh climates (Neggazi et al., 2016). Despite this, eiders in the present study lost far more of their initial body mass. However, it is also important to consider the initial body masses of the eiders when investigating total body mass loss. Bustnes et al. (2012); Fenstad et al. (2014) also reported initial body masses 24% lower than initial body masses in the present study. Furthermore, Svalbard eiders seem reach lower body masses with masses 12% lower than those reported in the present study (Bustnes et al., 2012; Fenstad et al., 2014). Therefore, Svalbard eiders may loose less body mass throughout incubation because they start incubation with less body mass compared to Baltic Sea eiders.

In the mass mortality events of 2007 and 2015 in Christiansø, Denmark, females found dead had mean body masses of 1145 g and 1040 g, respectively (Garbus, 2016). The critical level by which death is inevitable is 1100 g in eiders (Korschgen, 1977). None of the body masses reported in the present study in Christiansø were close to these values (see Appendix B.1).

Regardless, the overall body mass loss reported in the present study is considered high in waterfowl (Afton and Paulus, 1992; Bolduc and Guillemette, 2003). Additionally, Baltic Sea eiders seem to loose far more body mass than their counterparts in Northern Norway and in Svalbard. This may be due to a number of confounding factors as the Baltic/Wadden Sea flyway population has been plagued by anthropogenic pollutants (Sonne et al., 2012; Fenstad et al., 2016b, 2017; HELCOM, 2017b), diseases (Pedersen et al., 2003), parasites (Thieltges et al., 2006), and changes in the quality and quantity of mussel stocks (Camphuysen et al., 2002; Laursen and Møller, 2014). The overall health of this population therefore warrants further investigation.

4.2 Non-essential elements

One of the main goals of assessing concentrations of non-essential elements in blood of incubating eiders was to determine if exposure to these contaminants has decreased in the past two decades and to see how exposure to these contaminants differs on a spatial scale in the Baltic Sea. Furthermore, non-essential elements were assessed in

blood at both day 5 and day 25 of incubation to see how their concentrations changed throughout incubation and to see how these concentrations related to levels of essential elements. Non-essential elements were expected to increase in concentration throughout incubation and they were expected to be negatively correlated to body mass due to possible mobilization from soft tissues and/or due to their increased absorption in eiders feeding during incubation.

Although no recent data exists of non-essential elements in blood at the beginning of incubation in eiders, levels at the end of incubation are comparable to Fenstad et al. (2017) who took samples 6 years previously (in 2011) in the Finnish archipelago (the Northern Baltic Sea).

4.2.1 Arsenic

As concentrations in blood of incubating eiders in the present study were expected to be higher compared to blood samples taken by Fenstad et al. (2017) in incubating eiders in the Northern Baltic Sea because of the close proximity of Christiansø to chemical war fare dumping sites (Emelyanov et al., 2010). However, As concentrations in the present study (day 5: 13.6 µg/kg ww and day 25: 15.5 µg/kg ww) were similar to those reported by Fenstad et al. (2017) (16.24 ± 5.82 µg/kg ww) in the Northern Baltic Sea. This indicates the Southern Baltic Sea eider population is not more exposed to As compared to the Northern Baltic Sea eiders. Researchers have found As sediment concentrations decrease drastically with increasing distances from dumping sites, which are at very low depths (Emelyanov et al., 2010; HELCOM, 2013b). Thus, marine birds, like eiders, feeding closer to islands and at shallower areas may not be at a high risk of exposure.

Comparing blood As concentrations in the present study to other previous studies in Baltic Sea eiders becomes difficult as few of these studies exist. One study conducted 20 years ago, did not detect As in blood of any nesting eiders (Franson et al., 2000b). This may be due to advances in detection methods in recent years. In addition, main focuses in the past seems to have been on heavy metals such as Hg, Cd, and Pb (Wayland et al., 2001; Franson et al., 2000a; Franson and Pain, 2011). Hence, it is difficult to conclude how As exposure in Baltic Sea eiders has changed over time.

In the present study, As concentrations were expected to significantly increase between sampling days and be correlated to decreasing body masses. As concentrations did increase and were found to be positively correlated to body mass, however neither of these findings were significant. Fenstad et al. (2017) also reported that As concentrations were not significantly correlated with body mass in Baltic Sea (Finnish) eiders.

This indicates As may have not been mobilized from soft tissue during incubation. The possible mobilization of As into blood during incubation will greatly depend on its accumulation in certain tissues. Evidence suggests that lower trophic feeding birds, may accumulate As in muscle (Kunito et al., 2008). It is possible the eiders in the present study did not reach the protein catabolism phase of fasting and therefore did not mobilize As from muscle. Eiders have been shown to utilize mostly lipids throughout incubation (Parker and Holm, 1990). However, because the eiders in the present study loss so much body mass it is likely they entered into the protein catabolism phase of fasting.

Eiders were also expected to have increasing As concentrations because of increased feeding towards the end of incubation, as benthic feeding species tend to accumulate high concentrations of As (Neff, 1997; Rahman et al., 2012). The primary form of As is arsenobetaine in marine species (Neff, 1997). This form is thought to have a short half-life in blood compared to other non-essential elements (Neff, 1997). It is possible that when and if As had been ingested by the eiders it was quickly excreted in the present study. Evidence also suggests arsenobetaine is the least toxic form of As and thus does not present a risk to most marine species (Neff, 1997). Therefore, the toxicokinetics of As in fasting or incubating seabirds and the relevance of As as a hazardous marine pollutant warrants further research.

4.2.2 Cadmium

Cd concentrations in blood of incubating eiders in the present study were expected to be similar to blood concentrations reported by Fenstad et al. (2017) in incubating eiders in Northern Baltic Sea eiders as Cd concentrations show increasing trends in biota in the Baltic Sea (HELCOM, 2017b). However, Cd concentrations were found to be 65% and 47% lower on day 5 (0.8 ± 0.3 µg/kg ww) and day 25 (1.3 ± 0.5 µg/kg ww) of incubation compared to values reported by Fenstad et al. (2017) (2.4 ± 0.7 µg/kg ww). Contrary to this finding, Cd levels have been shown to be lower in sediment and in biota in the Finnish archipelago compared to Bornholm Basin (where Christiansø is located) (HELCOM, 2017b). The bioavailablility of Cd may be higher in the more brackish water of the Northern Baltic Sea (Dutton and Fisher, 2011). These blood levels are considered low. The threshold level for adverse effects in blood of ducks has been reported to be 20 and 11 times greater than the level reported here and by Fenstad et al. (2017) (<26 µg/dl (260 µg/kg ww) in mallard ducks) (Wayland and Scheuhammer, 2011)). In addition, blood may contain very low levels of Cd, as Cd tends to accumulate mainly in the liver and kidneys of seabirds (Scheuhammer, 1987).

Cd blood levels in the present study were found to significantly increase (by 60%), and were negatively and significantly correlated to body mass. This finding is in accordance with other studies showing an increase in Cd throughout incubation in fasting birds (Wayland et al., 2008). Other studies have focused on soft tissues and have found significantly higher Cd levels in the liver and kidneys of post incubation eiders compared to pre-incubation eiders (Wayland et al., 2001, 2005). This may indicate that Cd was not redistributed from soft tissues into the blood and perhaps the increase in Cd burden in these soft tissues was a result of feeding locally. Albeit, the eiders studied in Wayland et al. (2001, 2005) at the beginning of incubation had not yet laid eggs, which may be an important excretion route for Cd in eiders (Burger, 1994; Burger et al., 2008). Cd levels in the present study may have been initially lower because samples were taken after the eiders had laid their eggs.

Wayland et al. (2002) suggested that immobile non-essential elements like Cd may be more diluted in the body at the beginning of incubation compared to the end because eiders are bigger, have more tissue and circulating blood at the beginning of incubation. In the present study when the eiders lost body mass these non-essential elements may have become more concentrated in the blood. Yet, Cd was significantly correlated to other essential elements, which may indicate its increased absorption from feeding during incubation.

Cd concentrations increased with decreasing Ca concentrations throughout incubation. A dietary deficiency in Ca has been shown to increase the absorption of Cd in avian species (Scheuhammer, 1987, 1996; Wayland and Scheuhammer, 2011). It is possible that when eiders replenished more and more towards the end of incubation they were absorbing more and more Cd because of Cd's ability to mimic Ca.

Zn and Cu were expected to decrease in the present study and be negatively correlated to Cd. However, Cu was shown to significantly increase while Zn was shown to significantly decrease. Additionally, Zn was negatively (but not significantly) correlated to Cd while Cu was positively and significantly correlated to Cd. These findings may be related to MT. Studies have shown an induction of MT during fasting in birds as a result of oxidative stress (Kondoh et al., 2003). Debacker et al. (2001) showed common guillemots in worse body condition had a higher induction of MT compared to those in better body condition. These individuals also showed an increase in absorption of Cu and Cd, presumably due to a deficiency in Zn. This may explain why eiders in the present study had higher levels of Cd at the end of incubation. Eiders in worse condition may have absorbed more Cd along with Cu because of higher levels of MT due to oxidative stress.

In the graph of Cd and body mass presented in Section 3.3 Fig. 3.4, there seems to generally be a cluster of individuals with a body mass below 1500 g that have high Cd levels. These individuals may have induced higher levels of MT due to higher levels of oxidative stress brought on by fasting, therefore they may have absorbed more Cd when they were re-feeding. However, these ideas are merely speculative as MT was not quantified in the present study. In addition, these blood Cd levels are considered low.

4.2.3 Lead

Blood Pb concentrations in the present study were expected to be lower than in previous reports on eiders as levels in biota and sediments have shown decreasing trends in the Baltic Sea in the last two decades (Uścinowicz, 2011; HELCOM, 2017b). The present study generally confirms this hypothesis. Earlier studies conducted on eiders in the Northern Baltic Sea (Franson et al., 2000b) reported blood levels 54% and 35% higher in 1997 and 1998, respectively, than the eiders in the present study. However, in a more recent study conducted in 2011, Fenstad et al. (2017) showed similar concentrations (45.06 ± 17.07 µg/kg ww) to the present study (44.15 ± 25.00 µg/kg ww, without individual 26). This suggests Pb concentrations were high in the 1990's but were followed by a decrease and then plateau from 2011 to 2017. Lead gasoline was phased out in the European Union (EU) throughout the 1990's and completely banned in 2005 (EU-Commission et al., 1998). This may explain the overall decrease and then plateau of Pb levels found in eiders over the years as the concentrations reported by Fenstad et al. (2017) and in the present study (except for one individual) are considered to be below background levels of 200 µg/kg ww (Franson and Pain, 2011)

One individual in the present study had Pb concentrations exceeding 300 µg/kg ww. Other reports on blood of eiders have associated Pb concentrations exceeding 200 µg/kg ww with ingesting or being shot with lead bullets (Flint et al., 1997; Hollmén et al., 1998). It is therefore likely that the one individual in the present study with high Pb concentrations either ingested or was shot with a lead bullet. In some countries surrounding the Baltic and Wadden Seas lead bullets are still used (Kanstrup et al., 2016), which may explain the high concentrations of Pb seen in one individual in the present study.

Lead blood levels increased significantly (by 71%) throughout incubation and was negatively and significantly correlated with body mass. Other studies seem to support this finding. Franson et al. (2000b) and Wilson et al. (2007) showed eiders in later stages of incubation to have higher blood Pb levels compared those in earlier stages. While Wayland et al. (2008) showed Pb concentrations to be negatively correlated with body

condition of king eiders during incubation. However, the correlation between Pb and body condition or body mass seems to be inconclusive as other studies (Franson et al., 1998; Fenstad et al., 2017) have found that Pb concentrations to not be correlated with these parameters at the end of incubation. Pb's close association with essential elements may better explain the increase found throughout incubation in the present study.

Pb levels in the present study were found to be negatively and significantly correlated to Ca levels, which may be due to their ability to inhibit and/or mimic Ca (Scheuhammer, 1987; Tchounwou et al., 2012; Williams et al.). Studies generally show a mobilization of Ca from medullary bone during egg laying (Franson and Pain, 2011). Although blood samples in the present study were taken after eggs had been laid, studies have suggested that Ca stores in medullary bone may be utilized during incubation to meet nutritional needs brought on by fasting (Franson et al., 2000b; Wilson et al., 2007). However, some eiders in the present study had higher Pb blood levels at the end of incubation compared to others. Eiders are a long-lived species (Waltho and Coulson, 2015) and given the long half-life of Pb in bone (Skerfving and Bergdahl, 2014), older eiders may have released more Pb throughout incubation compared to younger eiders (Franson and Pain, 2011). Therefore, future studies should take into account the age of eiders when assessing Pb levels in blood throughout incubation.

Pb levels in the present study were expected to be negatively correlated to Fe levels due to Pb's inhibitory effects on ALAD (Abadin et al., 2007). They were found to be negatively correlated, however not significantly. Noteworthy was the individual with Pb concentrations exceeding 300 µg/kg ww as this individual had the lowest Fe concentrations. This suggests high concentrations of Pb may have an inhibitory effect on Fe. However, some researchers suggest that birds do not reach a state of anemia until Pb blood levels exceed 500 µg/kg ww (Pain, 1996). Studies have found Pb blood levels exceeding 200 µg/kg ww to be associated with decreasing ALAD activity in incubating eiders (Franson et al., 2000b) and suggest anemia may result if ALAD is inhibited over a long period of time (Franson and Pain, 2011). The individual in the present study with high blood levels of Pb may have had relatively low levels of Fe due to inhibition of ALAD over a long period of time. Although it is not known for sure if this individual had anemia nor if ALAD activities were inhibited. Therefore, the relationship between ALAD, Fe and sub-clinical levels of Pb (200 - 500 µg/kg ww (Franson and Pain, 2011)) warrants further investigation in this eider population and specifically in this individual.

4.2.4 Mercury

Hg concentrations in the present study were expected to be similar to previously reported concentrations in the Baltic Sea in eiders, as there has not been increasing or decreasing trends in biota in the past two decades (HELCOM, 2010, 2017b). The present findings support this hypothesis as concentrations of Hg (179.59 ± 62.89 µg/kg ww) in the present study were similar to concentrations reported by Fenstad et al. (2017) (174.22 ± 66.59 µg/kg ww) in blood of eiders in Finland in 2011. Additionally, Franson et al. (1998, 2000b) reported maximum blood Hg concentrations of 0.22 ppm and 0.31 ppm (220 and 310 µg/kg ww) in 1997 and 1998, respectively. The present study reported a maximum concentration of 318 µg/kg ww. This suggests that bioavailable Hg has not decreased in the last two decades.

In the present study, the blood molar ratio between Hg and Se (HgSe) was expected to increase throughout incubation because of decreasing Se levels due to fasting and increasing Hg levels. In addition, HgSe ratios were expected to approach 1, indicating a toxic effect of Hg (Luque-Garcia et al., 2013). Although the blood molar ratio between Hg and Se (HgSe) increased significantly, the HgSe molar ratio did not approach 1. Studies indicate that molar ratios of Hg to Se may only approach 1 in highly exposed birds (Scheuhammer, 1987; Kim et al., 1996) and many seabirds often have an excess of Se compared to Hg (Ikemoto et al., 2004; Lovvorn et al., 2013). This suggests the molar ratio of 1 may not be as relevant for most seabirds. Other studies report a similar finding in eiders in the Baltic Sea. Franson et al. (2000b) reported a molar ratio of 1:55 µmol/kg in incubating eiders from blood samples in 1997 and 1998 in Finland. This is a similar finding to the present study that reported a mean HgSe ratio of 0.85: 49.00 µmol/kg at the end of incubation. However, Franson et al. (2000b) did not specify the stage of incubation of the eiders and Hg to Se molar ratios may vary depending on body condition (Wayland et al., 2001). Fenstad et al. (2017) reported a molar ratio of 0.9:17 µmol/kg at the end of incubation from blood sampled in 2011 in incubating eiders in the Northern Baltic Sea. This indicates a much lower blood level of Se compared to levels in the present study despite these populations having similar Hg levels. Some studies suggest that Se levels may be higher in populations of less polluted areas (Shore et al., 2011), while others suggest that Se levels may be higher in populations that are more exposed to Hg (Scheuhammer et al., 1998). However, eiders sampled by Fenstad et al. (2017) have similar levels of Hg as the eiders in the present study. Se has also been known to interact with other pollutants (Zwolak and Zaporowska, 2012). The relationship be-

tween Hg and Se in birds is complex (Shore et al., 2011) and therefore requires further study.

4.3 DNA double strand breaks

One of the main goals of assessing DNA DSBs in blood in the present study was to assess how the level of DNA damage changed throughout the incubation period in female eiders and to give insight into the health of the Christiansø eider population at a molecular level. In addition, body mass and non-essential elements were assessed in blood to investigate their relationship to DNA DSBs on day 5 and day 25 of incubation. DNA DSBs were expected to increase throughout incubation in relation to decreasing body masses and increasing concentrations of non-essential elements.

4.3.1 Levels of DNA double strand breaks

In the present study, DNA DSBs (measured as DNA-FTM) in blood of incubating female eiders were found to significantly increase (by 27 ± 18%) from day 5 to day 25 of incubation. Fenstad et al. (2014) measured DNA-FTM in blood of incubating eiders in Svalbard at the beginning and end of incubation in 2008 and 2009 and found that DNA-FTM increased by an average of 60% in 2008 and 66% in 2009 throughout incubation. This is a much larger increase than what was found in the present study. However, the Svalbard eiders had a much lower DNA-FTM at the beginning (37 ± 20% in 2008 and 17 ± 9% in 2009) and end of incubation (60 ± 20 % in 2008 and 28 ± 18% in 2009), compared to the Baltic Sea eiders in the present study (63 ± 11% (day 5) and 79 ± 8% (day 25)) (see Table 4.1). This may indicate that Baltic Sea eiders in the present study were in a worse condition at the start of incubation, thus they had more DNA damage. This finding is consistent with Matson et al. (2004) who found incubating eiders in the Baltic Sea to have a higher level of chromosomal damage compared to incubating eiders in Svalbard.

Fenstad et al. (2016a) compared DNA-FTM in blood of eiders at the end of incubation in the Baltic Sea (Finland) and in Svalbard and found that DNA-FTM in the Baltic Sea (Finnish) eiders to be 14 ± 5% and in Svalbard eiders to be 13 ± 5% (see Table 4.1). Fenstad et al. (2016a) found no significant difference between DNA-FTM in Svalbard eiders and in Baltic Sea (Finnish) eiders. In the present study, the Southern Baltic Sea eiders had DNA-FTM levels six times higher than those reported by Fenstad et al. (2016a) in the Northern Baltic Sea (Finland) at the end of incubation.

Although DNA-FTM in the present study did not increase as drastically as in the Svalbard eiders, levels were much higher than previously reported levels in both Northern Baltic Sea (Finnish) eiders and Svalbard eiders.

The reasons for this may be multifaceted and closely related to the overall health of the Southern Baltic Sea eiders as mentioned above this population has suffered from disease (Pedersen et al., 2003), parasites (Christensen, 2008; Garbus, 2016) and environmental contaminants (HELCOM, 2013a, 2017b). In addition, the Christiansø eider population suffered mass mortality events in 2007 and 2015 and eiders found dead were in poor condition and had high quantities of parasites (Garbus, 2016). These findings may contribute to the overall high levels of DNA DSBs seen in the present study as the stress induced by these events may exacerbate the oxidative stress brought on by incubation and fasting.

4.3.2 DNA DSBs in relation to body mass and mercury

In the present study, the best explaining variables for DNA DSBs (measured as DNA-FTM) in blood of incubating eiders were Hg and body mass. Although there was no overall significant change in the concentrations of Hg, the difference in body mass was correlated (not significantly) with the difference in Hg. This may explain why Hg was positively correlated to DNA-FTM. Two previous studies (mentioned above) in Northern Baltic Sea eiders (Fenstad et al., 2016a) and in Svalbard eiders (Fenstad et al., 2014, 2016a) showed similar findings. The first study Fenstad et al. (2014) examined DNA-FTM in Svalbard eiders at the beginning and end of incubation in relation to body mass and POPs (PCBs, DDE, Hexachlorocyclohexane (HCH), Hexachlorobenzene (HCB) and chlordanes). They found that the level of POPs in the blood increased by 148 - 639%, body mass decreased by 21 - 24% and DNA-FTM increased by 60 - 66% from the beginning to end of incubation (Fenstad et al., 2014). However, only body mass best explained DNA-FTM at the end of incubation (see Table 4.1). In the second study, Fenstad et al. (2016a) examined DNA-FTM in Baltic Sea (Finland) and Svalbard eiders only at the end of incubation in relation to body mass, Hg, PCBs, DDE, HCH, HCB and chlordanes. The best explaining variables for DNA-FTM in Baltic Sea (Finnish) eiders was Hg and p,p'-DDE while in Svalbard eiders it was body mass (see Table 4.1). Although POPs were not quantified in the present study, both body mass and Hg best explained DNA-FTM similar to the findings reported by Fenstad et al. (2014, 2016a).

Table 4.1: Table showing DNA-FTM (%) in whole blood of incubating female common eiders (*Somateria mollissima*) in the present study, Fenstad et al. (2014) and Fenstad et al. (2016a). DNA-FTM is presented as the mean ± standard deviation (SD). The SDs are only presented if they were provided in the study. Each study is presented with the location, the year and if applicable, DNA-FTM at the beginning and end of incubation and the difference (%) between the two. The variables best explaining DNA-FTM from each study are also presented and include body mass, Hg (mercury) and p,p'-DDE (p,p'-Dichlorodiphenyldichloroethylene).

	Fenstad et al. (2014)		**Fenstad et al. (2016a)**		**The present study**
	Svalbard		**Baltic Sea (Finland)**	**Svalbard**	**Baltic Sea (Denmark)**
	2008	2009	2011	2011	2017
DNA-FTM (%) (Mean ± SD)					
Beginning of incubation	37.4 ± 19.8	16.7 ± 9.4			63.4 ±10.6
End incubation	60.2 ± 19.7	27.8 ± 17.6	14.3 ± 4.9	13.4 ± 4.5	79.1 ± 7.9
Difference (%)	60	66			27.4 ± 18.1
Best explanatory variable(s)	Body mass (end of incubation)		Hg + p,p'-DDE	Body mass	Body mass + Hg

In the present study, body mass was negatively and significantly correlated to DNA-FTM and included in the best model explaining DNA-FTM. This finding may be related to the increase in oxidative stress brought on by reproduction and fasting. Alonso-Alvarez et al. (2004) showed that body mass loss was greater for zebra finches with higher reproductive output and coincided with a decrease in antioxidant defense. In albatrosses, Costantini et al. (2014) showed that a combination of contaminants (POPs and Hg) and breeding effort was associated with higher levels of oxidative stress. In addition, a higher level of Hg in female albatrosses was associated with more oxidative damage (Costantini et al., 2014). However, few studies exist directly linking the oxidative stress brought on by fasting to DNA damage except for the previously mentioned studies by Fenstad et al. (2014, 2016a,c). However, studies have suggested that a high reproductive effort increases basal and field metabolic rates and because metabolism is greater free radicals are produced (Alonso-Alvarez et al., 2004; Costantini et al., 2014). These free radicals are then free to interact with DNA and cause damage (Speakman and Garratt, 2014) and when the level of damage is too great repair mechanisms fail (Valavanidis et al., 2006). This may explain why increasing DNA DSBs throughout incubation in the present study were associated with decreasing body masses (increasing reproductive outputs).

In the present study, Hg was negatively correlated (not significantly) to DNA-FTM and included in the best model explaining DNA-FTM. In addition to the previously mentioned studies about eiders, the genotoxicity of Hg has been confirmed in other species. Karouna-Renier et al. (2014) found bats along a Hg contaminated river to have higher blood levels of total Hg (17- 714 µg/kg ww) and they were found have higher mitochondrial DNA (mtDNA) damage compared to bats in uncontaminated rivers (5 - 55

µg/kg ww). Although these levels of Hg are similar to the present study, mtDNA damage may be higher compared to other cell organelles because of its role in cellular respiration (Cline, 2012). Moreover, mtDNA lacks some repair proteins that are found in the nucleus (Cline, 2012). A study on captive common loons feed methylmercury chloride (CH_3HgCl) showed signs of oxidative stress when fed with concentrations exceeding 0.4 µg/g ww (400 µg/kg ww) yet no effect at environmentally relevant concentrations (0.08 µg/g ww (80 µg/kg ww)) (Kenow et al., 2008). Additionally, no difference in chromosomal aberrations was found between the control and exposed groups (Kenow et al., 2008). However, chromosomal aberrations may be considered a more severe genotoxic event and DNA DSBs may lead to chromosomal aberrations (Pfeiffer, 1998). However, Kenow et al. (2008) did observe an increase in GSH in Hg exposed common loons. By binding to sulfhydryl groups Hg depletes the cell's major antioxidants like GSH (Ercal et al., 2001). These antioxidants are then not available to scavenge ROS produced by normal cellular respiration or by other stressors like pollutants leading to a state of oxidative stress (Ercal et al., 2001; Koivula and Eeva, 2010). In the present study, eiders may have experienced oxidative stress due to decreased level of available antioxidants brought on by Hg exposure.

However, Fenstad et al. (2016c) reported that neither total antioxidant capacity (TAC) nor total GSH (tGSH) were correlated with blood Hg levels in Finnish eiders at the end of incubation. In addition, even though Hg was the most important variable explaining DNA-FTM in the same eiders, TAC and tGSH were not (Fenstad et al., 2016a). However, there may be other mechanisms by which Hg is genotoxic. Hg has been shown to effect DNA repair mechanisms by directly binding to and inhibiting zinc finger proteins involved in DNA repair (Stohs and Bagchi, 1995). Also, free radicals produced by Hg exposure have been shown to induce conformational changes in proteins responsible for DNA repair (Tchounwou et al., 2012). The two previous studies were performed *in vitro* with human cells. Hg, especially MeHg, may also bind directly to DNA creating DNA adducts (Li et al., 2006). However, their study was conducted on isolated DNA so it is difficult to know if this effect is reproducible in cells. Regardless of the mechanism, MeHg *in vitro* has been shown to induce DSBs in human astrocytes (Pieper et al., 2014), human lymphocytes (Betti et al., 1992) and dolphin lymphocytes (Betti and Nigro, 1996). Precaution must be taken when comparing *in vitro* to *in vivo* studies as they lack the complete cellular and biochemical systems found in a living organisms (Saeidnia et al., 2015). Additionally, precaution must be taken when comparing mammalian genomes to avian genomes as they may differ greatly (Vleck et al., 2007).

Fenstad et al. (2016a) found a significant relationship with Hg and DNA DSBs ($p<0.01$) in Baltic Sea (Finnish) eiders and the present study found a nearly significant relationship ($p=0.07$) despite Hg concentrations being similar. This may be due to a number of factors. Se was found to be higher in the current study (2991.6 ± 982.3 μg/kg) compared to those reported by Fenstad et al. (2017) (1367.7 ± 663.4 μg/kg) at the end of incubation. This may indicate a more protective affect of Se on Hg in the eiders in the present study compared to those in Fenstad et al. (2016a, 2017). Given Se's role as a cofactor in GPx, this may also indicate a higher antioxidant capacity. However, this cannot be concluded as Se may provide protective effects against a number of pollutants (Zalewska et al., 2015). Even though GSH and TAC, were not measured in the the present study, the effect of these antioxidants on Hg and vice versa, cannot be ruled out, as they have been closely linked to Hg and ROS (Ercal et al., 2001; Crespo-López et al., 2009). Furthermore, there may be other factors at play in the present study in these Southern Baltic Sea eiders contributing to such a high levels of DNA DSBs. For example, POPs may affect DNA damage (Fenstad et al., 2016a) or they may be in worse condition due to parasites (Thieltges et al., 2006) and/ or diseases (Pedersen et al., 2003) thus increasing oxidative stress. Therefore, the relationship between genotoxic effects and antioxidants warrants further investigation in the Southern Baltic Sea eider population as other factors may be at play.

4.3.3 Other factors

Although Cd in interaction with body mass was included in the top models in the present study, it was not significant ($p=0.27$) in explaining DNA DSBs. As mentioned earlier Cd concentrations in the present study are considered low. Cd may have been a statistical artifact and increased the fit of the model because concentrations seem to have increased in birds that reached lower masses and higher DNA DSBs. Cd in the body is generally found complexed with MT making it less available to damage cells (Scheuhammer, 1987; Tokar et al., 2015). Due to decreasing amounts of Zn in the present study more sites on MT may have been available for Cd to bind.

Another factor that was not accounted for in the present study was POPs. A decreasing body mass in Svalbard eiders was associated with increasing POP levels in the blood, presumably because POPs were mobilized from lipophillic tissue and into the blood (Bustnes et al., 2010; Fenstad et al., 2016b). The Baltic Sea (Danish) eiders in the present study lost much more body mass (17-44%) than the Svalbard eiders (21-24%). Therefore, it is possible the change in POP levels over incubation is much greater in

Baltic Sea eiders. In addition, this release of POPs may have a much greater affect on DNA because more may be released from lipophillic tissue because the eiders in the present study lost much more body mass. Fenstad et al. (2016b) found HCH, PCBs and p,p'-DDE to be 26, 10 and 5 times higher in Baltic Sea eiders than Svalbard eiders with p,p'-DDE positively and significantly correlated to DNA-FTM in Baltic Sea (Finnish) eiders at the end of incubation (Fenstad et al., 2016a). However, the initial body masses of these Baltic Sea (Finnish) eiders is not known and therefore it is difficult to conclude if they are loosing a greater amount of body mass, and therefore redistributing a greater amount of POPs compared to the Svalbard eiders. Because the eiders in the present study lost more body mass than Svalbard eiders it would be interesting to see if the stress caused by a potential increase in POPs would cause an increase DNA DSBs and override the stress caused by body mass loss or vice versa. In addition, DDE, and TBT, a lipophillic contaminant not assessed by Fenstad et al. (2016b), have been shown to be in higher concentrations in both sediments and biota in the Bornholm Basin (Denmark) relative to the Archipelago Sea (Finland) (HELCOM, 2010, 2017a) where Fenstad et al. (2016a,b) took samples. Eiders in the present study (in Denmark) may therefore be more exposed to certain POPs.

Regardless, the DNA-FTM was high in the eiders in the present study compared to previously reported levels. In addition to contaminant exposure this population has suffered a number of ailments that could adversely effect their health. When stress is too great and damage to DNA becomes unmanageable, repair mechanisms fail leading to DNA DSBs, one of the most severe types of DNA damage (Mehta and Haber, 2014). If left unrepaired DSBs may lead to mutations, chromosomal aberrations, tumorigenesis, carcinogenesis, and/or apoptosis (Pfeiffer, 1998; van Gent et al., 2001; Vilenchik and Knudson, 2003; Bignold, 2009). The present study used whole blood to examine genotoxic effects of environmental contaminants. It is believed that contaminant levels in blood may reflect total body burden (Wayland et al., 2001). Therefore, exposure to environmental contaminants may be detrimental. Studies suggest that this exposure may lead to decreases in genetic diversity causing population bottlenecks and ultimately population crashes (Bickham et al., 2000). The DNA damage found in the present study may be reflective of the overall declining health of the Baltic Sea eider population and in turn their susceptibility to genotoxic agents.

4.4 Future prospects

As a long lived seabird eiders may accumulate non-essential elements. Older eiders may have higher levels of non-essential elements stored in soft tissue compared to younger eiders (for example Pb) (Franson and Pain, 2011). Consequently higher concentrations may be released into the blood during incubation (Franson and Pain, 2011). To account for this the age of the eiders should be estimated. Furthermore, it is challenging to make any firm conclusions about the effects of Hg in the present study as the speciation was unknown. Although eiders have been shown to contain primarily MeHg in whole blood (Wayland et al., 2001), this conclusion cannot be assumed. To better understand Hg and its relationship to fasting stress and DNA damage, both MeHg and total Hg should be measured. Further studies on DNA damage in Baltic Sea eiders should also include measurements of POPs as the eiders in the present study lost a great amount of body mass throughout incubation, therefore POPs may be released in great amounts from adipose tissue during incubation (Fenstad et al., 2014). Southern Baltic Sea eiders may also mobilize more POPs compared to Northern Baltic Sea eiders because they may be more exposed to POPs (HELCOM, 2017b). Finally, the overall health of the Southern Baltic Sea eider population warrants further investigated as the high frequency of DNA damage found at the molecular level in the present study may transcend into higher population levels, affecting the overall population.

5 | Conclusion

The main goal of the present study was to analyze body mass and non-essential elements in relation to DNA DSBs (measured as DNA-FTM) in blood of female common eiders (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation. In addition, the goal was to assess levels of non-essential elements levels and how they changed throughout incubation in relation to essential elements.

Concentrations of As were very similar to previous reports, indicating that seabirds in the Bornholm Basin may not be at greater risk of exposure due to chemical war fare dumping sites. As did not significantly increase throughout incubation. However, significantly increasing levels of Cd were associated with decreasing levels of Ca and Zn and increasing levels of Cu, which may demonstrate an increase in absorption of Cd from day 5 to day 25 of incubation. Significantly increasing levels of Pb were significantly correlated with decreasing levels of Ca, which may indicate that Pb was released from medullary bone during incubation. Although Hg did not significantly increase or decrease, the change in Hg was negatively correlated with the change in body mass, indicating a mobilization of Hg from tissue or increased feeding throughout incubation in eiders loosing more body mass.

The variables best explaining DNA-FTM throughout incubation were body mass and Hg. As increasing DNA-FTM levels throughout incubation were negatively and significantly correlated to body mass and positively correlated (not significantly) to Hg levels. This may indicate an increase in oxidative stress brought on by incubation and fasting and therefore an increased sensitivity to genotoxic pollutants. However, Hg was not significant in explaining DNA-FTM as in other Baltic Sea eider populations. This may indicate other pollutants or stressors are at play. Such high levels of DNA-FTM and drastic decreases in body mass found in the present study warrants further investigation as these parameters may be reflective of the overall declining health of the Baltic Sea eider population and in turn their susceptibility to genotoxic agents.

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A | Materials and methods

A.1 Materials DNA gel electrophoresis

Table A.1: List of solutions used in DNA DSB analyses including chemicals, producers, catalog numbers and purity

Solution	Chemicals	Producer	Catalog number	Purity
TE buffer (500ml pH 8)	Tris HCl pH 7.4 EDTA pH 8	Biorad Biorad	T3253 161-0729	
TBE buffer (1000ml pH 8)	Tris base boric acid EDTA pH 8	Sigma Sigma	T6066 B7901	≥99.9% ≥99.5%
Lysis buffer (200ml pH 7)	NaCl Tris EDTA SDS	Sigma Sigma Sigma Sigma	S3014 161-0301	≥98%
1% LMPA	TE buffer Low melt agarose	 Biorad	 162-0019	
0.6% agarose gel	agarose "for routine use" 0.5 x TBE buffer	Sigma	A9539	
Lambda DNA ladder	Lambda Hindiii digest 6X DNA loading dye	Fermentas Thermo scientific	SM0101 R0611	
Ethidium Bromide		Biorad	1610433	
ProteinaseK		Sigma-Aldrich	P2308	

Table A.2: List of concentrations, volumes and masses of chemicals used in DNA DSB analyses.

Solution	Chemicals	Amount
TE buffer (500ml pH 8)	Tris HCl pH 7.4	10mM (0.6057g)
	EDTA pH 8	1mM (1ml of 0.5M stock)
TBE buffer (1000ml pH 8)	Tris base	54g (45mM)
	boric acid	27.5g
	EDTA	1mM (1ml of 0.5M stock)
Lysis buffer (200ml pH 7)	NaCl	1.1688g (100mM)
	Tris	2ml (10mM)
	EDTA	4ml 0.5M (10mM)
	SDS	10ml 10% SDS (0.5%)
1% LMPA	TE buffer	20ml
	Low melt agarose	200mg
0.6% agarose gel	Agarose "for routine use"	0.6g
	0.5 x TBE buffer	100ml
Lambda DNA ladder	Lambda Hindiii digest	100ul
	lambda DNA	37ul
	6X DNA loading dye	24ul
Ethidium Bromide		0.1mg/l
ProteinaseK		1 mg/ml

A.2 Lambda DNA/ Hindiii Marker

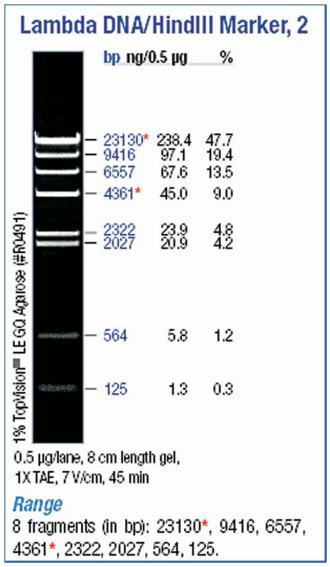

Figure A.1: A depiction of the molecular size marker λ DNA + Hindiii digest showing size of the fragments relative to its distance migrated. Image modified from Thermo Scientific (2016).

A.3 DNA-FTM example

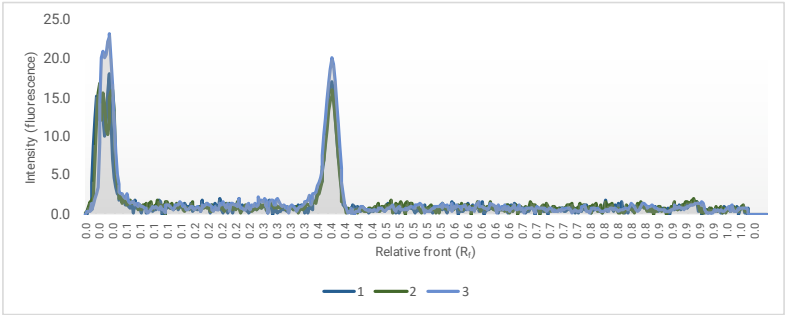

Figure A.2: Example of the intensity curves of DNA that remained in the well (left) and DNA that migrated into the gel (right). The intensity curves were compared to determine the percentage of DNA that had migrated into the gel (DNA-FTM)

A.4 ICP-MS

Table A.3: Method detection limits (MDL) of As (arsenic), Ca (calcium), Cd (cadmium), Co (cobalt), Cu (copper), Hg (mercury), Fe (iron), K (potassium), Pb (lead), Se (selenium) and Zn (zinc) determined by comparing the value of three times the standard deviation of the blanks and the instrument detection limit and choosing the highest value. The table presents the elements quantified, their isotope, resolution (LR, MR, HR = low, medium and high resolution respectively) and their method detection limits (MDL) (μg/kg).

Element	Isotope	Resolution	MDL
As	75	HR	1.02
Ca	44	MR	81.2 Cd
114	LR	0.08	
Co	59	MR	0.16
Cu	63	MR	0.00
Hg	57	LR	0.49
Fe	202	MR	93.66
K	39	MR	202.90
Pb	208	LR	0.08
Se	78	HR	6.09
Zn	67	MR	54.97

Table A.4: Measured levels in the reference material of of As (arsenic), Ca (calcium), Cd (cadmium), Co (cobalt), Cu (copper), Hg (mercury), Fe (iron), K (potassium), Pb (lead), Se (selenium) and Zn (zinc) used to determine the accuracy of the analysis of whole blood by HR-ICP-MS. Each element analyzed is listed with standard deviation (SD), the certified values, estimated uncertainty and percent recovery (recovery (%)).

Element	Isotope	Average (ug/l)	SD	Certified value (ug/l)	Estimated uncertainty	Recovery (%)
As	75	2.28	0.41	4.60	0.90	49.7%
Ca	44	17168.94		15000	109.88	90.2% Cd
114	0.23	0.03	0.28	0.11	81.9%	
Co	59	0.21	0.02	0.20	0.08	104.6%
Cu	63	527.48	10.04	130.00	130.00	405.8%
Fe	57	292063.92	1488.98	334000.00		87.4%
Hg	202	4.92	1.39	0.30	0.30	1641.1%
K	39	1005135.26	9199.85	1091000.00		92.1%
Pb	208	2.66	0.44	9.90	2.00	26.9%
Se	78	56.30	2.55	60.00	12.00	93.8%
Zn	67	3900.97	28.29	4300.00	900.00	90.7%

A.5 Additional R-packages

Table A.5: Additional R packages used in R (R Core Team (2015)) for statistical analyses.

Package name	Usage
AICcmodavg	AICc table
car	Variance inflation factor
carData	Variance inflation factor
factoextra	Principal component analyses (PCA)
FActoMinR	Principal component analyses (PCA)
ggplot2	Visualizing models and plotting graphs
lme4	Mixed linear effect models
MuMIn	Coniditional and Marginal R squared
nmle	Mixed linear effect models
Rcolorbrewer	Colors for graphs
visreg	Plotting and visualizing mixed linear effect models

B | Results

B.1 Body mass

Table B.1: Body mass (g) for each individual female common eider (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25. The first number before the decimal place represents the individual bird's identification number (ID) and the number after the decimal represents the time of incubation (1 for day 5 and 2 for day 25). Δ difference represents the difference between body mass on day 5 and day 25. The difference (%) was calculated as a percent decrease.

Individual	Body mass (g)	Difference (Δ)	Difference (%)
1.1	2305	745	32%
1.2	1560		
2.1	2455	745	30%
2.2	1710		
3.1	2050	580	28%
3.2	1470		
4.1	2450	640	26%
4.2	1810		
5.1	2230	702	31%
5.2	1528		
6.1	2280	755	33%
6.2	1525		
7.1	2525	420	17%
7.2	2105		
8.1	2665	1040	39%
8.2	1625		
9.1	2365	815	34%
9.2	1550		
10.1	2630	855	33%
10.2	1775		
11.1	2120	635	30%
11.2	1485		
12.1	2280	795	35%
12.2	1485		
13.1	2250	530	24%
13.2	1720		
14.1	2380	480	20%
14.2	1900		
15.1	2515	965	38%
15.2	1550		
16.1	2240	790	35%
16.2	1450		
17.1	2550	975	38%
17.2	1575		
18.1	2275	830	36%
18.2	1445		
19.1	2230	705	32%
19.2	1525		
20.1	2420	845	35%
20.2	1575		
21.1	2580	1125	44%
21.2	1455		
22.1	2580	1055	41%
22.2	1525		
23.1	2170	535	25%
23.2	1635		
24.1	2415	680	28%
24.2	1735		
25.1	2250	550	24%
25.2	1700		
26.1	2125	585	28%
26.2	1540		
27.1	2405	640	27%
27.2	1765		
28.1	2210	535	24%
28.2	1675		

B.2 Elements

Table B.2: Concentrations of elements for each individual female common eider (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation. All values are represented in μg/kg. The first number before the decimal place represents the individual bird's identification number (ID) and the number after the decimal represents the time of incubation (1 for day 5 and 2 for day 25). LR, MR and HR represent low, medium and high resolution, respectively, of the HR-ICP-MS.

Individual	As75(HR)		Ca44(MR)		Cd114(LR)		Co59(MR)		Cu63(MR)		Hg202(LR)	
	ug/kg	RSD%	g/kg	RSD. %	ug/kg	RSD%	ug/kg	RSD%	ug/kg	RSD%	ug/kg	RSD%
1.1	10.69	3.90	79795.78	1.35	0.79	4.35	1.27	10.20	287.77	1.55	219.95	1.30
1.2	6.81	8.70	54703.31	0.40	0.92	4.80	1.70	15.20	316.68	1.40	255.80	1.40
2.1	13.85	22.90	108901.08	0.50	0.82	3.20	1.90	19.60	196.30	2.60	125.04	1.50
2.2	22.50	5.10	51830.69	2.20	0.88	3.60	1.47	8.10	270.01	1.30	106.06	2.70
3.1	11.48	9.40	85771.27	1.40	1.08	3.00	1.25	4.80	439.04	1.40	158.97	0.60
3.2	14.21	8.10	54300.53	1.00	1.73	5.70	1.67	10.70	332.59	1.50	148.89	1.90
4.1	13.62	14.40	118332.67	1.10	0.80	5.00	1.83	13.30	392.15	2.90	185.22	1.90
4.2	23.66	5.70	48776.07	0.10	1.80	2.50	0.84	34.20	438.93	3.10	184.44	1.70
5.1	19.67	12.70	81316.53	1.80	0.76	20.60	1.59	27.80	349.29	2.10	106.06	3.60
5.2	25.29	5.80	48877.13	1.50	1.08	6.85	1.19	8.20	352.87	1.95	86.91	1.55
6.1	11.25	12.50	69175.17	1.20	1.23	3.90	0.68	7.30	282.30	1.10	183.14	0.80
6.2	10.24	5.20	48921.28	1.00	1.84	3.00	1.51	8.20	331.00	1.20	176.99	1.60
7.1	18.39	7.30	117872.36	0.50	0.71	5.80	1.86	16.80	459.69	2.40	186.78	1.60
7.2	20.37	14.20	54107.14	2.20	1.34	4.20	1.57	14.60	431.69	2.40	187.82	1.90
8.1	12.63	16.60	170233.53	0.80	0.56	6.00	1.59	6.40	386.96	1.80	92.15	1.90
8.2	11.16	21.00	49786.96	0.70	1.04	2.30	0.85	13.30	392.90	2.70	115.61	2.30
9.1	13.97	8.50	58115.14	1.00	0.73	6.80	0.56	5.50	284.82	1.80	255.87	0.70
9.2	11.15	10.20	44250.83	1.20	1.02	3.40	1.46	9.60	345.96	2.60	269.10	0.50
10.1	15.90	18.30	120958.41	0.90	0.72	15.40	1.58	29.10	433.02	1.90	159.51	2.00
10.2	23.19	5.70	52516.91	1.00	1.11	7.55	1.63	3.10	398.62	1.65	202.93	1.20
11.1	11.81	7.40	49496.52	1.30	0.90	16.40	2.05	15.20	411.00	0.90	145.56	1.70
11.2	15.53	6.90	52157.83	1.40	1.75	5.00	2.58	25.10	366.76	1.20	125.93	1.80
12.1	9.58	14.80	66622.50	3.00	0.93	2.90	2.47	12.10	343.74	3.40	173.59	1.20
12.2	14.42	11.50	54053.48	0.70	1.87	6.50	2.04	15.70	443.30	3.30	190.69	2.30
13.1	10.75	5.20	66913.09	2.20	0.49	15.40	0.98	16.10	270.97	1.10	211.28	1.90
13.2	13.78	8.90	44820.15	1.90	0.79	12.90	2.30	25.20	387.91	2.10	220.85	1.80
14.1	13.35	18.30	125652.60	1.20	1.14	3.90	1.45	18.40	375.38	0.60	233.89	1.70
14.2	21.03	12.40	59655.92	1.20	1.15	8.80	1.61	7.10	418.82	1.80	208.82	2.40
15.1	18.25	10.90	220548.56	2.10	0.56	11.60	1.31	12.70	312.76	1.20	161.36	2.30
15.2	13.83	10.60	50959.41	0.50	0.87	9.50	1.72	28.40	286.28	2.10	170.36	1.70
16.1	12.25	12.10	85629.69	2.60	1.15	4.00	1.02	17.30	329.44	4.80	170.07	2.60
16.2	14.50	2.60	52192.43	1.30	1.72	2.90	1.36	20.00	406.53	1.10	216.31	3.20
17.1	14.60	9.00	95106.92	1.30	1.58	9.80	0.89	16.20	330.67	1.60	139.45	0.70
17.2	13.36	11.30	50561.35	1.50	1.92	5.50	1.35	9.00	330.79	2.10	123.19	3.50
18.1	14.03	11.20	109140.22	0.30	0.97	4.10	1.90	8.70	352.19	1.70	192.38	1.00
18.2	9.94	20.60	56272.76	0.90	0.98	14.40	2.83	15.80	294.87	2.00	165.77	1.20
19.1	13.76	13.40	88836.93	1.40	0.77	6.70	0.90	23.30	357.87	3.40	102.04	2.10
19.2	15.21	8.50	51101.02	1.70	1.27	7.10	0.60	30.30	415.36	5.50	111.38	0.50
20.1	17.08	11.30	100858.73	1.70	0.72	7.10	1.37	8.90	306.12	1.00	277.30	0.40
20.2	19.49	10.80	54804.53	1.60	1.48	10.30	1.80	8.40	351.24	2.80	313.61	0.40
21.1	17.09	11.20	79836.42	2.60	0.80	14.60	1.17	19.60	355.44	2.20	114.49	2.50
21.2	12.03	0.60	53493.84	2.90	2.19	4.60	0.70	15.00	366.43	2.40	133.14	2.70
22.1	17.20	12.60	125208.57	2.90	1.02	6.00	1.39	14.30	389.03	2.90	231.63	1.70
22.2	11.84	6.80	44042.85	0.60	1.93	6.10	0.86	19.40	332.19	2.80	212.12	1.70
23.1	10.23	6.80	62266.40	2.00	0.50	3.30	2.05	14.00	278.58	0.60	171.40	1.60
23.2	11.62	6.50	43688.71	0.60	0.66	3.60	1.77	14.10	311.12	0.90	146.55	1.40
24.1	10.71	23.10	73091.93	0.70	0.52	0.60	1.40	4.80	298.43	1.20	149.97	2.50
24.2	15.05	7.90	53857.67	1.80	0.88	14.70	1.88	29.70	384.74	0.60	167.91	1.90
25.2	19.53	1.90	38932.69	0.40	0.77	7.50	0.91	18.40	318.12	1.40	63.41	1.40
26.1	8.51	14.40	75808.86	2.60	0.44	7.10	1.98	10.50	257.49	2.80	76.24	2.10
26.2	17.32	6.00	47749.62	1.60	1.00	4.00	1.38	34.80	334.30	2.80	66.42	1.40
27.1	13.67	11.30	85510.89	0.50	0.73	8.80	1.71	19.40	275.22	1.60	216.86	2.40
27.2	14.49	16.80	45223.78	4.30	0.78	9.00	2.44	70.60	277.88	3.60	163.16	1.60
28.1	13.39	12.20	62830.27	0.70	0.67	15.10	0.68	34.80	334.08	0.60	295.74	0.20
28.2	13.37	15.50	52037.24	1.10	0.69	13.50	0.85	20.70	350.58	2.00	239.67	1.00

Table B.3: Concentrations of elements for each individual eider on day 5 and day 25 of incubation in Christiansø, Denmark. All values are represented in µg/kg except HgSe which is a calculated molar ratio. The first number before the decimal place represents the individual bird's identification number (ID) and the number after the decimal represents the time of incubation (1 for day 5 and 2 for day 25). LR, MR and HR represent low, medium and high resolution, respectively, of the HR-ICP-MS.

Individual	Fe56(MR)		K39(MR)		Pb208(LR)		Se78(HR)		Zn66(MR)		HgSe	HgSe
	g/kg	RSD. %	g/kg	RSD. %	ug/kg	RSD%	ug/kg	RSD%	g/kg	RSD. %	molar ratio	molar ratio (umol/kg)
1.1	417982.4662	1.40	2299160.13	0.75	12.01	1.90	4517.09	5.60	6044.26	1.85	0.019	1.10:57.21
1.2	386988.4979	4.20	1754117.83	4.00	51.21	3.10	4043.22	5.00	5448.85	3.60	0.025	1.28:51.21
2.1	428341.2815	2.40	2537786.29	3.90	12.07	2.90	2904.27	4.20	7461.54	2.60	0.017	0.62:36.78
2.2	338617.1837	1.70	1826124.21	2.30	11.57	3.20	2136.99	2.90	3708.31	1.40	0.020	0.53:27.06
3.1	390096.4646	0.60	2030148.47	1.20	24.48	2.80	2745.41	3.50	6060.86	1.40	0.023	0.79:34.77
3.2	360798.6622	2.30	1650041.94	2.90	32.40	3.70	1926.51	7.50	3886.05	1.40	0.030	0.74:24.40
4.1	506156.2741	1.60	2585932.23	4.10	32.43	1.10	2928.33	4.00	8155.58	1.20	0.025	0.92:37.09
4.2	479850.3588	3.70	2672734.47	2.60	27.62	2.20	2387.78	5.00	5092.97	1.60	0.030	0.92:30.24
5.1	455133.6548	1.80	2197450.21	3.80	62.92	1.10	4446.22	4.10	5997.25	1.20	0.009	0.53:56.31
5.2	378821.1445	2.05	1765979.62	2.25	64.03	2.10	3463.09	3.00	3419.70	0.75	0.010	0.43:43.86
6.1	472920.1667	2.70	2144424.63	3.20	12.52	0.90	4952.05	4.60	5801.11	1.40	0.015	0.91:62.72
6.2	408107.0016	1.60	2050323.99	4.90	20.43	2.60	3966.30	3.00	5147.58	1.10	0.018	0.88:50.23
7.1	450183.1292	0.90	2271722.17	2.30	10.29	3.00	3269.06	4.30	7416.09	0.90	0.022	0.9:41.40
7.2	462870.3851	1.50	3169905.86	3.10	13.57	2.50	2301.36	5.30	4990.36	1.40	0.032	0.94:29.15
8.1	397127.3673	0.50	1860072.56	0.80	22.47	1.30	1718.38	9.00	10526.32	0.40	0.021	0.46:21.76
8.2	382997.8962	2.10	1886644.32	1.70	39.41	1.10	1946.75	5.20	5515.35	1.30	0.023	0.58:24.65
9.1	432370.774	0.10	2282186.98	1.70	10.68	1.30	4273.17	4.40	5410.47	1.20	0.024	1.28:54.12
9.2	396301.3424	2.40	1884156.61	2.10	28.45	2.00	3051.73	4.00	4847.99	2.70	0.035	1.34:38.65
10.1	352398.6791	0.70	1909307.01	3.50	25.04	3.70	4929.14	7.80	8083.40	1.60	0.013	0.80:62.43
10.2	434205.9891	0.85	2136509.53	2.35	72.38	2.55	6011.90	2.90	5114.68	1.65	0.013	1.01:76.14
11.1	473612.6447	1.50	3024098.81	2.50	34.32	1.90	5026.61	7.40	6177.50	2.10	0.011	0.73:63.66
11.2	381785.4539	3.00	3352737.16	1.40	39.27	2.40	3267.38	3.20	5821.36	0.40	0.015	0.63:41.38
12.1	401811.463	1.50	1850850.36	1.80	73.63	2.90	2701.75	2.30	5076.84	0.20	0.025	0.87:34.22
12.2	432102.3046	1.60	2119090.88	4.20	107.88	0.30	2770.44	5.00	5604.05	2.70	0.027	0.95:35.09
13.1	434255.4863	1.20	2193640.64	4.30	58.25	1.10	4023.71	4.00	5664.72	1.20	0.021	1.05:50.96
13.2	443420.1251	3.80	2212518.75	2.10	58.26	2.90	3455.71	6.60	5469.13	1.80	0.025	1.10:43.77
14.1	501025.4666	0.80	2863419.63	4.20	11.29	0.50	3865.05	3.90	7416.99	1.70	0.024	1.17:48.95
14.2	460458.0469	3.90	2524498.92	4.20	14.90	2.80	2617.90	0.60	5126.81	1.60	0.031	1.04:33.15
15.1	395705.1364	1.10	2086767.32	2.10	19.88	3.20	2485.28	8.40	8799.37	0.40	0.026	0.80:31.48
15.2	376852.6322	1.60	2072075.97	1.40	64.44	0.80	2290.12	1.40	4814.08	0.50	0.029	0.85:29.00
16.1	412074.7482	4.20	2286041.75	5.00	14.84	2.30	4639.03	4.50	6808.30	2.30	0.014	0.85:58.75
16.2	440857.8502	2.00	2159220.03	3.50	21.21	2.90	3701.76	4.00	6549.52	1.00	0.023	1.08:46.88
17.1	515023.7511	1.30	2481768.11	8.00	16.96	1.70	3892.47	6.10	8459.96	0.70	0.014	0.70:49.30
17.2	427376.079	4.40	2112929.01	6.30	35.65	1.00	2879.36	5.20	6140.29	0.70	0.017	0.61:36.47
18.1	498998.0623	0.90	2424073.22	3.80	12.21	1.10	6447.67	7.40	9047.20	1.30	0.012	0.96:81.66
18.2	362315.882	1.80	1923492.82	1.50	8.34	3.00	4728.50	2.60	5503.55	1.70	0.014	0.83:59.88
19.1	439363.4796	2.10	2163575.53	1.20	23.57	1.60	4110.32	3.70	7217.35	0.30	0.010	0.51:52.06
19.2	450839.8553	3.20	2116246.21	3.70	24.83	0.60	3514.00	4.80	6559.49	1.50	0.012	0.56:44.50
20.1	520644.4853	0.60	2554786.83	3.40	20.41	1.60	3945.43	7.70	9367.33	0.80	0.028	1.38:49.97
20.2	488426.4598	5.10	2270368.78	5.80	32.73	1.30	3132.61	3.30	6841.50	1.70	0.039	1.56:49.22
21.1	431058.3496	2.20	2104664.56	2.10	19.32	1.30	3886.50	7.70	6598.15	0.60	0.012	0.57:49.22
21.2	423858.4622	4.60	2076987.88	3.00	43.54	1.90	3252.49	2.60	5544.44	2.00	0.016	0.66:41.19
22.1	428268.4544	3.90	2172538.61	3.10	12.56	2.90	4339.85	7.30	8202.16	1.00	0.021	1.15:54.96
22.2	354500.8072	2.50	1888065.52	4.00	34.76	1.40	3025.13	5.50	4375.43	1.90	0.028	1.06:38.31
23.1	443650.2864	3.70	2088315.83	2.60	114.84	2.60	2261.24	3.60	6320.81	2.00	0.030	0.85:28.64
23.2	344773.984	3.40	1783745.40	3.20	70.45	3.00	1649.52	4.40	4772.58	1.30	0.035	0.73:20.89
24.1	407790.3568	1.40	2021917.63	2.30	45.15	2.90	2308.54	1.00	5578.41	0.80	0.026	0.75:29.24
24.2	449685.2629	2.20	3316059.36	2.20	74.92	5.30	2084.08	5.70	5195.31	1.80	0.032	0.84:26.39
25.2	335338.7163	3.80	1947175.97	3.70	41.40	0.80	1744.09	5.90	4333.21	2.50	0.014	0.32:22.09
26.1	328143.6888	1.30	1587858.45	1.00	354.93	0.40	2547.53	4.60	6068.99	0.30	0.012	0.38-32.26
26.2	336151.6967	3.80	1681099.63	1.40	366.09	3.00	1830.78	5.90	4809.22	0.70	0.014	0.33:23.19
27.1	464540.7768	0.60	3156427.00	1.30	22.81	2.60	5193.83	7.60	6320.58	0.60	0.016	1.08:65.78
27.2	321145.1383	1.50	2012831.77	1.30	51.16	1.60	2925.86	7.90	4564.84	2.40	0.022	0.81:37.05
28.1	494666.0177	3.50	2445975.98	3.20	44.69	3.80	6096.31	3.50	5454.33	0.10	0.019	1.47:77.21
28.2	399920.3614	1.60	2016720.45	2.20	85.17	0.80	3660.61	4.00	5041.88	1.30	0.026	1.19:46.36

B.3 Correlations between elements and body mass

Table B.4: Correlation tests ran between elements and body mass. The table shows the type of correlation test used (Spearman's rank correlation), the correlation coefficient (correlation coeff.) and the p-value.) The asterisks, *,**,***, represent a p-value of <0.05, <0.01 and <0.001 indicating a significant correlation between elements. Correlation tests were also performed without individual number 26 (without ID. 26).

	Elements	Correlation type	Correlation coeff.	p-value
Body mass	Ca***	Spearman	0.79	2.20×10^{-16}
	Cd***	Spearman	−0.56	1.85×10^{-5}
	Fe**	Spearman	0.36	0.0081
	K*	Spearman	0.34	0.013
	Pb**	Spearman	−0.39	0.0042
	Zn***	Spearman	0.63	7.3×10^{-7}
Without individual 26				
	Pb*	Spearman	−0.35	0.013

B.4 Correlations between non-essential and essential elements

Table B.5: Correlation tests ran between non-essential and essential elements. The table shows the type of correlation test used (either Pearson's product moment correlation and Spearman's rank correlation), the correlation coefficient (correlation coeff.) and the p-value.) The asterisks, *,**,**, represent a p-value of <0.05, <0.01 and <0.001 indicating a significant correlation between elements. Correlation tests were also performed without individual number 26 (without ID. 26).

Non-essential element	Essential element	Correlation type	Correlation coeff.	p-value
As	Cu**	Pearson	0.36	0.0070
	K*	Spearman	0.30	0.028
Cd	Ca**	Spearman	−0.37	0.0069
	Cu**	Pearson	0.37	0.0061
Hg	Fe***	Pearson	0.44	0.00095
	K***	Spearman	0.35	0.0089
	Se**	Pearson	0.37	0.0057
Pb	Ca***	Spearman	−0.47	0.00040
	Fe	Spearman	−0.25	0.068
	K**	Spearman	−0.36	0.0073
	Se*	Spearman	−0.38	0.015
	Zn**	Spearman	−0.42	0.0019
Without individual 26				
Pb	Ca***	Spearman	−0.47	0.00044
	Fe	Spearman	−0.16	0.26
	K*	Spearman	−0.36	0.038
	Se*	Spearman	−0.33	0.049
	Zn**	Spearman	−0.42	0.0024

B.5 DNA double strand breaks

DNA-FTM and MML raw data

Table B.6: Raw data of the DNA-fraction of total DNA that migrated into the gel (DNA-FTM (%)) and median molecular length (MML), measured in kilobase pairs (kbp), in each individual female common eider (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation. The first column represents the individual (IND.) and the first number before the decimal place represents the individual bird's identification number (ID) and the number after the decimal represents the time of incubation (1 for day 5 and 2 for day 25). Values are presented as an average of each gel and the raw data from each lane.

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
1.1	69.46 %	lane 1	69.26	308.77	306.00
		lane 2	71.53		306.00
		lane 3	66.04		306.00
		lane 4	78.58		314.32
		lane 5	81.25		314.32
		lane 6	75.34		314.32
		lane 7	55.89		306.00
		lane 8	64.20		306.00
		lane 9	63.07		306.00
1.1	73.83 %	lane 1	69.33	318.82	306.00
		lane 2	78.35		306.00
		lane 3	68.68		323.66
		lane 4	70.72		306.00
		lane 5	75.98		306.00
		lane 6	79.81		332.69
		lane 7	76.92		341.72
		lane 8	76.01		323.66
		lane 9	68.69		323.66
1.1	74.07 %	lane 1	72.96	309.24	324.65
		lane 2	75.58		324.65
		lane 3	75.93		324.65
		lane 4	73.50		287.75
		lane 5	68.54		278.43
		lane 6	68.04		278.43
		lane 7	78.63		315.33
		lane 8	79.01		324.65
		lane 9	74.44		324.65
1.2	81.51 %	lane 1	82.20	256.63	261.64
		lane 2	81.98		261.64
		lane 3	74.14		270.67
		lane 4	73.73		252.62
		lane 5	77.04		252.62
		lane 6	72.04		252.62
		lane 7	93.01		252.62
		lane 8	89.16		252.62
		lane 9	90.29		252.62
2.1	49.24 %	lane 1	52.63	341.35	374.67
		lane 2	51.11		374.67

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 3	55.91		374.67
		lane 4	50.60		331.93
		lane 5	47.14		323.17
		lane 6	42.93		314.76
		lane 7	49.90		323.17
		lane 8	44.32		323.17
		lane 9	48.64		331.93
2.1	41.66 %	lane 1	36.20	344.49	349.34
		lane 2	34.72		349.34
		lane 3	39.24		349.34
		lane 4	36.42		340.60
		lane 5	34.87		340.60
		lane 6	34.76		349.34
		lane 7	50.24		340.60
		lane 8	56.90		340.60
		lane 9	51.60		340.60
2.2	72.75 %	lane 1	82.26	255.55	246.09
		lane 2	88.63		246.09
		lane 3	85.56		246.09
		lane 4	79.15		271.67
		lane 5	79.45		271.67
		lane 6	67.58		271.67
		lane 7	60.48		246.09
		lane 8	59.47		246.09
		lane 9	52.17		254.50
3.1	73.16 %	lane 1	57.14	307.84	306.00
		lane 2	78.65		306.00
		lane 3	73.88		306.00
		lane 4	86.19		297.71
		lane 5	83.81		306.00
		lane 6	79.80		306.00
		lane 7	63.47		314.29
		lane 8	72.37		314.29
		lane 9	63.11		314.29
3.1	77.51 %	lane 1	69.61	333.59	332.71
		lane 2	76.70		323.54
		lane 3	81.13		323.54
		lane 4	75.58		332.71
		lane 5	80.96		332.71
		lane 6	62.80		341.48
		lane 7	79.19		341.48
		lane 8	89.33		341.48
		lane 9	82.29		332.71
3.2	74.69 %	lane 1	76.25	282.13	288.58
		lane 2	82.64		288.58
		lane 3	79.82		280.29
		lane 4	71.32		280.29
		lane 5	81.10		280.29
		lane 6	66.48		280.29
		lane 7	74.34		280.29
		lane 8	70.12		280.29

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 9	70.17		280.29
4.1	55.16 %	lane 1	48.76	320.60	313.73
		lane 2	53.09		321.46
		lane 3	47.98		321.46
		lane 4	46.72		313.73
		lane 5	52.98		321.46
		lane 6	64.58		336.93
		lane 7	68.42		329.20
		lane 8	57.85		313.73
		lane 9	56.04		313.73
4.1	57.40 %	lane 1	51.24	336.54	350.33
		lane 2	57.09		350.33
		lane 3	57.16		341.46
		lane 4	51.01		323.73
		lane 5	51.41		323.73
		lane 6	57.12		341.46
		lane 7	67.26		332.60
		lane 8	67.11		332.60
		lane 9	57.18		332.60
4.2	74.06 %	lane 1	75.43	195.89	180.43
		lane 2	64.22		187.07
		lane 3	53.11		272.81
		lane 4	76.75		167.43
		lane 5	74.63		180.43
		lane 6	76.51		200.34
		lane 7	82.61		187.07
		lane 8	82.96		193.71
		lane 9	80.31		193.71
5.1	58.54 %	lane 1	62.70	340.86	341.83
		lane 2	59.43		333.08
		lane 3	54.21		333.08
		lane 4	47.72		350.58
		lane 5	47.67		350.58
		lane 6	46.38		350.58
		lane 7	72.37		333.08
		lane 8	69.44		333.08
		lane 9	66.99		341.83
5.1	54.99 %	lane 1	60.73	285.19	297.74
		lane 2	65.09		297.74
		lane 3	66.85		297.74
		lane 4	51.78		281.56
		lane 5	46.88		281.56
		lane 6	51.60		281.56
		lane 7	49.04		273.64
		lane 8	52.83		273.64
		lane 9	50.13		281.56
5.1	68.57 %	lane 1	71.50	299.43	298.60
		lane 2	72.88		298.60
		lane 3	71.77		298.60
		lane 4	66.44		291.21
		lane 5	69.29		298.60

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 6	67.22		298.60
		lane 7	67.36		306.00
		lane 8	67.03		306.00
		lane 9	63.65		298.60
5.2	82.38 %	lane 1	83.48	291.35	292.84
		lane 2	85.88		286.26
		lane 3	81.05		286.26
		lane 4	83.64		292.84
		lane 5	85.08		292.84
		lane 6	81.89		299.12
		lane 7	85.36		292.84
		lane 8	79.03		292.84
		lane 9	76.05		286.26
5.2	61.81 %	lane 1	65.70	290.80	297.95
		lane 2	69.80		297.95
		lane 3	67.44		297.95
		lane 4	30.61		297.95
		lane 5	43.74		289.91
		lane 6	33.68		281.86
		lane 7	80.24		281.86
		lane 8	83.38		281.86
		lane 9	81.67		289.91
5.2	59.95 %	lane 1	60.73	264.80	297.74
		lane 2	65.09		297.74
		lane 3	66.85		297.74
		lane 4	51.78		281.56
		lane 5	46.88		281.56
		lane 6	51.60		281.56
		lane 7	49.04		273.64
		lane 8	52.83		273.64
		lane 9	50.13		281.56
6.1	71.72 %	lane 1	72.16	226.77	223.06
		lane 2	82.50		223.06
		lane 3	64.10		223.06
		lane 4	61.82		223.06
		lane 5	65.09		223.06
		lane 6	69.93		231.39
		lane 7	78.67		231.39
		lane 8	81.54		231.39
		lane 9	69.63		231.39
6.2	88.59 %	lane 1	82.02	217.32	217.32
		lane 2	86.50		217.32
		lane 3	83.95		217.32
		lane 4	92.74		217.32
		lane 5	93.29		217.32
		lane 6	92.62		226.27
		lane 7	90.39		208.37
		lane 8	91.51		217.32
		lane 9	84.32		217.32
7.1	59.23 %	lane 1	44.88	311.97	314.89
		lane 2	60.86		322.98

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 3	59.87		322.98
		lane 4	65.98		322.98
		lane 5	72.53		322.98
		lane 6	68.39		322.98
		lane 7	53.50		298.32
		lane 8	56.25		289.83
		lane 9	50.83		289.83
7.1	60.66 %	lane 1	56.95	299.34	306.00
		lane 2	64.01		306.00
		lane 3	59.14		297.20
		lane 4	67.93		306.00
		lane 5	69.16		306.00
		lane 6	66.99		306.00
		lane 7	58.55		306.00
		lane 8	51.27		280.41
		lane 9	51.94		280.41
7.2	69.79 %	lane 1	54.32	297.29	298.32
		lane 2	66.15		306.40
		lane 3	63.31		314.89
		lane 4	59.39		289.83
		lane 5	78.24		289.83
		lane 6	81.83		298.32
		lane 7	74.73		289.83
		lane 8	79.41		298.32
		lane 9	70.77		289.83
8.1	51.04 %	lane 1	54.43	312.53	341.28
		lane 2	58.33		341.28
		lane 3	50.01		323.44
		lane 4	56.01		323.44
		lane 5	56.46		323.44
		lane 6	57.12		323.44
		lane 7	47.36		278.83
		lane 8	43.98		278.83
		lane 9	35.62		278.83
8.1	58.36 %	lane 1	59.03	319.60	314.77
		lane 2	58.29		314.77
		lane 3	53.47		314.77
		lane 4	59.16		323.54
		lane 5	57.81		323.54
		lane 6	55.21		314.77
		lane 7	60.61		331.91
		lane 8	63.51		323.54
		lane 9	58.10		314.77
8.2	79.31 %	lane 1	87.63	205.20	184.65
		lane 2	80.30		184.65
		lane 3	73.52		192.85
		lane 4	80.21		184.65
		lane 5	84.83		184.65
		lane 6	81.15		176.81
		lane 7	80.51		176.81
		lane 8	77.69		176.81

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 9	67.99		168.61
9.1	85.48 %	lane 1	85.03	265.65	260.52
		lane 2	89.00		260.52
		lane 3	84.62		268.21
		lane 4	86.67		268.21
		lane 5	90.03		268.21
		lane 6	84.57		268.21
		lane 7	88.47		260.52
		lane 8	89.41		268.21
		lane 9	71.53		268.21
9.2	91.60 %	lane 1	95.15	246.46	233.81
		lane 2	94.30		258.00
		lane 3	93.46		266.18
		lane 4	90.16		242.00
		lane 5	89.99		242.00
		lane 6	86.58		242.00
		lane 7	91.89		250.18
		lane 8	93.63		242.00
		lane 9	89.28		242.00
10.1	44.83 %	lane 1	40.20	380.73	379.64
		lane 2	46.96		379.64
		lane 3	46.16		379.64
		lane 4	40.63		379.64
		lane 5	44.15		379.64
		lane 6	42.98		379.64
		lane 7	48.36		370.71
		lane 8	48.41		389.01
		lane 9	45.64		389.01
10.2	76.02 %	lane 1	72.86	254.19	274.98
		lane 2	76.35		274.98
		lane 3	76.06		267.05
		lane 4	73.20		259.48
		lane 5	86.16		259.48
		lane 6	71.78		259.48
		lane 7	74.78		236.04
		lane 8	77.83		228.10
		lane 9	75.17		228.10
11.1	51.92 %	lane 1	42.78	355.75	350.73
		lane 2	45.48		350.73
		lane 3	47.69		359.76
		lane 4	44.98		359.76
		lane 5	48.96		359.76
		lane 6	48.49		359.76
		lane 7	64.46		359.76
		lane 8	63.16		350.73
		lane 9	61.26		350.73
11.1	52.68 %	lane 1	48.36	325.67	323.70
		lane 2	47.86		323.70
		lane 3	48.95		332.55
		lane 4	56.48		332.55
		lane 5	56.23		332.55

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 6	55.05		323.70
		lane 7	54.52		323.70
		lane 8	56.71		323.70
		lane 9	50.01		314.85
11.2	74.98 %	lane 1	68.85	278.06	289.26
		lane 2	77.16		297.45
		lane 3	63.58		280.71
		lane 4	74.27		280.71
		lane 5	76.30		280.71
		lane 6	72.06		280.71
		lane 7	78.95		264.33
		lane 8	84.10		264.33
		lane 9	79.53		264.33
12.1	78.00 %	lane 1	59.84	259.43	246.24
		lane 2	65.32		237.41
		lane 3	70.81		246.24
		lane 4	83.53		263.08
		lane 5	89.00		263.08
		lane 6	83.78		263.08
		lane 7	84.54		271.91
		lane 8	84.22		271.91
		lane 9	80.97		271.91
12.1	78.52 %	lane 1	65.19	253.86	246.22
		lane 2	69.38		254.81
		lane 3	67.77		263.41
		lane 4	84.52		254.81
		lane 5	84.20		246.22
		lane 6	85.24		263.41
		lane 7	85.34		272.01
		lane 8	83.42		246.22
		lane 9	81.63		237.62
12.2	89.28 %	lane 1	91.10	198.68	193.78
		lane 2	91.73		215.82
		lane 3	89.15		204.80
		lane 4	84.59		193.78
		lane 5	90.77		193.78
		lane 6	87.51		204.80
		lane 7	87.58		193.78
		lane 8	90.20		193.78
		lane 9	90.88		193.78
12.2	72.46 %	lane 1	58.52	145.93	151.66
		lane 2	71.98		151.66
		lane 3	69.49		143.06
		lane 4	69.84		143.06
		lane 5	75.63		143.06
		lane 6	70.23		143.06
		lane 7	81.23		143.06
		lane 8	79.80		143.06
		lane 9	75.39		151.66
12.2	73.99 %	lane 1	81.33	171.63	177.25
		lane 2	76.12		168.83

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 3	67.94		177.25
		lane 4	68.94		168.83
		lane 5	67.82		177.25
		lane 6	69.75		168.83
		lane 7	77.05		168.83
		lane 8	80.85		168.83
		lane 9	76.12		168.83
13.1	59.15 %	lane 1	47.72	294.85	280.90
		lane 2	52.98		280.90
		lane 3	55.73		289.27
		lane 4	64.41		306.00
		lane 5	65.15		297.63
		lane 6	64.39		297.63
		lane 7	58.11		306.00
		lane 8	63.94		297.63
		lane 9	59.93		297.63
13.2	72.48 %	lane 1	69.62	279.12	281.85
		lane 2	77.17		281.85
		lane 3	71.89		273.67
		lane 4	60.36		281.85
		lane 5	74.31		273.67
		lane 6	57.46		273.67
		lane 7	84.89		281.85
		lane 8	84.94		281.85
		lane 9	71.71		281.85
14.1	71.85 %	lane 1	71.46	388.03	372.63
		lane 2	72.53		383.65
		lane 3	76.10		395.17
		lane 4	73.60		406.19
		lane 5	72.07		395.17
		lane 6	73.16		395.17
		lane 7	70.07		383.65
		lane 8	65.77		372.63
		lane 9	-		-
14.2	90.20 %	lane 1	86.58	315.30	316.52
		lane 2	88.52		316.52
		lane 3	87.51		316.52
		lane 4	87.51		305.50
		lane 5	90.84		316.52
		lane 6	90.64		316.52
		lane 7	93.46		316.52
		lane 8	94.46		316.52
		lane 9	92.27		316.52
15.1	60.54 %	lane 1	51.91	350.86	330.56
		lane 2	67.12		338.86
		lane 3	69.52		338.86
		lane 4	60.98		355.48
		lane 5	54.01		338.86
		lane 6	56.82		347.17
		lane 7	63.05		363.78
		lane 8	62.13		372.09

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 9	59.33		372.09
15.1	48.25 %	lane 1	45.55	287.43	287.34
		lane 2	39.99		296.88
		lane 3	35.66		306.00
		lane 4	60.62		287.34
		lane 5	54.03		287.34
		lane 6	49.59		287.34
		lane 7	51.66		278.22
		lane 8	49.21		278.22
		lane 9	47.91		278.22
15.2	70.67 %	lane 1	72.12	263.18	265.80
		lane 2	66.31		257.83
		lane 3	70.64		265.80
		lane 4	69.49		274.13
		lane 5	74.23		265.80
		lane 6	71.92		265.80
		lane 7	74.29		257.83
		lane 8	72.23		257.83
		lane 9	64.81		257.83
16.1	58.90 %	lane 1	58.72	297.95	297.95
		lane 2	67.82		289.91
		lane 3	59.65		289.91
		lane 4	54.73		289.91
		lane 5	47.88		281.86
		lane 6	57.34		289.91
		lane 7	63.85		314.05
		lane 8	61.35		314.05
		lane 9	58.73		314.05
16.1	58.19 %	lane 1	58.44	358.93	347.79
		lane 2	62.38		356.15
		lane 3	60.48		356.15
		lane 4	57.50		372.86
		lane 5	58.17		364.50
		lane 6	55.18		364.50
		lane 7	54.24		356.15
		lane 8	60.61		364.50
		lane 9	56.75		347.79
16.1	63.71 %	lane 1	54.26	316.04	314.21
		lane 2	60.60		314.21
		lane 3	55.76		314.21
		lane 4	70.18		322.43
		lane 5	67.37		322.43
		lane 6	61.61		314.21
		lane 7	67.80		314.21
		lane 8	70.34		314.21
		lane 9	65.46		314.21
16.2	88.22 %	lane 1	89.16	194.24	193.34
		lane 2	85.77		193.34
		lane 3	84.54		185.30
		lane 4	82.15		193.34
		lane 5	87.26		193.34

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 6	87.89		193.34
		lane 7	93.43		193.34
		lane 8	93.47		193.34
		lane 9	90.34		209.44
16.2	89.15 %	lane 1	89.09	218.22	222.79
		lane 2	92.41		222.79
		lane 3	88.02		222.79
		lane 4	87.23		214.57
		lane 5	90.86		222.79
		lane 6	84.45		222.79
		lane 7	94.82		214.57
		lane 8	90.01		214.57
		lane 9	85.42		206.36
16.2	85.50 %	lane 1	87.04	188.15	223.22
		lane 2	88.33		214.86
		lane 3	87.00		223.22
		lane 4	87.25		173.47
		lane 5	77.43		173.47
		lane 6	60.08		206.50
		lane 7	87.93		165.11
		lane 8	89.56		156.75
		lane 9	79.46		156.75
17.1	65.55 %	lane 1	54.83	336.81	322.98
		lane 2	57.99		322.98
		lane 3	63.88		331.28
		lane 4	71.82		356.16
		lane 5	72.89		356.16
		lane 6	72.14		356.16
		lane 7	71.08		322.98
		lane 8	65.08		331.28
		lane 9	60.19		331.28
17.2	81.51 %	lane 1	83.27	140.13	156.71
		lane 2	86.39		140.13
		lane 3	85.89		140.13
		lane 4	74.04		140.13
		lane 5	86.36		140.13
		lane 6	85.44		140.13
		lane 7	76.67		131.83
		lane 8	83.84		123.54
		lane 9	71.68		148.42
18.1	53.97 %	lane 1	53.34	388.72	367.94
		lane 2	48.69		367.94
		lane 3	57.05		385.75
		lane 4	53.52		385.75
		lane 5	53.23		385.75
		lane 6	48.32		385.75
		lane 7	52.65		403.57
		lane 8	53.95		403.57
		lane 9	64.99		412.48
18.2	86.07 %	lane 1	91.33	289.17	288.18
		lane 2	80.23		288.18

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 3	76.51		279.27
		lane 4	85.73		288.18
		lane 5	89.64		288.18
		lane 6	87.52		288.18
		lane 7	85.85		297.09
		lane 8	89.00		288.18
		lane 9	88.83		297.09
19.1	72.95 %	lane 1	67.84	308.89	314.68
		lane 2	75.32		314.68
		lane 3	77.25		314.68
		lane 4	70.07		306.00
		lane 5	78.83		306.00
		lane 6	71.78		306.00
		lane 7	69.15		306.00
		lane 8	73.45		306.00
		lane 9	72.83		306.00
19.2	85.97 %	lane 1	86.07	114.32	117.22
		lane 2	89.09		108.53
		lane 3	85.82		117.22
		lane 4	79.59		125.90
		lane 5	88.06		117.22
		lane 6	84.91		125.90
		lane 7	84.43		108.53
		lane 8	89.42		99.85
		lane 9	86.32		108.53
20.1	73.41 %	lane 1	66.57	161.90	172.54
		lane 2	69.49		172.54
		lane 3	71.36		163.99
		lane 4	67.79		163.99
		lane 5	68.69		163.99
		lane 6	65.44		163.99
		lane 7	83.93		155.01
		lane 8	87.01		155.01
		lane 9	80.42		146.02
20.2	88.68 %	lane 1	90.76	155.01	146.02
		lane 2	90.08		155.01
		lane 3	89.48		155.01
		lane 4	89.73		146.02
		lane 5	92.06		163.99
		lane 6	90.81		163.99
		lane 7	82.83		155.01
		lane 8	87.78		155.01
		lane 9	84.61		155.01
20.2	91.27 %	lane 1	89.49	206.76	212.00
		lane 2	83.89		212.00
		lane 3	90.78		204.08
		lane 4	91.26		212.00
		lane 5	93.29		204.08
		lane 6	93.54		196.52
		lane 7	92.32		212.00
		lane 8	93.19		204.08

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 9	93.68		204.08
21.1	69.30 %	lane 1	-	321.64	314.98
		lane 2	75.64		314.98
		lane 3	75.65		306.41
		lane 4	63.44		340.69
		lane 5	70.09		332.12
		lane 6	71.15		349.26
		lane 7	59.37		306.41
		lane 8	69.41		314.98
		lane 9	69.62		314.98
21.2	90.11 %	lane 1	93.28	199.35	203.16
		lane 2	92.63		194.59
		lane 3	90.67		194.59
		lane 4	87.19		194.59
		lane 5	91.02		194.59
		lane 6	88.50		194.59
		lane 7	90.17		203.16
		lane 8	90.11		211.73
		lane 9	87.41		203.16
22.1	61.01 %	lane 1	51.65	297.72	297.59
		lane 2	60.98		297.59
		lane 3	56.72		297.59
		lane 4	72.03		314.41
		lane 5	77.35		314.41
		lane 6	79.73		314.41
		lane 7	51.28		281.17
		lane 8	52.44		281.17
		lane 9	46.87		281.17
22.2	80.03 %	lane 1	77.44	183.32	181.45
		lane 2	81.23		181.45
		lane 3	81.14		181.45
		lane 4	80.28		181.45
		lane 5	76.12		181.45
		lane 6	69.25		181.45
		lane 7	86.74		181.45
		lane 8	86.92		189.86
		lane 9	81.19		189.86
22.2	78.53 %	lane 1	74.90	290.24	217.13
		lane 2	71.48		217.13
		lane 3	71.66		217.13
		lane 4	75.56		208.01
		lane 5	76.77		208.01
		lane 6	80.06		208.01
		lane 7	86.22		217.13
		lane 8	84.05		217.13
		lane 9	86.04		217.13
23.1	61.12 %	lane 1	48.82	261.77	289.57
		lane 2	54.11		289.57
		lane 3	52.92		297.59
		lane 4	47.23		256.33
		lane 5	62.31		256.33

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 6	43.17		247.93
		lane 7	73.98		239.52
		lane 8	86.01		239.52
		lane 9	81.50		239.52
	62.81 %	lane 1	77.49	309.75	297.17
		lane 2	73.08		297.17
		lane 3	71.72		297.17
		lane 4	68.86		306.00
		lane 5	65.02		306.00
		lane 6	55.70		306.00
		lane 7	58.51		331.71
		lane 8	52.93		323.27
		lane 9	41.99		323.27
23.2	72.76 %	lane 1	81.05	251.43	254.21
		lane 2	64.99		254.21
		lane 3	61.70		254.21
		lane 4	72.74		245.88
		lane 5	74.09		254.21
		lane 6	71.37		245.88
		lane 7	70.42		254.21
		lane 8	83.76		254.21
		lane 9	74.72		245.88
24.1	54.21 %	lane 1	45.78	247.05	243.43
		lane 2	43.87		251.39
		lane 3	53.26		266.94
		lane 4	62.31		235.84
		lane 5	63.62		235.84
		lane 6	55.57		235.84
		lane 7	54.21		251.39
		lane 8	57.08		251.39
		lane 9	52.21		251.39
24.1	50.56 %	lane 1	48.22	319.16	306.31
		lane 2	47.62		306.31
		lane 3	44.41		306.31
		lane 4	36.85		328.36
		lane 5	41.28		320.91
		lane 6	38.89		328.36
		lane 7	66.70		328.36
		lane 8	67.46		328.36
		lane 9	63.62		320.91
24.2	71.15 %	lane 1	69.60	289.13	275.50
		lane 2	64.22		283.20
		lane 3	57.16		283.20
		lane 4	76.75		283.20
		lane 5	79.90		290.91
		lane 6	70.37		283.20
		lane 7	70.41		290.91
		lane 8	70.85		290.91
		lane 9	81.07		321.09
24.2	68.59 %	lane 1	57.55	212.72	210.26
		lane 2	69.89		217.52

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 3	52.90		210.26
		lane 4	76.38		210.26
		lane 5	72.98		203.00
		lane 6	79.22		210.26
		lane 7	71.77		225.12
		lane 8	75.95		217.52
		lane 9	60.68		210.26
25.1	75.77 %	lane 1	72.21	306.50	305.63
		lane 2	76.85		305.63
		lane 3	72.82		305.63
		lane 4	71.04		305.63
		lane 5	71.52		305.63
		lane 6	78.56		313.49
		lane 7	79.73		297.77
		lane 8	77.23		305.63
		lane 9	81.97		313.49
25.2	91.88 %	lane 1	90.94	250.23	250.98
		lane 2	94.35		250.98
		lane 3	92.59		250.98
		lane 4	94.11		250.98
		lane 5	94.35		258.84
		lane 6	93.52		258.84
		lane 7	89.95		243.50
		lane 8	90.26		243.50
		lane 9	86.89		243.50
26.1	68.02 %	lane 1	56.56	290.93	284.74
		lane 2	51.74		284.74
		lane 3	63.90		291.71
		lane 4	75.39		299.03
		lane 5	86.82		299.03
		lane 6	86.93		299.03
		lane 7	58.12		291.71
		lane 8	64.01		291.71
		lane 9	68.74		284.74
26.2	80.12 %	lane 1	79.65	264.09	263.15
		lane 2	87.27		263.15
		lane 3	77.95		254.66
		lane 4	78.56		271.64
		lane 5	73.45		271.64
		lane 6	80.60		263.15
		lane 7	83.10		263.15
		lane 8	80.94		263.15
		lane 9	79.61		263.15
26.2	76.20 %	lane 1	75.72	237.67	234.57
		lane 2	81.42		234.57
		lane 3	73.58		234.57
		lane 4	75.41		234.57
		lane 5	75.81		234.57
		lane 6	66.95		241.54
		lane 7	78.27		241.54
		lane 8	81.70		241.54

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 9	76.90		241.54
27.1	47.40 %	lane 1	46.21	334.16	338.74
		lane 2	42.43		338.74
		lane 3	46.09		338.74
		lane 4	55.40		338.74
		lane 5	50.63		338.74
		lane 6	49.99		338.74
		lane 7	41.86		330.66
		lane 8	46.36		322.17
		lane 9	47.65		322.17
27.2	68.06 %	lane 1	70.27	238.31	247.39
		lane 2	72.54		239.30
		lane 3	66.90		239.30
		lane 4	71.61		239.30
		lane 5	74.57		239.30
		lane 6	71.13		239.30
		lane 7	67.44		230.81
		lane 8	59.10		230.81
		lane 9	58.96		239.30
28.1	62.40 %	lane 1	58.25	294.89	298.43
		lane 2	58.62		290.47
		lane 3	57.68		298.43
		lane 4	69.97		298.43
		lane 5	72.21		298.43
		lane 6	69.25		298.43
		lane 7	57.41		290.47
		lane 8	59.58		290.47
		lane 9	58.62		290.47
28.1	77.90 %	lane 1	77.18	336.92	335.98
		lane 2	78.55		343.74
		lane 3	74.22		335.98
		lane 4	78.20		328.58
		lane 5	79.96		328.58
		lane 6	73.76		320.82
		lane 7	80.42		343.74
		lane 8	80.29		351.15
		lane 9	78.51		343.74
28.1	60.00 %	lane 1	72.11	292.67	290.15
		lane 2	72.38		297.72
		lane 3	47.21		297.72
		lane 4	50.44		290.15
		lane 5	41.78		290.15
		lane 6	41.07		290.15
		lane 7	69.84		297.72
		lane 8	72.22		290.15
		lane 9	72.96		290.15
28.2	71.35 %	lane 1	74.80	296.52	298.24
		lane 2	58.39		290.48
		lane 3	66.78		283.07
		lane 4	69.79		298.24
		lane 5	79.19		298.24

IND.	DNA FTM avg. (%)	Lane	DNA-FTM (%) raw data	MML (kbp) avg.	MML(kbp) raw data
		lane 6	73.54		298.24
		lane 7	77.46		305.65
		lane 8	70.89		298.24
		lane 9	71.35		298.24

B.6 Standard deviation and coefficient of variation gels

Table B.7: DNA-fraction of total DNA that migrated into the gel (DNA-FTM (%)) and median molecular length (MML), measured in kilobase pairs (kbp) in each individual female common eider (*Somateria mollissima*) in Christiansø, Denmark on day 5 and day 25 of incubation. The first column represents the individual (IND.) and the first number before the decimal place represents the individual bird's identification number (ID) and the number after the decimal represents the time of incubation (1 for day 5 and 2 for day 25). Averages are presented between one samples in one gel. Standard deviations (SD) and coefficient of variations (CV) are presented between all lanes, triplicates and parallels in one gel.

DNA FTM (%)								MML (kpb)								
IND.	DNA-FTM	SD all	CV all			CV trip.	SD parallels	CV parallels	MML	SD all	CV	SD trip.		CV trip.	SD parallels	CV parallels
2.1	69.46	8.10	11.67	1st 3 lines	2.76	4.01 %	8.68	0.12	308.77	4.16	1.35	1st 3 lines	0.00	0.00 %	4.80	1.56 %
				2nd 3 lines	2.96	3.77 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	4.50	7.38 %						3rd 3 lines	0.00	0.00 %		
2.1	73.83	4.44	6.02	1st 3 lines	5.40	7.49 %	1.69	0.02	318.82	13.44	4.21	1st 3 lines	10.20	3.27 %	9.52	2.99 %
				2nd 3 lines	4.56	6.05 %						2nd 3 lines	15.41	4.89 %		
				3rd 3 lines	4.51	6.11 %						3rd 3 lines	10.42	3.16 %		
2.1	74.07	3.87	5.22	1st 3 lines	1.62	2.17 %	3.72	0.05	309.24	21.17	6.85	1st 3 lines	0.00	0.00 %	24.05	7.78 %
				2nd 3 lines	3.02	4.31 %						2nd 3 lines	5.38	1.91 %		
				3rd 3 lines	2.54	3.28 %						3rd 3 lines	5.38	1.67 %		
2.2	81.51	7.85	9.63	1st 3 lines	4.59	5.78 %	8.47	0.10	256.63	6.56	2.56	1st 3 lines	5.21	1.97 %	6.95	2.71 %
				2nd 3 lines	2.54	3.42 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.98	2.18 %						3rd 3 lines	0.00	0.00 %		
3.1	49.24	4.04	8.20	1st 3 lines	2.45	4.61 %	3.46	0.07	341.35	25.51	7.47	1st 3 lines	0.00	0.00 %	28.89	8.46 %
				2nd 3 lines	3.84	8.19 %						2nd 3 lines	8.58	2.66 %		
				3rd 3 lines	2.93	6.15 %						3rd 3 lines	5.06	1.55 %		
3.1	41.66	8.73	20.95	1st 3 lines	2.30	6.27 %	9.77	0.23	344.49	4.61	1.34	1st 3 lines	0.00	0.00 %	4.45	1.29 %
				2nd 3 lines	0.93	2.63 %						2nd 3 lines	5.05	1.47 %		
				3rd 3 lines	3.52	6.65 %						3rd 3 lines	0.00	0.00 %		
3.2	72.75	1.31	1.80	1st 3 lines	3.19	3.73 %	1.42	0.20	255.55	12.39	4.85	1st 3 lines	0.00	0.00 %	14.03	5.49 %
				2nd 3 lines	0.21	0.28 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	4.53	7.90 %						3rd 3 lines	4.85	1.95 %		
5.1	73.16	10.06	13.76	1st 3 lines	11.30	16.17 %	8.94	0.12	307.84	5.53	1.80	1st 3 lines	0.00	0.00 %	5.76	1.87 %
				2nd 3 lines	3.23	3.88 %						2nd 3 lines	4.79	1.58 %		
				3rd 3 lines	5.25	7.91 %						3rd 3 lines	0.00	0.00 %		
5.1	77.51	7.69	9.93	1st 3 lines	5.81	7.67 %	5.45	0.07	333.59	7.00	2.10	1st 3 lines	5.29	1.62 %	6.23	1.87 %
				2nd 3 lines	9.33	12.76 %						2nd 3 lines	5.06	1.51 %		
				3rd 3 lines	5.19	6.21 %						3rd 3 lines	5.06	1.50 %		
5.2	74.69	5.63	7.53	1st 3 lines	3.21	4.03 %	4.28	0.06	282.13	3.66	1.30	1st 3 lines	4.79	1.68 %	3.19	1.13 %
				2nd 3 lines	7.45	10.21 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	2.42	3.39 %						3rd 3 lines	0.00	0.00 %		
7.1	55.16	7.46	13.53	1st 3 lines	2.75	5.51 %	5.43	0.10	320.60	8.15	2.54	1st 3 lines	4.46	1.40 %	2.98	0.93 %
				2nd 3 lines	9.06	16.54 %						2nd 3 lines	11.81	3.64 %		
				3rd 3 lines	6.68	11.00 %						3rd 3 lines	8.93	2.80 %		
7.1	57.40	6.19	10.78	1st 3 lines	3.40	6.16 %	5.68	0.10	336.54	10.02	2.98	1st 3 lines	5.12	1.47 %	9.50	2.82 %
				2nd 3 lines	3.42	6.43 %						2nd 3 lines	10.24	3.11 %		

DNA FTM (%)									MML (kpb)							
IND.	DNA-FTM	SD all	CV all			CV trip.	SD parallels	CV parallels	MML	SD all	CV	SD trip.		CV trip.	SD parallels	CV parallels
				3rd 3 lines	5.78	9.05 %						3rd 3 lines	0.00	0.00 %		
7.2	74.06	9.63	13.01	1st 3 lines	11.16	17.37 %	9.00	0.12	195.89	30.39	15.52	1st 3 lines	51.53	24.14 %	15.82	8.07 %
				2nd 3 lines	1.16	1.53 %						2nd 3 lines	16.58	9.07 %		
				3rd 3 lines	1.44	1.76 %						3rd 3 lines	3.83	2.00 %		
8.1	58.54	10.01	17.10	1st 3 lines	4.28	7.28 %	11.17	0.19	340.86	8.12	2.38	1st 3 lines	5.05	1.50 %	8.42	2.47 %
				2nd 3 lines	0.76	1.61 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	2.70	3.88 %						3rd 3 lines	5.05	1.50 %		
8.1	54.99	7.31	13.28	1st 3 lines	3.15	4.90 %	8.00	0.15	285.19	9.95	3.49	1st 3 lines	0.00	0.00 %	11.18	3.92 %
				2nd 3 lines	2.78	5.55 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.95	3.84 %						3rd 3 lines	4.57	1.65 %		
8.1	68.57	3.01	4.39	1st 3 lines	0.74	1.02 %	3.12	0.05	299.43	4.44	1.48	1st 3 lines	0.00	0.00 %	3.77	1.26 %
				2nd 3 lines	1.47	2.18 %						2nd 3 lines	4.27	1.44 %		
				3rd 3 lines	2.05	3.11 %						3rd 3 lines	4.27	1.41 %		
8.2	82.38	3.25	3.94	1st 3 lines	2.41	2.89 %	1.94	0.02	291.35	4.32	1.48	1st 3 lines	3.80	1.32 %	3.30	1.13 %
				2nd 3 lines	1.60	1.91 %						2nd 3 lines	3.63	1.23 %		
				3rd 3 lines	4.75	5.93 %						3rd 3 lines	3.80	1.31 %		
8.2	61.81	20.62	33.36	1st 3 lines	2.06	3.05 %	23.43	0.38	290.80	7.47	2.57	1st 3 lines	0.00	0.00 %	6.75	2.32 %
				2nd 3 lines	6.87	19.07 %						2nd 3 lines	8.05	2.78 %		
				3rd 3 lines	1.57	1.92 %						3rd 3 lines	4.65	1.63 %		
8.2	59.95	11.17	18.64	1st 3 lines	3.54	6.06 %	12.18	0.20	264.80	2.75	1.04	1st 3 lines	0.00	0.00 %	1.59	0.60 %
				2nd 3 lines	2.73	3.75 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	5.87	12.07 %						3rd 3 lines	4.77	1.81 %		
9.1	71.72	7.66	10.67	1st 3 lines	9.22	12.65 %	5.60	0.08	226.77	4.39	1.94	1st 3 lines	0.00	0.00 %	4.24	1.87 %
				2nd 3 lines	4.08	6.22 %						2nd 3 lines	4.81	2.13 %		
				3rd 3 lines	6.22	8.12 %						3rd 3 lines	0.00	0.00 %		
9.2	88.59	4.40	4.96	1st 3 lines	2.25	2.67 %	4.37	0.05	217.32	4.47	2.06	1st 3 lines	0.00	0.00 %	2.98	1.37 %
				2nd 3 lines	3.55	0.38 %						2nd 3 lines	5.17	2.35 %		
				3rd 3 lines	3.87	4.36 %						3rd 3 lines	5.17	2.41 %		
10.1	59.23	8.86	14.96	1st 3 lines	8.96	16.23 %	7.95	0.13	311.97	14.92	4.78	1st 3 lines	4.67	1.46 %	16.78	5.38 %
				2nd 3 lines	3.31	6.00 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	2.71	3.93 %						3rd 3 lines	4.90	1.67 %		
10.1	60.66	6.72	11.08	1st 3 lines	3.61	6.02 %	7.08	0.12	299.34	11.11	3.71	1st 3 lines	5.08	1.68 %	9.12	3.05 %
				2nd 3 lines	1.08	1.59 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	4.02	7.46 %						3rd 3 lines	14.77	5.11 %		
10.2	69.79	9.60	13.75	1st 3 lines	6.18	10.08 %	7.45	0.11	297.29	8.79	2.96	1st 3 lines	8.29	2.70 %	8.01	2.70 %
				2nd 3 lines	12.06	16.48 %						2nd 3 lines	4.90	1.67 %		
				3rd 3 lines	4.32	5.77 %						3rd 3 lines	4.90	1.67 %		
12.1	51.04	7.56	14.82	1st 3 lines	4.16	7.68 %	7.63	0.15	312.53	26.31	8.42	1st 3 lines	10.30	3.07 %	29.79	9.53 %
				2nd 3 lines	0.56	0.99 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	6.04	14.28 %						3rd 3 lines	0.00	0.00 %		
12.1	58.36	2.89	4.95	1st 3 lines	3.02	5.30 %	2.08	0.04	319.60	6.27	1.96	1st 3 lines	0.00	0.00 %	4.41	1.38 %
				2nd 3 lines	2.01	3.50 %						2nd 3 lines	5.06	1.58 %		

DNA FTM (%)									MML (kpb)						
IND.	DNA-FTM	SD all	CV all			CV trip.	SD parallels	CV parallels	MML	SD all	CV	SD trip.		CV trip.	SD parallels	CV parallels
				3rd 3 lines	2.71	4.45 %						3rd 3 lines	8.57	2.65 %		
12.2	79.31	5.82	7.33	1st 3 lines	7.06	8.77 %	3.48	0.04	205.20	6.48	3.16	1st 3 lines	4.90	2.32 %	5.77	2.81 %
				2nd 3 lines	2.45	2.98 %						2nd 3 lines	4.68	2.30 %		
				3rd 3 lines	6.57	8.71 %						3rd 3 lines	4.68	2.33 %		
13.1	85.48	5.65	6.61	1st 3 lines	2.42	2.81 %	2.08	0.02	265.65	3.85	1.45	1st 3 lines	4.44	1.69 %	2.57	0.97 %
				2nd 3 lines	2.75	3.16 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	10.06	12.10 %						3rd 3 lines	4.44	1.67 %		
13.2	91.60	2.80	3.06	1st 3 lines	0.85	0.90 %	2.70	0.03	246.46	9.97	4.05	1st 3 lines	16.83	6.66 %	5.54	2.25 %
				2nd 3 lines	2.02	2.28 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	2.19	2.39 %						3rd 3 lines	4.73	1.93 %		
14.1	44.83	3.07	6.84	1st 3 lines	3.69	8.31 %	2.47	0.05	380.73	5.53	1.45	1st 3 lines	0.00	0.00 %	1.89	0.50 %
				2nd 3 lines	1.79	4.20 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.58	3.34 %						3rd 3 lines	10.56	2.76 %		
14.2	76.02	4.25	5.59	1st 3 lines	1.94	2.58 %	0.98	0.01	254.19	18.72	7.37	1st 3 lines	4.58	1.68 %	21.30	8.38 %
				2nd 3 lines	7.92	10.29 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.66	2.19 %						3rd 3 lines	4.58	1.99 %		
15.1	51.92	8.53	16.44	1st 3 lines	2.46	5.42 %	9.63	0.19	355.75	4.75	1.34	1st 3 lines	5.21	1.47 %	3.47	0.98 %
				2nd 3 lines	2.17	4.58 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.61	2.56 %						3rd 3 lines	5.21	1.47 %		
15.1	52.68	3.80	7.20	1st 3 lines	0.55	1.13 %	3.88	0.07	325.67	5.90	1.81	1st 3 lines	5.11	1.56 %	4.51	1.38 %
				2nd 3 lines	0.76	1.36 %						2nd 3 lines	5.11	1.55 %		
				3rd 3 lines	3.42	6.36 %						3rd 3 lines	5.11	1.59 %		
15.2	74.98	6.15	8.21	1st 3 lines	6.85	9.80 %	5.54	0.07	278.06	11.70	4.21	1st 3 lines	8.37	2.89 %	12.62	4.54 %
				2nd 3 lines	2.12	2.86 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	2.82	3.49 %						3rd 3 lines	0.00	0.00 %		
16.1	78.00	10.11	12.96	1st 3 lines	5.48	8.39 %	11.04	0.14	259.43	12.94	4.99	1st 3 lines	5.09	2.09 %	14.65	5.65 %
				2nd 3 lines	3.09	3.61 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.97	2.37 %						3rd 3 lines	0.00	0.00 %		
16.1	78.52	8.44	10.75	1st 3 lines	2.11	3.13 %	9.61	0.12	253.86	10.91	4.30	1st 3 lines	8.60	3.37 %	1.65	0.65 %
				2nd 3 lines	0.53	0.63 %						2nd 3 lines	8.60	3.37 %		
				3rd 3 lines	1.86	2.23 %						3rd 3 lines	17.89	7.10 %		
16.2	89.28	5.43	6.09	1st 3 lines	1.35	1.49 %	1.54	0.02	198.68	11.82	5.95	1st 3 lines	11.02	5.38 %	5.61	2.82 %
				2nd 3 lines	3.09	3.52 %						2nd 3 lines	6.36	3.22 %		
				3rd 3 lines	1.74	1.94 %						3rd 3 lines	0.00	0.00 %		
16.2	72.46	6.75	9.31	1st 3 lines	7.16	10.74 %	6.09	0.08	145.93	4.30	2.95	1st 3 lines	4.96	3.34 %	2.87	1.96 %
				2nd 3 lines	3.23	4.49 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	3.04	3.86 %						3rd 3 lines	4.96	3.40 %		
16.2	73.99	5.45	7.36	1st 3 lines	6.75	8.98 %	4.69	0.06	171.63	4.21	2.45	1st 3 lines	4.86	2.79 %	2.81	1.64 %
				2nd 3 lines	0.97	1.41 %						2nd 3 lines	4.86	2.83 %		
				3rd 3 lines	2.50	3.21 %						3rd 3 lines	0.00	0.00 %		
17.1	59.15	6.08	10.28	1st 3 lines	4.07	780.43 %	6.39	0.11	294.85	9.35	3.17	1st 3 lines	4.83	1.70 %	9.66	3.28 %
				2nd 3 lines	0.44	67.46 %						2nd 3 lines	4.83	1.61 %		
				3rd 3 lines	2.98	491.62 %						3rd 3 lines	4.83	1.61 %		

DNA FTM (%)									MML (kpb)							
IND.	DNA-FTM	SD all	CV all			CV trip.	SD parallels	CV parallels	MML	SD all	CV	SD trip.		CV trip.	SD parallels	CV parallels
17.2	72.48	9.46	13.05	1st 3 lines	3.88	5.32 %	8.24	0.11	279.12	4.09	1.46	1st 3 lines	4.72	1.69 %	2.72	0.98 %
				2nd 3 lines	9.01	14.07 %						2nd 3 lines	4.72	1.71 %		
				3rd 3 lines	7.63	9.47 %						3rd 3 lines	0.00	0.00 %		
21.1	71.85	5.66	7.88	1st 3 lines	2.43	3.31 %	0.61	0.01	388.03	11.93	3.07	1st 3 lines	11.27	2.94 %	20.87	5.38 %
				2nd 3 lines	0.79	1.08 %						2nd 3 lines	6.36	1.60 %		
				3rd 3 lines	10.98	14.81 %						3rd 3 lines	36.00	10.07 %		
21.2	90.20	2.83	3.14	1st 3 lines	0.59	0.67 %	2.82	0.03	315.30	3.67	1.17	1st 3 lines	6.36	2.03 %	2.12	0.67 %
				2nd 3 lines	1.57	1.72 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.55	1.66 %						3rd 3 lines	0.00	0.00 %		
22.1	60.54	5.76	9.51	1st 3 lines	9.55	15.19 %	2.91	0.05	350.86	15.60	4.45	1st 3 lines	4.80	1.43 %	16.92	4.82 %
				2nd 3 lines	3.51	6.12 %						2nd 3 lines	8.31	2.39 %		
				3rd 3 lines	1.94	3.15 %						3rd 3 lines	4.80	1.30 %		
22.1	48.25	7.37	15.28	1st 3 lines	4.96	12.28 %	7.27	0.15	287.43	9.28	3.23	1st 3 lines	9.33	3.14 %	9.26	3.22 %
				2nd 3 lines	5.55	10.13 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.90	3.84 %						3rd 3 lines	0.00	0.00 %		
22.2	70.67	3.29	4.65	1st 3 lines	3.02	4.33 %	1.11	0.02	263.18	5.72	2.17	1st 3 lines	4.60	1.75 %	5.37	2.04 %
				2nd 3 lines	2.37	3.30 %						2nd 3 lines	4.81	1.79 %		
				3rd 3 lines	4.98	7.07 %						3rd 3 lines	0.00	0.00 %		
23.1	58.90	5.61	9.53	1st 3 lines	5.01	8.07 %	4.84	0.08	297.95	12.72	4.27	1st 3 lines	4.65	1.59 %	14.19	4.76 %
				2nd 3 lines	4.89	9.16 %						2nd 3 lines	4.65	1.62 %		
				3rd 3 lines	2.56	4.18 %						3rd 3 lines	0.00	0.00 %		
23.1	58.19	2.64	4.54	1st 3 lines	1.97	3.26 %	1.94	0.03	358.93	8.36	2.33	1st 3 lines	4.83	1.37 %	7.37	2.05 %
				2nd 3 lines	1.57	2.75 %						2nd 3 lines	4.83	1.31 %		
				3rd 3 lines	3.20	5.60 %						3rd 3 lines	8.36	2.35 %		
23.1	63.71	5.97	9.38	1st 3 lines	3.31	5.82 %	5.97	0.09	316.04	3.62	1.15	1st 3 lines	0.00	0.00 %	3.16	1.00 %
				2nd 3 lines	4.37	6.58 %						2nd 3 lines	4.74	1.48 %		
				3rd 3 lines	2.44	3.60 %						3rd 3 lines	0.00	0.00 %		
23.2	88.22	3.83	4.34	1st 3 lines	2.40	2.77 %	3.65	0.04	194.24	6.29	3.24	1st 3 lines	4.65	2.44 %	4.10	2.11 %
				2nd 3 lines	3.14	3.66 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.80	1.94 %						3rd 3 lines	9.29	4.68 %		
23.2	89.15	3.30	3.71	1st 3 lines	2.28	2.54 %	1.42	0.16	218.22	5.97	2.73	1st 3 lines	0.00	0.00 %	3.16	1.00 %
				2nd 3 lines	3.21	3.67 %						2nd 3 lines	4.74	2.16 %		
				3rd 3 lines	4.70	5.22 %						3rd 3 lines	4.74	2.24 %		
23.2	85.50	9.45	11.05	1st 3 lines	0.76	0.87 %	6.78	0.08	188.15	28.38	15.08	1st 3 lines	4.83	2.19 %	30.61	16.27 %
				2nd 3 lines	13.76	18.36 %						2nd 3 lines	19.07	10.34 %		
				3rd 3 lines	5.42	6.33 %						3rd 3 lines	4.83	3.02 %		
24.1	65.55	6.81	10.39	1st 3 lines	4.59	7.80 %	6.69	0.10	336.81	14.95	4.44	1st 3 lines	4.79	1.47 %	16.82	4.99 %
				2nd 3 lines	0.55	0.76 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	5.45	8.33 %						3rd 3 lines	4.79	1.46 %		
24.2	81.51	5.77	7.08	1st 3 lines	1.67	1.96 %	3.91	0.05	140.13	9.27	6.62	1st 3 lines	9.58	6.57 %	5.53	3.95 %
				2nd 3 lines	6.86	8.37 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	6.11	7.90 %						3rd 3 lines	12.67	9.41 %		
25.1	53.97	4.92	9.12	1st 3 lines	4.19	7.90 %	2.87	0.05	388.72	15.43	3.97	1st 3 lines	10.29	2.75 %	16.53	4.25 %

DNA FTM (%)									MML (kpb)							
IND.	DNA-FTM	SD all	CV all			CV trip.	SD parallels	CV parallels	MML	SD all	CV	SD trip.		CV trip.	SD parallels	CV parallels
				2nd 3 lines	2.92	5.66 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	6.78	11.86 %						3rd 3 lines	5.14	1.27 %		
25.2	86.07	4.80	5.58	1st 3 lines	7.71	9.33 %	2.93	0.03	289.17	5.35	1.85	1st 3 lines	5.14	1.80 %	4.54	1.25 %
				2nd 3 lines	1.96	2.23 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.78	2.02 %						3rd 3 lines	5.14	1.75 %		
26.1	72.95	3.69	5.06	1st 3 lines	4.97	6.76 %	0.99	0.01	308.89	4.34	1.41	1st 3 lines	0.00	0.00 %	5.01	1.62 %
				2nd 3 lines	4.64	6.31 %						2nd 3 lines	0.00	0.00 %	8.68	1.25 %
				3rd 3 lines	2.32	3.24 %						3rd 3 lines	0.00	0.00 %		
26.2	85.97	2.97	3.45	1st 3 lines	1.82	2.10 %	1.55	0.02	114.32	8.68	7.60	1st 3 lines	5.01	4.39 %	8.68	1.25 %
				2nd 3 lines	4.28	5.09 %						2nd 3 lines	5.01	4.08 %		
				3rd 3 lines	2.52	2.90 %						3rd 3 lines	5.01	4.75 %		
27.1	73.41	8.13	11.07	1st 3 lines	2.41	3.49 %	9.03	0.12	161.90	8.60	5.31	1st 3 lines	4.94	2.91 %	9.02	5.57 %
				2nd 3 lines	1.68	2.50 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	3.30	3.94 %						3rd 3 lines	5.19	3.41 %		
27.2	88.68	3.07	3.47	1st 3 lines	0.48	0.54 %	3.42	0.04	155.01	6.35	4.10	1st 3 lines	5.19	3.41 %	2.99	1.93 %
				2nd 3 lines	0.89	0.97 %						2nd 3 lines	10.37	6.56 %		
				3rd 3 lines	2.51	2.95 %						3rd 3 lines	0.00	0.00 %		
27.2	91.27	3.12	3.41	1st 3 lines	3.66	4.16 %	2.79	0.03	206.76	5.52	2.67	1st 3 lines	4.57	2.18 %	2.58	1.25 %
				2nd 3 lines	1.25	1.35 %						2nd 3 lines	7.74	3.79 %		
				3rd 3 lines	0.69	0.74 %						3rd 3 lines	4.57	2.21 %		
29.1	69.30	5.58	8.05	1st 3 lines	0.00	0.01 %	5.00	0.07	321.64	15.32	4.76	1st 3 lines	4.95	1.59 %	16.49	5.13 %
				2nd 3 lines	4.18	6.12 %						2nd 3 lines	8.57	2.52 %		
				3rd 3 lines	5.86	8.86 %						3rd 3 lines	4.95	1.59 %		
29.2	90.11	2.12	2.35	1st 3 lines	0.46	0.49 %	2.01	0.02	199.35	6.23	3.12	1st 3 lines	4.95	2.51 %	5.95	2.98 %
				2nd 3 lines	1.78	1.98 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.58	1.77 %						3rd 3 lines	4.95	2.40 %		
35.1	61.01	12.31	20.19	1st 3 lines	4.67	8.28 %	13.67	0.22	297.72	14.39	4.83	1st 3 lines	0.00	0.00 %	16.62	5.58 %
				2nd 3 lines	3.94	5.16 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	2.94	5.85 %						3rd 3 lines	0.00	0.00 %		
35.2	80.03	5.42	6.77	1st 3 lines	2.16	2.70 %	4.87	0.06	183.32	3.71	2.02	1st 3 lines	0.00	0.00 %	3.24	1.76 %
				2nd 3 lines	5.57	7.41 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	3.26	3.83 %						3rd 3 lines	4.85	2.59 %		
35.2	78.53	5.81	7.40	1st 3 lines	1.92	2.65 %	6.44	0.08	290.24	4.56	1.57	1st 3 lines	0.00	0.00 %	5.26	1.81 %
				2nd 3 lines	2.33	3.01 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.20	1.41 %						3rd 3 lines	0.00	0.00 %		
36.1	61.12	15.75	25.77	1st 3 lines	2.77	5.34 %	16.79	0.27	261.77	23.89	9.13	1st 3 lines	4.63	1.58 %	27.31	10.43 %
				2nd 3 lines	10.08	19.81 %						2nd 3 lines	4.85	1.91 %		
				3rd 3 lines	6.08	7.55 %						3rd 3 lines	0.00	0.00 %		
36.1	62.81	11.41	18.17	1st 3 lines	3.02	4.07 %	11.48	0.18	309.75	13.06	4.22	1st 3 lines	0.00	0.00 %	14.82	4.78 %
				2nd 3 lines	6.77	10.71 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	8.41	16.44 %						3rd 3 lines	4.87	1.49 %		
36.2	72.76	6.94	9.54	1st 3 lines	10.24	14.95 %	3.53	0.05	251.43	4.16	1.66	1st 3 lines	0.00	0.00 %	2.78	1.10 %
				2nd 3 lines	1.36	1.87 %						2nd 3 lines	4.81	1.93 %		

DNA FTM (%)									MML (kpb)							
IND.	DNA-FTM	SD all	CV all			CV trip.	SD parallels	CV parallels	MML	SD all	CV	SD trip.		CV trip.	SD parallels	CV parallels
				3rd 3 lines	6.81	8.92 %						3rd 3 lines	4.81	1.91 %		
39.1	54.21	7.00	12.91	1st 3 lines	4.97	10.43 %	6.44	0.12	247.05	10.37	4.20	1st 3 lines	11.96	4.71 %	9.79	3.96 %
				2nd 3 lines	4.32	7.15 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	2.45	4.40 %						3rd 3 lines	0.00	0.00 %		
39.1	50.56	11.88	23.49	1st 3 lines	2.05	4.38 %	14.50	0.29	319.16	10.94	3.43	1st 3 lines	0.00	0.00 %	12.08	3.78 %
				2nd 3 lines	2.22	5.69 %						2nd 3 lines	4.30	1.32 %		
				3rd 3 lines	0.54	0.80 %						3rd 3 lines	0.00	0.00 %		
39.2	71.15	7.53	10.59	1st 3 lines	6.24	9.80 %	6.53	0.09	289.13	13.03	4.51	1st 3 lines	4.45	1.59 %	10.57	3.66 %
				2nd 3 lines	4.86	6.42 %						2nd 3 lines	4.45	1.56 %		
				3rd 3 lines	6.03	8.14 %						3rd 3 lines	17.42	5.79 %		
39.2	68.59	9.28	13.54	1st 3 lines	8.78	14.61 %	8.08	0.12	212.72	6.37	2.99	1st 3 lines	4.19	1.97 %	4.90	2.30 %
				2nd 3 lines	3.13	4.10 %						2nd 3 lines	4.19	2.02 %		
				3rd 3 lines	7.89	11.36 %						3rd 3 lines	7.43	3.41 %		
40.1	75.77	3.98	5.26	1st 3 lines	2.52	3.41 %	3.36	0.04	306.50	4.72	1.54	1st 3 lines	0.00	0.00 %	1.51	0.49 %
				2nd 3 lines	4.21	5.71 %						2nd 3 lines	4.54	1.47 %		
				3rd 3 lines	2.37	2.98 %						3rd 3 lines	7.86	2.57 %		
40.2	91.88	2.56	2.79	1st 3 lines	1.71	1.84 %	2.56	0.03	250.23	5.99	2.39	1st 3 lines	0.00	0.00 %	6.40	1.25 %
				2nd 3 lines	0.43	0.45 %						2nd 3 lines	4.54	1.77 %		
				3rd 3 lines	1.86	2.09 %						3rd 3 lines	0.00	0.00 %		
42.1	68.02	12.71	18.69	1st 3 lines	6.12	10.67 %	13.38	0.20	290.93	5.95	2.05	1st 3 lines	4.02	1.40 %	6.34	2.18 %
				2nd 3 lines	6.63	7.98 %						2nd 3 lines	0.00	0.00 %	3.55	1.25 %
				3rd 3 lines	5.32	8.37 %						3rd 3 lines	4.02	1.39 %		
42.2	80.12	3.76	4.69	1st 3 lines	4.96	6.08 %	2.25	0.03	264.09	5.10	1.93	1st 3 lines	4.90	1.88 %	4.32	1.64 %
				2nd 3 lines	3.69	4.75 %						2nd 3 lines	4.90	1.82 %		
				3rd 3 lines	1.76	2.17 %						3rd 3 lines	0.00	0.00 %		
42.2	76.20	4.41	5.79	1st 3 lines	4.05	5.27 %	2.19	0.03	237.67	3.67	1.55	1st 3 lines	0.00	0.00 %	3.55	1.25 %
				2nd 3 lines	5.00	6.88 %						2nd 3 lines	4.02	1.70 %		
				3rd 3 lines	2.47	3.26 %						3rd 3 lines	0.00	0.00 %		
44.1	47.40	4.19	8.85	1st 3 lines	2.15	4.78 %	3.99	0.08	334.16	7.30	2.18	1st 3 lines	0.00	0.00 %	7.94	2.37 %
				2nd 3 lines	2.96	5.69 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	3.04	6.71 %						3rd 3 lines	4.90	1.51 %		
44.2	68.06	5.64	8.28	1st 3 lines	2.84	4.06 %	5.54	0.08	238.31	5.01	2.10	1st 3 lines	4.67	1.93 %	4.26	1.79 %
				2nd 3 lines	1.87	2.58 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	4.86	7.86 %						3rd 3 lines	4.90	2.10 %		
48.1	62.40	6.14	9.84	1st 3 lines	0.47	0.81 %	7.00	0.11	294.89	4.19	1.42	1st 3 lines	4.59	1.55 %	4.05	1.37 %
				2nd 3 lines	1.54	2.19 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.09	1.86 %						3rd 3 lines	0.00	0.00 %		
48.1	77.90	2.46	3.16	1st 3 lines	2.22	2.89 %	1.63	0.02	336.92	9.63	2.86	1st 3 lines	4.48	1.32 %	10.21	3.03 %
				2nd 3 lines	3.20	4.13 %						2nd 3 lines	4.48	1.37 %		
				3rd 3 lines	1.07	1.34 %						3rd 3 lines	4.28	1.24 %		
48.1	60.00	14.40	24.00	1st 3 lines	14.46	22.63 %	14.03	0.23	292.67	3.78	1.29	1st 3 lines	4.37	1.48 %	2.52	0.86 %
				2nd 3 lines	5.22	11.75 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	1.63	2.28 %						3rd 3 lines	4.37	1.49 %		

DNA FTM (%)									MML (kpb)							
IND.	DNA-FTM	SD all	CV all			CV trip.	SD parallels	CV parallels	MML	SD all	CV	SD trip.		CV trip.	SD parallels	CV parallels
48.2	71.35	6.19	8.68	1st 3 lines	8.65	12.68 %	2.93	0.04	296.52	6.82	2.13	1st 3 lines	7.58	2.61 %	5.27	1.78 %
				2nd 3 lines	6.47	9.00 %						2nd 3 lines	0.00	0.00 %		
				3rd 3 lines	3.31	4.47 %						3rd 3 lines	4.28	1.42 %		