NTNU – Trondheim
Norwegian University of
Science and Technology

Metal Levels in Blood and Feather from Incubating Female Common Eiders (*Somateria mollissima*) in Svalbard.

Kjersti Lervik

Teacher Education with Master of Science
Submission date: May 2012
Supervisor: Åse Krøkje, IBI
Co-supervisor: Anette Fenstad, IBI

Norwegian University of Science and Technology
Department of Biology

Acknowledgements

This Master's thesis was written at the Department of Biology, NTNU, Trondheim as a part of the Programme for Teacher Education, from August 2011 to May 2012. Financial support was provided by *Kong Haakon den 7des utdannelsesfond for norsk ungdom* at the University of Tromsø.

First of all I would like to thank my supervisors Associate Professor Åse Krøkje (NTNU) and PhD candidate Anette Fenstad (NTNU), for good help and guidance. Special thanks to Åse for all the help throughout this period, with good advise, good spirits, and solid expertise. Thanks to Anette for letting me use the material that was collected at Svalbard during the spring of 2011, good guidance, all the extra help with statistics, and nice conversations about the thesis. I would also like to thank Syverin Lierhagen at the Department of Chemistry, NTNU, for conducting the metal analysis with the ICP-MS.

I would like to give a special thanks to my sisters in Trondheim, Eli Ane and Nora, for all fun, support, patience and cheerfulness.

Lene and Magnus, my nieces Signe Elise and Matilde Oline, and my nephew Jakob Emrik for being fantastic to come home to and visit, you bright up even the greyest day.

My parents deserve a large thanks – relaxation in Loddotrøa, great trips and building of the cabin is something I really appreciate, and has been invaluable throughout my study period.

To all my wonderful friends, thanks for always being there – no matter what.
A special thanks to Ingunn, for five years trough storms and sunshine, I could not have done this without you! Jenny is great, thanks for diversions, master talks, and great dinners, Kari and Olav for your nice visits, Kristin for all advice about studies and life, Tonje and Emil for being so great, Jeroen you wonderful tullebok, to Silje, Mari and Marianne who I see to seldom, but is just a phone call away.

Trondheim, 22th of May 2012

Kjersti Lervik

Front page: Incubating common eider (*Somateria mollissima*), Kongsfjorden, Svalbard.
Photo: Anette Fenstad

Abstract

In the present study, the concentration of selected metals and metalloids were determined in blood and feathers of 29 incubating female common eiders (*Somateria mollissima*). The birds were sampled at the island Storholmen in Kongsfjorden, Svalbard, at the end of the nesting season of 2011. In order to increase the knowledge of metal levels of the female common eiders nesting in Svalbard, concentration of the non-essential metals mercury (Hg), lead (Pb), arsenic (As), and cadmium (Cd) and the essential metals selenium (Se), manganese (Mn), copper (Cu), zinc (Zn), calcium (Ca), and iron (Fe) were determined by inductively coupled plasma mass spectrometry (ICP-MS).

The present findings suggest that concentration of the non-essential metals in blood and feathers from female common eiders nesting in Kongsfjorden, were below toxicity threshold. However, in some individuals, concentrations of Se in feathers were close to the toxicity threshold. All together, these results indicate that the levels of metals do not seem to be an immediate threat to common eider colonies nesting in Svalbard. At the same time, it is important to recognize that the birds sampled in the present study represent the most successful individuals within the population; having completed a long migration, found a partner and produced eggs. It is therefore a possibility that birds with high enough levels of non-essential metals to affect reproduction were not breeding during the season of 2011. Metal concentrations in blood may provide a picture of recent dietary exposure from areas close to the breeding ground, while concentrations in feathers represent blood levels at the time of molt, when the female common eiders migrate away from Svalbard. The non-essential metals with the highest concentration in blood were Hg (117 μg/kg ± 31.9), followed by Pb (56.3 μg/kg ± 38.9), while in feather the highest concentrations were found for As (1.10 μg/g ± 1.36) and Hg (0.969 μg/g ± 0.328). These findings suggest that the female common eiders are subject to variations in metal exposures throughout the year. Only two metals (Se [$r = 0.51$, $p = 0.005$] and Cd [$r = 0.467$, $p = 0.011$]) showed significant positive correlation between blood and feather. Indicating that blood samples should be used when investigating recent dietary uptake, while feather samples should be used to represent metal levels on wintering grounds. No sign of metal mimicry was observed when investigating feather samples. This may be related to the close homeostatic control of essential metals, and may indicate that the levels of essential metals in female common eiders were sufficient, since mimicry most often become visible at low dietary levels of essential metals.

Sammendrag

I dette studiet ble konsentrasjonen av utvalgte metaller og halvmetaller bestemt i blod og fjær hos 29 rugende ærfuglhunner (*Somateria mollissima*). Prøvene ble tatt fra fugler på øya Storholmen i Kongsfjorden, Svalbard, på slutten av hekkesesongen i 2011. For å øke kunnskapsnivået om metallnivået i hunnærfugler som hekker på Svalbard ble konsentrasjonen av de ikke-essensielle metallene kvikksølv (Hg), bly (Pb), arsenikk (As) og kadmium (Cd), og de essensielle metallene selen (Se), mangan (Mn), kobber (Cu), sink (Zn), kalsium (Ca) og jern (Fe) bestemt ved hjelp av induktiv koblet plasma-massespektrometri (ICP-MS).

Funnene i dette studiet indikerer at konsentrasjonen av de ikke-essensielle metallene i blod og fjær hos hekkende ærfuglhunner i Kongsfjorden, var under den toksiske terskelverdien. Hos noen individ, var imidlertid konsentrasjonen av Se, nær den toksiske terskelverdien. Sammen indikerer disse resultatene at metallnivået ikke innebærer en umiddelbar trussel mot ærfuglkoloniene som hekker på Svalbard. På samme tid er det viktig å legge merke til at fuglene som ble undersøkt i dette studiet, representerer de mest suksessfulle individene i populasjonen; de har fullført en lang migrasjon, funnet en partner og produsert egg. Det er derfor en mulighet for at fugler som hadde høye nok nivå av ikke-essensielle metaller til at det påvirket reproduksjonen, ikke hekket under sesongen i 2011. Metallkonsentrasjoner i blod representerer i hovedsak nylig eksponering via dietten, fra områder nærliggende hekkeområdet, mens konsentrasjonene i fjær representerer blodnivået ved myting, når ærfuglene migrerer bort fra Svalbard. De ikke-essensielle metallene med høyest konsentrasjon i blod var Hg (117 µg/kg ± 31,9) etterfulgt av Pb (56,3 µg/kg ± 38,9), mens i fjær ble de høyeste konsentrasjonene funnet for As (1,10 µg/g ± 1,36) og Hg (0,969 µg/g ± 0,328). Disse funnene antyder at ærfuglhunnene er utsatt for variasjoner i metalleksponeringer i løpet av året. Bare to metaller (Se [$r = 0,51$, $p = 0,005$] og Cd [$r = 0,467$, $p = 0,011$]) viste signifikant positiv korrelasjon mellom fjær og blod. Dette indikerer at blodprøver bør brukes når en ønsker å undersøke nylig diettopptak, mens fjærprøvene kan brukes for å representere metallnivået fra vinterområdene. Det ble ikke observert tegn til mimikry mellom ikke-essensielle og essensielle metaller når fjærene ble undersøkt. Dette kan være relatert til den strenge homeostatiske kontrollen av essensielle metaller, og kan indikere at nivået av essensielle metaller i hunnærfugl var tilstrekkelig høyt, siden mimikry som oftest sees ved lave diettnivå av essensielle metall.

List of abbreviations

As	Arsenic
Ca	Calcium
Cd	Cadmium
Cd-MT complex	Cadmium-metallothionein complex
Cu	Copper
Fe	Iron
GI	Gastrointestinal
Hg	Mercury
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma mass spectrometry
IDL	Instrument detection limit
ln	Natural logarithm
MeHg	Methylmercury
Mn	Manganese
MT	Metallothionein
Ni	Nickel
NTNU	Norwegian University of Science and Technology
Pb	Lead
Pearson	Pearson product moment coefficient
SD	Standard deviation
POPs	Persistent organic pollutants
Se	Selenium
SH-groups	Sulfhydryl groups
Spearman	Spearman´s rank correlation coefficient
Zn	Zinc

Table of content

Acknowledgements ..... **i**
Abstract ..... **ii**
Sammendrag ..... **iii**
List of abbreviations ..... **iv**
Table of content ..... **v**

1. Introduction **1**
1.1. Metals in the Arctic 1
1.2. Metals and metalloids in birds 1
1.2.1 Metal exposure and toxicity in marine birds 1
1.2.2 Absorption, distribution, metabolism and elimination of metals in birds 2
1.2.3. The distribution of metals in blood 4
1.2.4. The excretion of metals through feather 4
1.2.5. Non-essential toxic metals 5
1.2.6. Essential metals with the potential for toxicity 7
1.3. Study species: Common eider (*Somateria mollissima*) in Svalbard 7
1.4. Objectives 9
2. Methods **11**
2.1. Sampling 11
2.2. Chemical element analysis 12
2.2.1. Preparation and acid digestion of samples 12
2.2.2. Principles of inductive coupled plasma mass spectrometry 12
2.2.3. Quantification 13
2.3. Statistics 14
3. Results **15**
3.1. Metal concentrations 15
3.1.1. Blood samples 15
3.1.2. Feather samples 16
3.2. Relationship between metal concentrations in blood and feather 16
3.3. Relationships of metals within feathers 18
4. Discussion **19**
4.1. Metal concentrations in female common eiders 19
4.1.1. Blood samples 19
4.1.2. Feather samples 20
4.1.3. Toxic relevance of metal exposure 21
4.1.4. Comparison of blood and feather 24
4.2. Relationships between metals in blood and feather 24
4.3. Relationships of metals within feather 25
5. Conclusions **29**
References **31**

Appendices

1. Introduction

1.1. Metals in the Arctic

Metals are unique among pollutants in that they occur naturally in all ecosystems (Liu et al., 2008). In addition, they are released into the environment from a variety of anthropogenic sources, which greatly enhances metal distribution in the global environment (Burger et al., 2008). Human activity is responsible for the majority of the global distribution of mercury (Hg), lead (Pb), cadmium (Cd), zinc (Zn), and manganese (Mn) (Walker, 2006). Metals and metalloids can appear in different chemical forms; metal toxicity is related to oxidative state, reactivity with other compounds, or other metals (Scheuhammer, 1987). Metals and metalloids have long residual times, hence, they may cause harmful effects long after the metal pollution itself has occurred (Dauwe et al., 2004).

Despite limited human activities, the Arctic is subject to metal pollution from industrialized areas far from the Arctic area. With its fragile ecosystems and unique conditions this poses an increasing concern for Arctic wildlife (Steffen et al., 2008; Hargreaves et al., 2011). Metals released into the environment from remote, mid-latitude industrialized areas, can be deposited in the Arctic via various routes. A major pathway is long-range atmospheric transport, in addition long-range transport in water, via rivers, and ocean currents (Brunström and Halldin, 2000). Since the long-range transport mechanisms of metals are so complex, it is difficult to distinguish between natural, background, and anthropogenic levels of metals in the Arctic (Malinga et al., 2010).

1.2. Metals and metalloids in birds

1.2.1 Metal exposure and toxicity in marine birds

Marine birds are useful as bioindicators or biomonitors of metals, due to their wide distribution and relatively high tropic level position (Burger et al., 2008; Malinga et al., 2010). Compared to terrestrial ecosystems, chemical levels are usually elevated in coastal ecosystems, due to river influxes, runoff, point-source pollution, and deposition of long-range transported pollutants (Burger and Gochfeld, 2009a). Bird species foraging in aquatic

environments are therefore particularly vulnerable to metal pollution (Burger and Gochfeld, 2009b). The main exposure route of metals in wild birds is via ingestion of food and water, marine birds may, in addition, be exposed via direct ingestion of contaminated sediments (Figure 1) (Hargreaves et al., 2011). Ambient medium such as soil, sediment, water, and atmosphere also represent exposure routes to bird via dermal contact and the respiratory tract (Figure 1) (Beyer et al., 2004). There are several factors influencing the toxic potential related to metal exposure. The exposure-related factors such as chemical form, dose, exposure site, duration and frequency of exposure are of great importance. The state of the exposed individual is also important, e.g. age at exposure, gender, and the individual's capacity for metal detoxification (Liu et al., 2008).

Metals can be grouped into non-essential and essential metals. The non-essential metals are often toxic and demonstrate no biological requirements in birds (Sterner, 2010). Exposure to non-essential metals such as Hg, Pb, Cd, and the metalloid arsenic (As) is generally associated with toxicity, even at relatively low concentrations (Elliott et al., 1992; Goyer, 1997). Non-essential metals can be toxic to organisms by different mechanisms, such as molecular or ionic mimicry, where the non-essential metals mimic the function of essential metals. Also, interruptions of other functions in the body may occur, such as enzyme systems, when metals bind to sulfhydryl (SH) groups in enzymes. Tissue damage after metal induced oxidative stress may also occur (Cuypers et al., 1999; Manahan, 2010). In contrast to the non-essential metals, animals require essential metals, such as Mn, Zn, copper (Cu), iron (Fe), calcium (Ca), and selenium (Se), for normal growth and reproduction (Walker, 2006). These metals may have the capacity to modify health risks from exposure to non-essential toxic metals (Goyer, 1997); Se can reduce the toxicity of certain metals such as As, Cd, and Hg by forming inert compounds (Högberg and Alexander, 2007; Liu et al., 2008). However, even essential metals have a potential for toxicity when present in excess amounts (Elliott et al., 1992). Birds have, as other animals, developed a variety of homeostatic mechanisms for the regulation of levels of essential metals, which usually involve the control of gastrointestinal (GI) absorption. Toxic effects are therefore less likely to occur with essential metals compared to non-essential metals (Clarkson, 1986).

1.2.2 Absorption, distribution, metabolism and elimination of metals in birds

There is no uniform mechanism for the absorption, distribution, detoxification and excretion of metals. This is due to great variations in chemical properties, and toxic endpoints of

different metals (Liu et al., 2008). However some general mechanisms are shared; a basic overview of metal dynamics in birds is shown in figure 1. After exposure, metals may be excreted directly or absorbed from the exposure site into the bloodstream. Once absorbed, metals are distributed in the body by the circulatory system, irrespective of their chemical form. Metals are initially distributed to a variety of organs and tissues, followed by redistribution to target organs, or other tissues for storage or inactivation. For as long as the uptake exceeds the rate of excretion, the metals tend to accumulate in storage compartments (AMAP, 1998; Burger and Gochfeld, 2009b). In general, the major factor underlying the biochemical properties of metals with regard to their transportation, distribution, and elimination in organisms is their high affinity to sulphur and SH groups of proteins. Sulfhydryl groups are ubiquitous in organisms, occurring in plasma proteins, membrane proteins, and enzymes. Therefore, metals accumulate primarily in tissues rich in protein (Davidson et al., 2007).

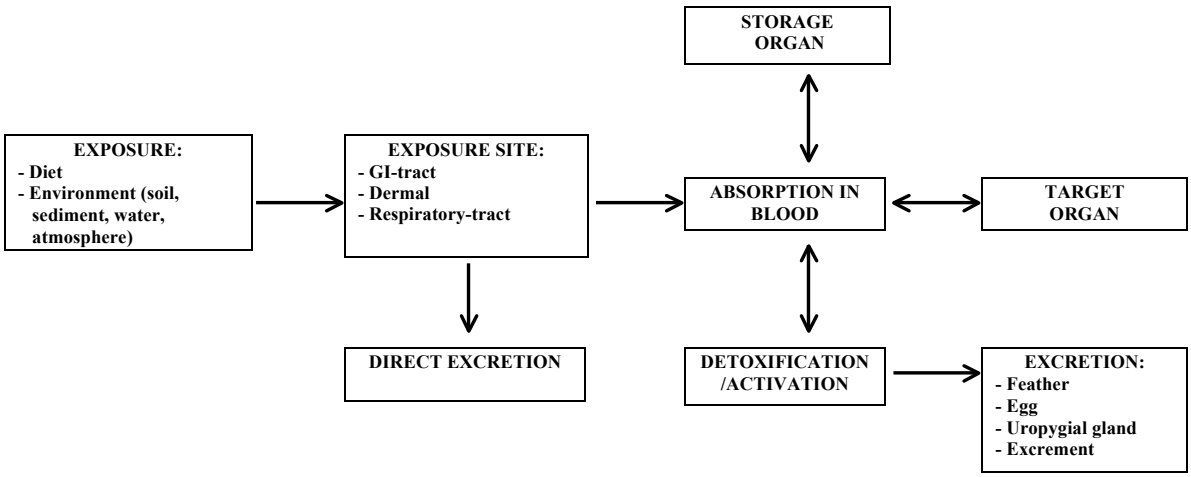


Figure 1: Simplified model for metal dynamics in birds. Refer to the text for details.

Metals are non-degradable, which means that they can not be metabolized to nontoxic forms (Sodhi, 2005). However, they may be transformed to insoluble forms, which are biologically unavailable, in inactivation or detoxification processes, unless they are again converted into more soluble substances (Baird and Cann, 2008). Detoxification processes of metals includes methylation and demethylation, where metal-carbon bonds are formed or broken down (AMAP, 1998). Methylation is important as a detoxifying process for As in most animals, and demethylation is an important step in the detoxification and elimination after exposure to methylmercury (MeHg) (Beckett et al., 2007). Another metal detoxification process is the change in oxidation state, which affects the chemical activity of metals and therefore toxicity (AMAP, 1998). Formation of inert complexes also plays an important role in metal

inactivation, such as between metallothionein (MT), a specific low molecular weight, high affinity metal-binding protein (Klaassen et al., 1999), and metals such as Cd, Zn, Cu, and Hg. Formation of such complexes may prevent the acute and short-term toxic effects of these metals, however it usually result in retention of the metal in body tissues (Clarkson, 1986). The only mechanism by which metals can be removed from a birds body is by excretion (Baird and Cann, 2008). Excretion routes in birds are via excrement, deposition to feathers (Braune, 1987; Lewis and Furness, 1991) or via the uropygial gland, females can also sequester metals in their eggs (Fimreite et al., 1974; Dauwe et al., 2000).

1.2.3. The distribution of metals in blood

Metal levels in the birds' blood are often closely related to the levels in their prey, and can vary spatially within sites, depending on habitat differences in contaminant levels (Hargreaves et al., 2011). Since absorbed metals are transported in the circulatory system, the metal concentrations in blood may provide a picture of short-term exposure from recent dietary intake (Monteiro and Furness, 1995). Physiological factors such as mobilization of reserves for egg production, fasting, or increases in blood volume at the start of molt may also influence metal concentrations in blood (Furness, 1993; Monteiro and Furness, 1995; Wayland et al., 2003). For certain metals, the total body burden, or levels in organs such as liver and kidney, may influence the metal levels in blood. This is related to the continual process of equilibrium and redistribution between metals in the blood and various soft tissues where metals are stored (Wayland et al., 2007).

1.2.4. The excretion of metals through feather

Metal concentrations in feathers reflect metal levels in the blood during the short period of feather growth, when the feathers are connected to the blood vessels (Dauwe et al., 2000). After feather formation, the blood supply atrophies and the feather become completely isolated from the rest of the body (Burger and Gochfeld, 1993). There is usually a high correlation between metal levels in birds diet and levels in their feathers. This is related to the relatively high proportion of the body burden of certain metals being excreted to feathers, due to the metals affinity for SH rich feather keratin protein (Burger and Gochfeld, 1992; Monteiro and Furness, 1995). Metals can accumulate in feathers both endogenously, during feather growth, and exogenously from the environment (Hargreaves et al., 2011). Exogenous contamination onto feather surfaces may be caused by atmospheric deposition and/or originate from secretion products of the uropygial gland that is smeared onto the feathers

during preening (Jaspers et al., 2004). The plumage of marine birds is usually renewed once or twice every year, in a process called molting, where both body and wing feathers are renewed (Lorentsen and Røstad, 1990). During molting, the metals that have accumulated in soft tissues between molts, may mobilize and be excreted into the growing feathers (Monteiro and Furness, 1995). Metals in feathers therefore represent circulatory concentrations in the blood during the few weeks of feather formation, which, in turn, represent both local exposure and mobilization from internal tissues (Braune, 1987; Lewis and Furness, 1991).

1.2.5. Non-essential toxic metals

Mercury occurs in the environment as elemental, organic and inorganic compounds with quite different biological properties, affecting the capacity of absorption in birds (AMAP, 1998). A large amount of Hg in the environment is unavailable for organisms, as it is strongly bound to sediment, or other organic material. Inorganic forms of Hg can be methylated by microorganisms, and transformed to highly toxic MeHg, which is readily absorbed and accumulated in organisms (Liu et al., 2008). Methylmercury is lipid-soluble and may therefore be stored in lipid-rich tissues, and has the capacity of biomagnification (Furness and Camphuysen, 1997). Birds are primarily exposed to MeHg, as it is preferentially accumulated in the tissues of their prey (Jaspers et al., 2004). Methylmercury is readily absorbed into the circulatory system from the GI-tract (Liu et al., 2008), and bird studies have shown that after absorption, MeHg is distributed evenly in tissues (AMAP, 1998). Methylmercury is bound to thiol-containing molecules such as cysteine, which mimic methionine and is therefore able to cross the blood-brain barrier (Liu et al., 2008). In the liver, parts of the MeHg can be detoxified by demethylation and converted into inorganic Hg, while the remaining MeHg remains as a body pool until it can be excreted in processes such as feather formation and egg-laying (Bond and Diamond, 2009). Feathers are widely recognized as a major excretory pathway for Hg, allowing adult birds to sequester from 70 - 93 % of their Hg body burden during molt (Honda et al., 1986; Braune and Gaskin, 1987).

Environmental Pb exists in several different forms. Elemental Pb is not an environmental problem to most life forms, however, its ionic species poses larger threats (Baird and Cann, 2008). The stable ion of Pb is the divalent specie, which is considered to be the most bioavailable for organisms (AMAP, 1998). Lead has the capacity to enter cells by mimicking essential metals such as Ca, Fe and Zn, and may therefore disrupt normal metal homeostasis (Walker, 2006). Laboratory and field studies conducted on birds indicate that blood

concentrations of Pb are extremely dynamic, thus Pb concentrations in blood serves as a stronger indicator of recent exposure than other tissues (Wilson et al., 2007). It is suggested that the molecular mechanism of Pb toxicity is that it binds to sulphur and nitrogen groups in proteins and other macromolecules (Sterner, 2010). In birds, Pb is distributed via the blood to kidney and bone tissue (Koivula and Eeva, 2010). Once Pb is deposited in bone, it is relatively stable having a biological half-life of many years (Elliott et al., 1992). Since absorbed Pb normally becomes firmly bound in bone, it is thought to enter feathers only in trace amounts (Furness and Camphuysen, 1997).

The metalloid As exists in nature both organic and inorganic forms. Inorganic As in trivalent and pentavalent forms are considered more toxic than organic forms (Baird and Cann, 2008). Arsenic primarily accumulates in body tissues such as liver and kidney (Koivula and Eeva, 2010). Concerning feathers, exogenous contamination appears to alter the concentrations of As after feather formation (Jaspers et al., 2004).

The transition metal Cd is mainly absorbed directly by organisms from water in its free ionic form. The most common ion of Cd is the divalent form, although many of its inorganic salts are soluble in water (e.g., acetate, chloride and sulphate), they do not appear to be absorbed by organisms (AMAP, 1998; Baird and Cann, 2008). Cadmium has the capacity to enter target cells by interacting with membrane transporters involved in the uptake of essential metals such as Ca, Fe and Zn (Bridges and Zalups, 2005). Therefore Cd absorption can be increased by dietary deficiencies of essential metals (Liu et al., 2008). Cadmium transportation into cells is mediated through ionic mimicry via Ca channels and via molecular mimicry (Liu et al., 2008). It is rapidly taken up by tissues and is primarily deposited in the kidney and to a lesser extent in the liver (Garciá-Fernández et al., 1996). Cadmium is transported in the circulatory system by binding to albumin and other larger molecular-weight proteins, for example by MT, forming a cadmium-metallothionein complex (Cd-MT complex) that is very stable. Once bound to MT, Cd has a very long biological half-life, which accounts for its tendency to accumulate with age (Elliott et al., 1992; Klaassen et al., 1999). Cadmium is stored in the liver primarily as a Cd-MT complex, however, it may be released from the liver and transported via blood to the kidney, where it is reabsorbed and degraded in the lysosomes of the renal tubules. This process releases Cd, and may lead to induction of more Cd-MT complex, or cause renal toxicity (Liu et al., 2008). Once ingested, Cd is often firmly bound in the kidney, and is expected to enter feathers in trace amounts

(Furness and Camphuysen, 1997). As blood concentrations of Cd, under steady-state conditions, are expected to equilibrate with concentrations in both liver and kidney, changes in liver-kidney ratios, in for example fasting birds, will likely also influence blood-kidney ratios (Wayland et al., 2001).

1.2.6. Essential metals with the potential for toxicity

The metalloid Se is widely distributed in the environment, it is usually present as the hexavalent and divalent form, and only rarely as elemental Se (Bridges and Zalups, 2005). The availability and toxic potential of Se compounds are related to their chemical forms, and most importantly, to solubility. Selenium has the capacity to form insoluble complexes with various metals such as Hg, As, and Cd (Liu et al., 2008). Due to the extremely high affinity of Se to Hg, Se sequesters Hg and reduces its biological availability in organisms (Högberg and Alexander, 2007). Most Se compounds are water-soluble and are thus readily available for dermal and GI uptake. Concentrations of Se generally are highest in the kidney, but are also found in liver and muscle (AMAP, 1998).

Manganese is an essential metal required for many metabolic and cellular functions such as being a cofactor for a number of enzymatic reactions. Manganese is found as many ionic species, with the divalent form as the most predominant. Once in the body, the divalent Mn may be oxidized to the more reactive and toxic trivalent form (Liu et al., 2008).

Copper is an essential element widely distributed in the environment (Liu et al., 2008). Once absorbed, Cu is initially transported to the liver, brain, and muscle tissue. Under certain circumstances Cu may be released back into the circulatory system and redistributed various other tissues for storage or excretion (AMAP, 1998).

1.3. Study species: Common eider (*Somateria mollissima*) in Svalbard

The common eider is a large diving duck (50 - 71 cm, 1200 - 2800 g, Figure 2) (Svensson et al., 2004). Common eiders feed on invertebrates, especially mussels, snails and crustaceans that they collect by diving in shallow waters (Bustnes and Erikstad, 1988; Røv et al., 1992). Female common eiders start breeding when they are 2 - 3 years old, the highest known age of a common eider ringed in Norway (included Svalbard) is 22 years (Kovacs and Lydersen, 2006).

The common eider is connected to the marine environment throughout the entire year (Røv et al., 1992). It has a circumpolar distribution, and breeds mostly on small coastal islands in the Arctic and boreal zones of the Northern Hemisphere (Bustnes and Tertiski, 2000; Burger and Gochfeld, 2009b). The common eider is a stationary species, which means that it normally winters within breeding range (Strøm, 2011). Exceptions are populations connected to areas that freeze up during winter; these populations leave their breeding areas in the fall and return the following spring (Røv et al., 1992). There is little detailed knowledge on where the Svalbard populations winter. However, the majority most likely winters along the Norwegian coast and on Iceland. It is also proposed that they may winter along the ice-free areas along the coast of southwest Spitsbergen (Bustnes and Tertiski, 2000; Bakken and Strøm, 2004; Kovacs and Lydersen, 2006). It is during the winter migration that the female common eider molt body and wing feather (Bustnes and Tertiski, 2000).

Figure 2: Incubating female common eider. Photo: Anette Fenstad

In Svalbard, the common eider population is estimated to 80 000 - 140 000 individuals, and the majority breed in colonies on small islands along the west- and north coast of Spitsbergen. The islands are good areas for nesting because of shallow waters with good food availability, and low predator rates once the fjords surrounding the islands are ice-free (Mehlum et al., 1990). Hence, the establishment of the nesting site generally takes place as soon as the snow and sea ice has disappeared, depending on weather, sea and ice conditions (Waldeck et al., 2011). In a period of 4 - 6 weeks before egg-laying, female common eiders feed heavily near the nesting islands, increasing fat reserves and body weight by approximately 20 % above winter levels (Parker and Holm, 1990; Kovacs and Lydersen, 2006). The common eider normally lays 3 - 6 eggs, with the incubation starting after the second or third egg (Bustnes

and Tertiski, 2000; Waldeck et al., 2011). The female incubates without help from the male, and thus, rarely leave their nests during the incubation period, attending the nests for 90 - 95 % of the time. Therefore, during the average 26 days of incubation they abstain from feeding, and rely merely on their stored resources for the nutrition and energy required for producing and incubating a clutch of eggs, this result in a weight loss of 30 - 45 % (Korschgen, 1977; Parker and Holm, 1990; Gabrielsen et al., 1991). Thus, the common eider is an ideal species for studying how fasting affect circulating levels of contaminants. At the end of the incubation period, the body reserves of the female common eiders are depleted and their immune system is severely suppressed (Hanssen et al., 2003; 2005). Whereas there are several studies on effects and levels of persistent organic pollutants (POPs) in incubating female common eiders (Hanssen et al., 2003; Bustnes et al., 2010), there is to our knowledge, no available information about metal levels in blood at the critical incubation period.

Traditionally, most studies on metals in wild birds have been conducted on internal tissues, usually liver or kidney, a sample collection method that requires that birds have to be killed (Wayland et al., 2001). However, the number of studies making use of non-destructive methods, like measuring the concentrations in feathers, excrement, blood and eggs, has increased over the years (Jaspers et al., 2004). These non-invasive methods of sampling make it possible to assess metal contamination in endangered or threatened species (Burger and Gochfeld, 2009a). It also makes studies of survival rates, and long-term trends of metal pollution possible (Appelquist et al., 1985; Wayland et al., 2001). To our knowledge, few non-invasive studies have been conducted to investigate metal levels in blood and feathers of female common eiders in Svalbard. Furthermore, there is limited information of the relationships between certain metals in blood and feathers at the time of incubation of the female common eiders.

1.4. Objectives

The main objective of this master project has been to increase the knowledge of levels of certain metals and metalloids in female common eiders breeding in Kongsfjorden, Svalbard, using non-invasive sampling methods. Concentrations of Hg, Pb, As, Cd, Se, Mn, Cu, Zn, Ca, and Fe in blood and feather are reported. In an effort to examine whether female common eiders are exposed to different levels of metals throughout the year, comparisons of metal concentrations in blood and feather were conducted. To examine the feasibility of whether

feather samples can be used as a means of monitoring metals in blood of female common eider, the relationship between concentration in blood and feather was examined. Feathers provide information about blood levels during feather formation. By looking at relationships between metals within feathers one may get an insight into possible mimicry of non-essential and essential metals in the common eider.

2. Methods

2.1. Sampling

The sampling was conducted by PhD candidate Anette Fenstad, during the breeding season of 2011 (June 26th-30th), on the island Storholmen in Kongsfjorden, (Ny-Ålesund, 79^{o} N, 13^{o} E, Svalbard, Figure 3). The common eider colony at Storholmen comprises ~ 800 breeding pairs. During the incubation period, the nests were kept under surveillance and sampling was conducted when the eggs were haching or when the chick was hached. Thus, all the females had been fasting for 26 days when sampled. Common eiders (females, n = 29) were caught on the nest using a fishing pole with a nylon snare at the end. Blood (8 - 10 ml) was sampled from the jugular vein using a heparinsed syringe and kept in eppendorf vials (1.5 ml) in a thermos containing a mixture of ice and salt (~ 5 ts salt/l snow, ~ -10 ^{o}C). The blood was transferred to a freezer (-20 ^{o}C) within 6 hours and transported to the Norwegian University of Science and technology (NTNU) at the end of the field season, where it was kept on -80 ^{o}C until analysis. Levels of metals may be different in different types of feather as a result of molting patterns (Monteiro and Furness, 1995), therefore it is essential to sample a consistent feather area from all birds, and using body feathers are the most adequate. During sampling, about 10 body feathers from the same area of the birds back were collected; the feathers that were least exposed externally were taken to avoid high levels of external contamination. The feathers were kept in airtight, blank plastic bags until analysis to avoid contamination.

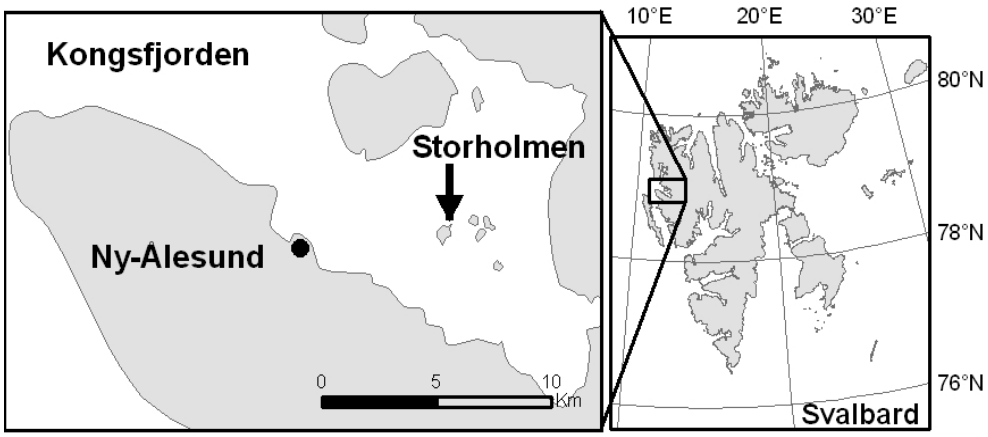


Figure 3: Overview of the sample location for common eider in Kongsfjorden, Svalbard. The common eider colony was located on the island Storholmen (79^{o} N, 13^{o} E) (Fenstad, 2011).

2.2. Chemical element analysis

The process of metal analysis involved preparation, digestion and metal quantification of feather and blood samples. The digestion was performed using UltraCLAVE and the final quantification of metal content was performed by inductive coupled plasma mass spectrometry (ICP-MS).

The analysis of metals was performed at the Department of Chemistry, NTNU. The following metals were subject for analysis: Hg, Pb, As, Cd, Se, Mn, Cu, Zn, Ca, and Fe.

2.2.1. Preparation and acid digestion of samples

Before digestion approximately 0.5 ml blood, and 30 mg feather (exact volume was noted) were transferred to acid washed Teflon tubes designed for the UltraCLAVE (Milestone UltraClave, EMLS, Leutkirch, Germany). Blood was added 0.5 ml concentrated nitric acid (HNO_3, in unit Supur from Milestone, ultra pure grade, distilled at Department of Chemistry), whereas 2 ml 50 % HNO_3 was added to the feather samples. Blood and feather samples were then digested over two hours using UltraCLAVE, a high-pressure microwave system. During the process the temperature gradually increased to a maximum of 240 ^{o}C with a pressure of 160 bar within one hour, followed by a cooling step that return the temperature to the initial value. Procedure and temperature program is described in appendix I.

2.2.2. Principles of inductive coupled plasma mass spectrometry

The description of the principles of ICP-MS are based on the following papers Smith (2004), Thomas (2004), and Gellein (2008). Inductive coupled plasma mass spectrometry is an analytical technique used for elemental determinations. It is an extremely precise instrument for quantification of trace elements. Inductive coupled plasma mass spectrometry makes analysis of elements, even at extremely low concentrations, possible. Figure 4 shows the basic components making up the ICP-MS system. The method is based upon combining a high-temperature inductively coupled plasma (ICP) source with a mass spectrometer (MS). The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the MS. The samples are usually in liquid form but are transferred into aerosols in the instrument. The fine droplets of the aerosols (1-2 % of the sample) are separated from larger droplets in a spray chamber that prevent all the larger aerosols from proceeding. The fine aerosol is then transported into the plasma torch where the temperature

normally is very high. Here the sample is ionized (positive ions are formed). The plasma consists of a partly ionized gas with a high concentration of free charged particles in the form of electrons, protons and ions. In the ICP-MS, the plasma is produced via a flow of gas, usually argon, through a concentrical quartz tube, where it interacts with an intense magnetic field, produced by radiofrequency passing through a copper coil. Plasma with a very high temperature (~ 10,000 K) has the capacity to ionize all elements in the sample, which is necessary for detection in the MS. Once the ions are produced in the plasma, they are directed towards the detection. In this transition, the ions must go from the plasma with a high pressure (760 Torr) to the mass spectrometer with very low pressure (10^{-6} Torr). To ensure that the necessary pressure fall takes place before the ions enter the mass spectre, they pass an interface region that is maintained at vacuum (10^{-3} Torr). In the mass spectrometer, the ions are separated by their mass-to-charge ratio in a mass separation device. There are many different mass separation devices quadruple, magnetic sector and high resolution are the most popular. However all separation devices serve the same purpose; to allow analyte ions of a particular mass-to-charge ratio through to the detector and to filter out all the nonanalyte, interfering and matrix ions.

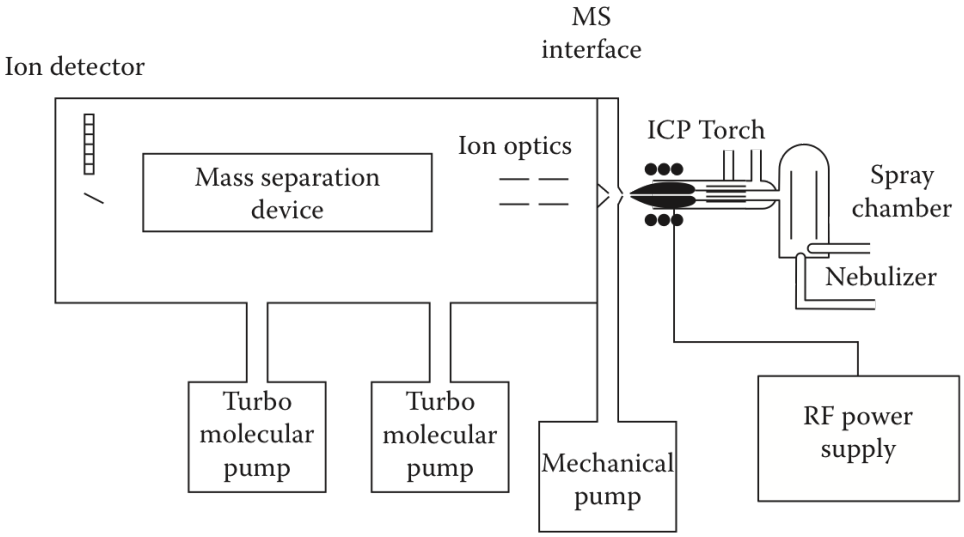


Figure 4: Overview of the basic instrumental components of the ICP-MS (Thomas, 2004). Refer to the text for details.

2.2.3. Quantification

After digestion, the samples were diluted with Milli-Q water (18.2mohm); feather samples to a final volume of 24 ml resulting in 0.6 M HNO_3, and blood samples to 12 ml resulting in 0.6 M HNO_3. Thereafter, the samples were transferred to 12 ml trace metal free tubes for final

analysis. Analyses by ICP-MS was carried out by Syverin Lierhagen, with a Thermo Fisher model ELEMENT 2 instrument (Bremen, Germany). The accuracy of the method was verified by analysing the certified reference material of blood serum (SeronormTM Trace Elements Whole Blood L-1, Sero) and tealeaves (GBW-07605, Langfang, China, Chem Scan), Detection limits were calculated from the instrument detection limit (IDL). Estimation of IDL was done by subsequent analysis of solutions containing decreasing solutions of the element. Finally, the concentration resulting in a relative standard deviation of approximately 25 % (n = 3 scans) was chosen as IDL with baseline corrections applied for these values. All operational parameters are presented in appendix II (Table A1 and A2).

2.3. Statistics

The statistical analyses of the data were conducted using SPSS version 19.0. (IBM Company, Chicago, IL, USA). All data were checked for normal distribution using Shapiro-Wilk, and if necessary, transformed using natural logarithm (ln) to obtain normal distribution. The relationship between the variables was examined using Pearson product moment coefficient (Pearson) or the nonparametric correlation test Spearman's rank correlation coefficient (Spearman) for variables that did not obtain normal distribution. To test for the relationship between independent variable (blood) and dependent variable (feather), linear regression was employed. In the linear regression, all variables were ln-transformed to meet model requirements. Variables are presented as mean ± standard deviation (SD), and significance level were set to $p < 0.05$.

3. Results

3.1. Metal concentrations

The concentrations of the selected metals Hg, Pb, As, Cd, Se, Mn, Zn, Cu, Ca and Fe were measured in the blood and feather at day 26 of the incubation period, in 29 female common eiders. A complete overview of metal concentrations in blood and feather of the birds is given in appendix III.

3.1.1. Blood samples

Mercury was the most abundant non-essential metal in blood, with a total concentration of 117 µg/kg ± 31.9 (range 62 - 201 µg/kg, n = 29, Table 1). In most of the individuals the concentration were between 90 and 130 µg/kg, four birds exhibited Hg concentrations above 150 µg/kg (individual 4, 5, 19, and 24, Appendix III). Following Hg, Pb exhibited the second highest concentration of the non-essential metals in blood, with a mean of 56.3 µg/kg ± 38.9 (range 19.9 - 198 µg/kg, n = 29, Table 1). 86.7 % of the birds had blood levels under 80 µg/kg (Appendix III). Cadmium concentration was low compared to the other non-essential metals (5.29 µg/kg ± 2.79, n = 29, Table 1). The essential metals that were found in high concentrations were Fe (437163 µg/kg ± 39089, n = 29, Table 1), and Ca (58736 µg/kg ± 5420, n = 29, Table 1). Blood concentrations of Mn (15.1 µg/kg ± 5.86, n = 29, Table 1) and Cu (420 µg/kg ± 75.2, n = 29, Table 1) were low compared to the other essential metals.

Table 1: Blood (µg/kg) and feather (µg/g) concentrations of metals of female common eiders (*Somateria mollissima*) from Kongsfjorden, Svalbard, June 2011. n denotes number of individuals, concentrations are given as mean ± standard deviation (SD), minimum and maximum.

	Blood concentration (µg/kg)			**Feather concentration (µg/g)**		
Element	*n*	Mean±SD	Min-max	*n*	Mean±SD	Min-max
Hg	29	117±31.9	62.0-201	29	0. 969±0.328	0.462-1.91
Pb	29	56.3±38.9	19.9-198	29	0.172±0.0830	0.0584-0.354
As	29	21.7±7.35	7.45-38.2	29	1.10±1.36	0.0753-6.07
Cd	29	5.29±2.79	2.22-12.1	29	0.00924±0.00302	0.00530-0.0167
Se	29	4321±2342	1046-10511	29	2.24±0.988	0.923-4.48
Mn	29	15.1±5.86	9.37-34.3	29	0.543±0.157	0.305-0.954
Zn	29	5800±570	4700-7211	29	151±9.31	131-165
Cu	29	420±75.2	282-625	29	26.1±6.13	17.1-39.2
Ca	29	58736±5420	45571-72290	29	826±138	579-1124
Fe	29	437163±39089	368427-517746	29	22.0±9.15	12.3-60.6

3.1.2. Feather samples

Arsenic was the most abundant non-essential metal in feather of the female common eider, with a mean concentration of 1.10 µg/g ± 1.36 (range 0.0753 - 6.07 µg/g, n = 29, Table 1). 60 % of the birds exhibited concentrations under 0.5 µg/g (Appendix III). Mercury concentration (0.969 µg/g ± 0.328, n = 29, Table 1) showed the second highest concentration of the non-essential metals, Hg ranged between 0.462 - 1,91 µg/g, where 30 % of the samples exhibited concentrations over 1.0 µg/g (Appendix III). As seen with blood, Cd concentrations (0.00924 µg/g ± 0.00302, n = 29, Table 1) were low compared to the other non-essential metals. Concentrations of the most abundant essential metals in feather were Ca (826 µg/g ± 138, n = 29, Table 1) and Zn (151 µg/g ± 9.31, n = 29, Table 1). Concentrations of Mn and Se were low compared to the other essential metals (Table 1), where mean Ca and Zn levels were more than 1500 and 270 times higher than Mn, and 360 and 60 times higher than Se (Appendix III).

3.2. Relationship between metal concentrations in blood and feather

Correlation tests were used to examine whether the concentrations of metals in feathers reflect the concentrations in blood. Results from Pearson and Spearman correlation tests are presented in appendix IV. There was a significant positive correlation between Se (Pearson, r = 0.51, p = 0.0047, Appendix IV) and Cd (Pearson, r = 0.467, p = 0.011, Appendix IV). Regression analyses were conducted in order to study the relationship further (Figure 5 and 6). There was a significant positive relationship between Se (linear regression, slope = 0.394 ± 0.256, $F_{1,27}$ = 9.472, p = 0.005, r^2 = 0.260, Figure 5) and Cd (linear regression, slope = 0.300 ± 0.218, $F_{1,27}$ = 7.547, p = 0.011, r^2 = 0.218, Figure 6) in blood and feather. For all other measured metals, no significant correlations between the metal concentration of blood and feather were found (Pearson, p > 0.05, Spearman, p > 0.05).

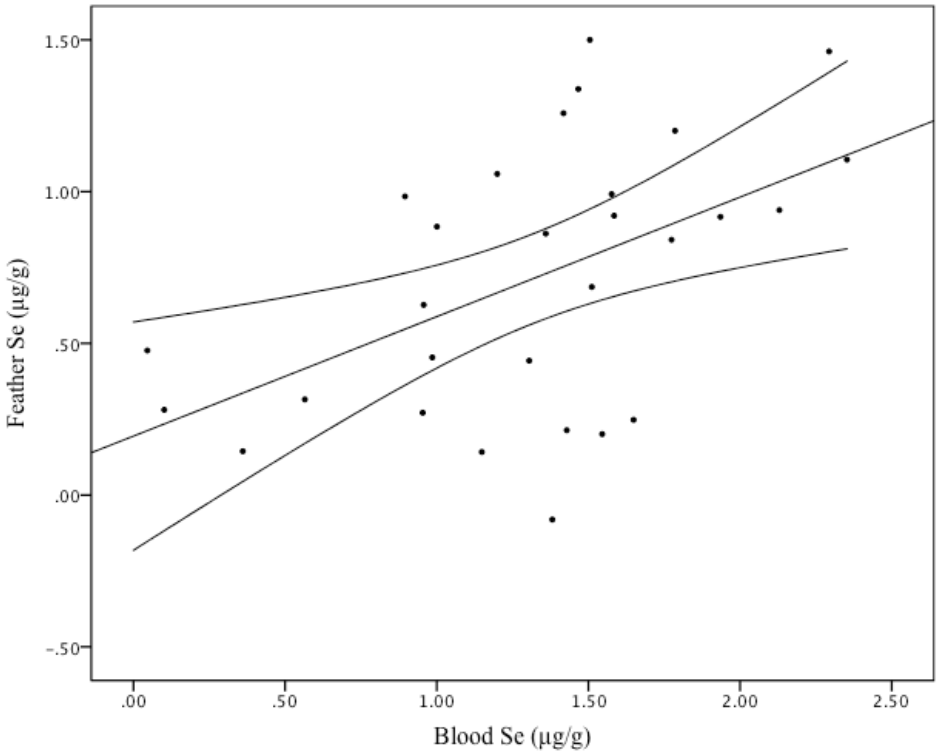


Figure 5: Linear relationship between Se concentrations (µg/g) in blood and feather of female common eiders (*Somateria mollissima*) (n = 29), sampled in Kongsfjorden, Svalbard, June 2011. All values are ln-transformed, linear regression line represents the correlation between feather and blood (slope = 0.394 ± 0.256, $F_{1,27} = 9.472$, $p = 0.005$, $r^2 = 0.260$). Curved lines denote the 95 % confidence interval.

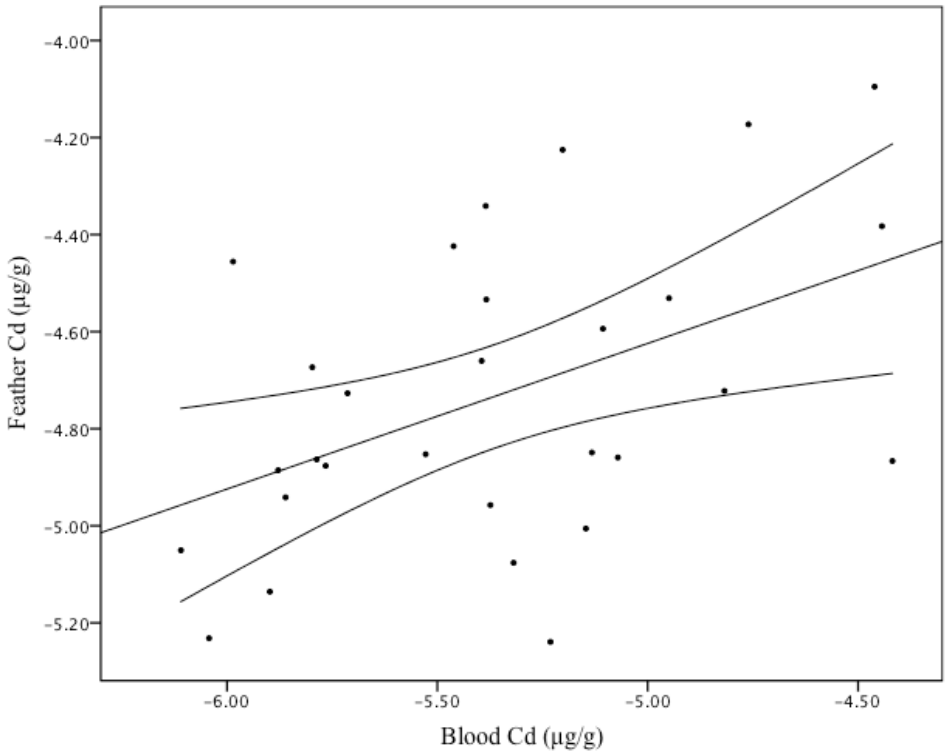


Figure 6: Linear relationship between Cd concentrations (µg/g) in blood and feather of female common eiders (*Somateria mollissima*) (n = 29), sampled in Kongsfjorden, Svalbard, June 2011. All values are ln-transformed, linear regression line represents the correlation between feather and blood (slope = 0.300 ± 0.218, $F_{1,27} = 7.547$, $p = 0.011$, $r^2 = 0.218$). Curved lines denote the 95 % confidence interval.

3.3. Relationships of metals within feathers

Correlation tests were performed to obtain information on the blood-levels at the time of feather formation, when the birds are feeding. At the same time the correlations can be used to investigate and illustrate possible mimicry between non-essential and essential metals. Statistical significant relationships between the metals in feathers are presented in appendix IV. Several significant correlations were observed and the strongest include Fe-Mn (Spearman, r = 0.76, p = 0.0000012), Cd-Ca (Pearson, r = 0.67, p = 0.000054), and Mn-Ca (Pearson, r = 0.66, p = 0.000086). Also, positive correlations were observed between Cd-Mn (Pearson, r = 0.62, p = 0.00033), Pb-Cd (Pearson, r = 0.39, p = 0.036), and Cd-Fe (Spearman, r = 0.42, p = 0.023). A borderline positive correlation was observed between As-Se (Pearson, r = 35, p = 0.065). Negative correlations were observed between As-Mn (Pearson, r = -0.47, p = 0.011), As-Fe (Spearman, r = -0.45, p = 0.015), Cu-Ca (Pearson, r = -0.43, p = 0.021), Cu-Mn (Pearson, r = -0.44, p = 0.017) Cd-As (Pearson, r = -0.49, p = 0.0073), and Cu-Fe (Spearman, r = -0.49, p = 0.0073).

4. Discussion

4.1. Metal concentrations in female common eiders

4.1.1. Blood samples

The female common eiders that were sampled in this study had been incubating for ~ four weeks, a period where they are known to fast, and consequently are expected to lose about 30-45 % of their body mass (Gabrielsen et al., 1991). This reduction may alter blood-organ ratios of total metal levels (Wayland et al., 2003). Hence, the blood concentrations of metals are thought to reflect both mobilization of metals stored or bound in tissues, as well as recent dietary, dermal or respiratory exposure from areas close to the breeding location (Burger et al., 2008; Rattner et al., 2008). Gabrielsen et al. (1991) presented a daily weight loss of 17.7 g in breeding common eiders in Svalbard, where 11 g were lost as fat, 1.8 g as protein, and 4.8 g as water. Metals bind to proteins, and Gabrielsen with co-workers (1991) suggested that the loss of protein would be related to redistribution of metals in the blood. Thus, redistributed metals may represent a large part of the metal levels in blood detected in the present study.

Regarding the non-essential metals, concentrations of Hg and Pb were high compared to Cd (Table 1). Differences in atmospheric contamination in Svalbard may be the explanatory factor; Berg et al. (2004) presented concentrations of metals measured between 1994-2002 at the Zeppelin atmospheric research station (Ny-Ålesund, Svalbard). Annual averages of Hg ranged between 1.50 - 1.79 ng m^{-3}, Pb varied between 0.48 and 0.83 ng m^{-3}, whereas Cd averages ranged from 0.01 - 0.03 ng m^{-3}.

The essential metals with the highest concentration in blood were Fe and Ca (Table 1). Iron is an essential dietary trace mineral and is necessary for haemoglobin production as well as in numerous enzymes, including those of cellular respiration (Osofsky et al., 2001). Body Fe is distributed into a number of different compartments, where blood-haemoglobin contains the largest amount (Tietz et al., 2008). Consequently, it is not surprising that Fe was the most abundant metal in blood, with a mean concentration of 437163 µg/kg reported here (Table 1). In female birds, medullary bone acts as labile reservoir for Ca which is released and used in eggshell formation (Dacke et al., 1993). The release of Ca from medullary bone might explain high Ca levels in blood of the female common eiders. Also Pb levels may be related to medullary bone. Lead is chemically similar to Ca, and may therefore easily assimilate in

medullary bone (Wilson et al., 2007). Lead is normally stored in bone in an inert form. However, release from bone under certain physiological conditions may lead to poisoning result (Clarkson, 1986). In a study of incubating Pacific common eiders Wilson et al. (2007) found high blood Pb levels in some individuals after egg production, and speculated in that common eiders may continue to utilize medullary storages to meet the nutritional demands of the incubation fast. If this is true, metabolic release of stored Pb in medullary bone could be responsible for a share of the Pb levels observed in blood of the female common eiders in the present study.

4.1.2. Feather samples

Metals enter feathers during the few weeks of feather formation, when they are connected to the circulatory system. Feathers are keratin structures rich in SH-groups, which are capable of binding metals available in the blood. Metal concentrations in feathers therefore represent metals present in the blood during feather formation (Burger and Gochfeld, 1997). As breeding female common eiders most likely leave Svalbard in the fall, and migrate to molt sites, concentrations of metals in feather will most likely reflect both winter dietary uptake, as well as mobilization of metals stored in internal tissues due to the stressful molting period (Evers et al., 1998).

Arsenic, followed by Hg are the non-essential metals in this study with the highest mean concentrations (Table 1). This may indicate that feathers are a significant excretory pathway for As and Hg. Braune and Gaskin (1987) found that in adult Bonaparte's gulls *(Larus philadelphia)*, the Hg in the plumage could account for as much as 93 % of the body burden. In addition, Hg is not concentrated in the uropygial gland (Scheuhammer, 1987), therefore the amount of external contamination is probably minimal. As in blood, feather concentrations of Cd were low compared to other non-essential metals. This may indicate that excretion of Cd to feather is a less important pathway than for Hg and As. However, Scheuhammer (1987) argued that feathers may be a good indicator of dietary Cd levels, since it had been shown experimentally that young wood ducks (*Aix sponsa*) could accumulate Cd in their growing feathers. The lower concentration of Cd may therefore be a result of low Cd exposures at the wintering grounds for the female common eiders.

For the essential metals, Ca concentration was the highest, followed by Zn (Table 1). Zinc is an essential metal that is required for normal feather formation (Jaspers et al., 2004), which

may explain the relatively high deposition rate of Zn into the feathers reported in the present study.

When using feathers to look at metal concentrations, it is important to recognize the possible influence of external contamination. Jaspers et al. (2004) suggested that concentrations of most metals increase with the age of the feather, indicating that exogenous contamination could be an important metal source. However, external contamination plays a lesser role in remote areas such as the Arctic because it is normally considered to be less polluted (Burger and Gochfeld, 2009b). In the present study the feathers were collected from an area of the birds back that was little exposed to the surroundings. In light of these factors, the level of external contamination in the sampled feathers is considered to be limited.

4.1.3. Toxic relevance of metal exposure

In general, Hg, Cd, Pb and As are of primary concern in marine environments, as they are non-essential and toxic. Also Se was present at relatively high concentrations, which can lead to toxic effects. Concentrations and toxicity thresholds for each of these metals will be discussed below. When looking at toxicity thresholds, it is important to consider the fact that the thresholds presented may not apply directly to the female common eiders sampled in the present study. Avian species vary considerably in their sensitivity to toxic elements, therefore factors such as taxonomic differences, physiology, and other environmental and physical stressors may exist and have to be taken into consideration when comparing avian species (Wenzel and Gabrielsen, 1995; Hargreaves et al., 2011). It is also important to note that feathers represent a route of excretion and not a target organ. Once the metal is bound in the feather, it is not physiologically available for redistribution to target organs (Wolfe et al., 1998), threshold concentration for metals in feather must therefore be used with caution.

In a study by Hargreaves et al. (2011), blood Hg level of 3000 µg/kg, represents the threshold for adverse effect in common loons *(Gavia immer)*. Incubating female common eiders sampled in the present study exhibited concentrations (117 µg/kg ± 31.9, range 62 - 201 µg/kg, Table 1) far below the toxicity threshold presented in Hargreaves et al. (2011). Feather concentrations of 5 µg/g are associated with adverse reproductive effects of birds (Burger and Gochfeld, 2000). The threshold for sublethal toxic effects in common loons are by Evers et al. (2008) set to 40 µg/g. Mercury concentration of feather in the present study (0.969 µg/g ± 0.328, range 0.462 - 1.91 µg/g, Table 1) is far below the levels associated with toxic effects.

Wayland et al. (2001) presented Hg concentration in blood of 0.14 - 0.37 μg/g in a study of female common eiders in the Canadian Arctic during June 1997 and July 1998. Which reflects the levels found in the common eiders in the present study (Table 1). In most other studies on metal contamination of common eider from Svalbard, invasive methods such as analysing liver and kidney samples have been conducted. Saunes (2011) examined liver tissues of female common eider from Kongsfjorden and Leifdefjorden, Svalbard, sampled in July of 2008 and 2009. In that study the liver concentration of Hg (1.61 μg/g dw ± 0.76) was found to be below the threshold levels connected to acute toxicity in seabirds. Savinov et al. (2003) also studied hepatic tissues on several seabird species at different locations in the Barents Sea in 1991-1992, including Ny-Ålesund. They found that Hg concentrations of seabirds in the Barents Sea were too low to cause toxic effects. However, the study showed that seabirds inhabiting the Ny-Ålesund area displayed slightly higher concentrations than in the other areas in the study. In northern fulmar (*Fulmarus glacialis*) the hepatic concentrations were close to the critical level. In summary, the levels of Hg found in the blood and feather of the female common eiders in the present study were all below toxicity thresholds, indicating that the common eiders in Svalbard are not in risk of exhibiting toxic effects from Hg. This is in agreement with concentrations reported in previous studies from Arctic areas.

The lower limit for subclinical effects of Pb in blood of birds in general is approximately 200 μg/kg (Hargreaves et al., 2011). Mean Pb level of the female common eiders sampled in the present study was 56.28 μg/kg ± 38.9 (range 19.9 - 198 μg/kg, Table 1), where three birds had levels higher than 100 μg/kg (individual 12, 15, and, 24, Appendix III). These three birds may have a Pb level close to subclinical Pb toxication. In feathers, adverse effects in birds occur at Pb levels of 4 μg/g, although seabirds can often tolerate higher levels (Burger and Gochfeld, 2000). The mean concentration in feather of the common eider is well below this threshold for toxic response (0.172 μg/g ± 0.0830, Table 1). The bird with the highest level of Pb exhibited a concentration of 0.354 μg/g (individual 3, Appendix III). Hollmén et al. (1998) investigated blood Pb levels in incubating female common eiders along the coast of south-western Finland. The mean concentration was found at 370 μg/kg (range 110 - 630 μg/kg). These concentrations are above the threshold for toxic response, indicating differences in Pb exposure in the Gulf of Finland compared to Svalbard. Burger and Gochfeld (2009a) found Pb levels of 0.993 μg/g ± 0.132 when studying breast feathers of 26 adult common eiders in

the Aleutian Chain of Alaska. These levels are higher than the levels presented in this study; however, they are well below the toxicity threshold.

Feather concentrations related to adverse effects of Cd have not been determined from laboratory studies. Conversion factors developed from Burger (1994) suggest that feather levels associated with adverse effects would range from 0.1 µg/g (shearwaters) to 2 µg/g (terns). The Cd level found in this study with a mean of 0.00924 µg/g (Table 1) is far below these levels. However, the wide variety in thresholds between different species makes comparisons to common eiders in the present study difficult.

The mean As concentration in feather of birds sampled was 1.10 µg/g ± 1.36 (range 0.0753-6.07 µg/g, Table 1). Arsenic showed large individual differences in feather, where two birds, individual 20 (6.07 µg/g, Appendix III), and individual 25 (4.004 µg/g, Appendix III) exhibited significantly higher concentrations than the rest of the individuals. 60 % of the birds showed feather concentrations under 0.5 µg/g (Appendix III). Savinov et al. (2003) investigated levels of As in liver samples from different seabird species in the Barents Sea. They found significantly higher levels of As in the northern part of the Barents Sea (10.2 mg/kg) compared to levels in liver from the same bird species from Ny-Ålesund (3.7 mg/kg). The authors argued that toxic effects of such As levels were not believed to be a problem for seabirds, because mainly the organic form of As was found in the bird's tissue. This may also be a possibility in the present study.

Despite the fact that Se may demonstrate protective properties toward Hg, As, and Cd toxicity, it can at high levels cause behavioural abnormalities, reproductive deficits, and ultimately mortality (Heinz et al., 1990). Burger and Gochfeld (2000) suggested that Se levels in feather of 3.8 - 26 µg/g is associated with severe adverse effects. The levels in this study ranged from 0.923 - 4.48 µg/g (Table 1), three individuals had concentrations over the lowest toxicity threshold (individual 16, 20, and 28, Appendix III). The results from the present study are similar to the study of Saunes (2011), where some of the female common eiders studied in Kongsfjorden were found to have hepatic Se levels exceeding the toxicity threshold.

It is important to note that the birds sampled in this study represent the most successful individuals in the population, having completed a long migration, found a partner and produced eggs. Contaminant burden accrued over winter could influence body condition in spring thereby reducing the number of birds attempting to breed (Grand et al., 2002). Hence,

it is possible that the females with high enough levels of toxic non-essential metals to affect reproduction were not breeding during the season of 2011. Non-breeding in some years has been observed in common eiders, and is considered to be a strategy by birds in poor condition to reduce the risk of mortality, or to avoid the risks associated with reproduction during years when less favourable environmental conditions exist (Coulson, 1984).

4.1.4. Comparison of blood and feather

By comparing levels in blood and feather one may get an indication of variation in exposure of metals throughout the year. Since blood concentrations of metals in birds are closely related to the concentrations in their prey, the blood concentration is expected to vary according to habitat (Hargreaves et al., 2011). In this study the blood samples give information of local metal concentrations in Kongsfjorden during the spring of 2011, while the feathers represent the blood level during molt, at wintering ground. Body feather molt in many seabirds is poorly known; the body feather may be molted at different times, hence, metal concentrations found in feather may represent different years in different individuals (Bond and Diamond, 2008). The toxic metals with the highest concentrations in blood were Hg and Pb, and in feather As and Hg. Arsenic concentrations in feather of common eiders may be a result of feeding on contaminated food in the coastal areas during winter migration, while Pb may be more prominent in areas close to the breeding sites in Svalbard.

4.2. Relationships between metals in blood and feather

The only metals that showed significant correlations between blood and feather were Se and Cd (Appendix IV), while no statistically significant correlations were observed for the other metals. Hence, with the exception of Se and Cd, the mean levels of metals present during feather formation do not reflect the metals present in the blood of female common eiders after approximately four weeks of fasting. The fact that few metals showed correlation between feather and blood, imply that use of feather samples to reflect blood levels of incubating common eider must be done with caution. This is related to the fact that feathers are grown at different times of the year, than the time of sampling; hence feathers represent the blood levels during the few weeks of feather growth. Metals circulating in the blood at the time of sampling, when the female common eider had been incubating for 26 days, are different from those that were available at the time when body feathers were formed. This is especially true for migratory species that molt away from the breeding grounds (Burger et al., 2008). In a

study conducted by Geens et al. (2010) on great tits (*Parus major*) South of Antwerp, possible correlations were examined between blood and feather for eight metals. Correlations were only found between Cd ($r = 0.37$, $p < 0.01$) and Pb ($r = 0.76$, $p < 0.001$). The authors suggested that a reason for the general discrepancy between blood and feather metal concentrations could be that blood metal concentrations reflect the immediate (i.e. dietary) conditions, while feather concentrations may be affected by exogenous contamination and reflect conditions at wintering ground. Geens et al. (2010) proposed that essential metals did not correlate because of a generally high homeostatic regulation of these metals in blood; they would therefore not be reflected in feather concentrations. This may be a plausible explanation in the present study, where blood concentrations of the essential metals Zn, Cu, Ca, Mn, and Fe did not reflect feather concentrations.

The few correlations that exist in this project may not be easily explained. During molting the birds normally sequester large amounts of metals in feather, and the positive correlations found between blood and feather for Cd and Se may indicate that the bird rapidly come in contact with these metals after molting; hence the blood and feather level show positive correlation. Another plausible possibility is related to redistribution of metals stored in other tissues into the blood during fasting, to cope with metabolic needs during incubation. Wayland et al. (2005) found in a study of incubating common eiders from the Canadian Arctic a depletion of hepatic Se during the incubation period, parallel to the decrease in liver mass. It was suggested that the decrease in hepatic Se was connected to redistribution of Se stored in liver, to meet nutritional needs. Hence, the correlations in this study may be an indication of Cd and Se being released into the blood during fasting to a higher degree than other metals.

4.3. Relationships of metals within feather

Common eiders molt at wintering ground, a period where the birds are expected to have access to food, thereby being exposed to both non-essential and essential metals. By looking at correlations between metals in feathers, one may gain information of metal exposure and also how metals are distributed from the blood to the feather of the common eider. Several metals showed significant positive correlation (Appendix IV). This is seen between Cd-Ca, Cd-Ma, Cd-Pb, and Cd-Fe, these positive correlations indicate co-variation in exposure as well as absorption to the circulatory system, and transfers from blood to feather. On the other

hand negative correlation was observed between Mn-As and Mn-Cu (Appendix IV). This may indicate that individuals with high Mn concentrations in feathers, are exposed to high levels of Mn and low levels of As and Cu via diet, and the environment (e.g. soil, sediment, water, and atmosphere). Another plausible explanation for the negative correlations is related to possible differences in metal absorption, due to variation in chemical properties of the metals (Beckett et al., 2007). Metal absorption in animals depend on a variety of factors, often directly related to the physiology of the animal (Debacker et al., 2000), but also which metal, and in which chemical form the metal is present (AMAP, 1998).

Selenium is known to have the capacity of reducing toxicity of certain metals such as Hg, As, and Cd, by forming inert compounds (AMAP, 1998). Positive correlation between Se and As was observed (Appendix IV), indicating possible As-Se complexes in the feathers of female common eider. However, no correlations were observed between Se and Hg nor between Se and Cd. Selenium and Hg concentrations are often positively correlated, assumingly because of the formation of Hg-Se complexes that are biologically immobile; hence the complex decreases the bioavailability of Hg in the organism (Burger et al., 2008; Sørmo et al., 2010). Scheuhammer et al. (2008) reported correlations between Hg and Se concentrations in livers and kidneys of several fish-eating avian species, including common loons. However, Leonzio et al. (1986) suggested that non-correlations between Hg and Se may be a result of too-low Hg levels. Since the Hg levels in the present study are low when compared to toxicity thresholds, the explanation presented Leonzio et al. (1986) might be credible also in the present study.

By looking at correlations within feather samples one may get an insight into possible metal mimicry between non-essential and essential metals. No obvious signs of mimicry were observed in the present study. However, it may be relevant to mention the fact that the non-essential metal As was negatively correlated to the essential metals Mn and Fe. Also, Cd was negatively correlated to Cu. No proof of mimicry between these metals has been reported in literature (Clarkson, 1993; Ballatori, 2002; Bridges and Zalups, 2005). The negatively correlated metals may therefore possibly be explained by differences in exposure, e.g. the female common eiders might feed on diets containing high levels of some metals and at the same time low concentrations of others. However, also in this context one cannot rule out possible differences in metal absorption of female common eiders.

Potential reasons for no sign of metal mimicry may be related to the homeostatic control of essential metals. The accumulation of essential metals is regulated homeostatically to keep concentrations in internal tissues physiologically adequate (Clarkson, 1986). Dauwe et al. (2004) conducted a study on feathers and excreta of nestling great tits collected from four study sites along a pollution gradient in the south of Antwerp, Beligum. When the authors compared the level of essential metals between the different study sites, no significant differences were found between the essential metals Cu, Zn, and nickel (Ni) in feathers. In excreta however, it was found increased excretion of the same essential metals in the most polluted study sites. The authors suggest that these findings were related to homeostatic regulation of essential metals in the great tit nestlings. Therefore, it is plausible that when levels of essential metals are sufficient to meet metabolic needs, no sign of mimicry is observed. Only when the levels of essential metals are low, non-essential metals will be taken up in the place of the essential. Petering (1978) proposed that diets deficient in Ca, Zn and Fe result in an increased intestinal uptake of Cd and enhanced Cd toxicity. Similarly Pb toxication has been related to low levels of Ca. Previous studies of adult mallard ducks (*Anas platyrhynchos*) were reviewed by Scheuhammer (1987); two groups of ducks were examined. The first group was fed to a diet low in Ca, and was exposed to a high dose of Pb (721 mg Pb per body weight). Within 30 days all birds died, after having developed signs of Pb intoxication. The second group of ducks was fed the same diet supplemented with additional Ca. Only 50 % mortality was observed within the second group, and the surviving mallard ducks were able to recover after experiencing some signs of Pb intoxication. Sufficient levels of essential metal nutritients such as Zn, Cu, and Fe could therefore protect against the toxicity of Cd and Pb. In light of these studies, it is possible that the female common eiders in the present study exhibited sufficient levels of essential metals during feather formation, and therefore were able to withstand mimicry.

5. Conclusions

During the present study, concentrations of potentially toxic metals and metalloids were examined in incubating female common eiders, using non-invasive techniques. Levels of most elements in blood and feather of female common eider were well below the toxicity thresholds known to present adverse effects; the exception being Se in feather where some individuals were close to the toxicity threshold. Based on the results, metal pollution in the Arctic does not seem to be an immediate threat to common eider colonies nesting in Svalbard. However, it is important to recognize that the birds sampled in the present study represent the most successful individuals within the population. Hence, it is possible that birds with metal concentrations high enough to affect reproduction were not breeding during the season of 2011. By looking at the differences between the non-essential metals with highest concentrations in blood vs. feather, we can see indications of different metal exposures throughout the year. In blood, the highest concentrations observed were of Hg and Pb, while in feathers, As and Hg exhibited the highest levels. These observations indicate varying metal exposures at the breeding site in Kongsfjorden, Svalbard and at molting sites at wintering ground. The relationships between levels of the selected metals in blood and feather were generally weak. In light of these observations, feather concentrations do not seem to represent blood concentrations in fasting common eider, with the exception of Se and Cd. Hence, blood samples should be used when wanting to look at recent dietary uptake, while feather samples may be used as an indicator of metal levels on wintering ground. There were no sign of metal mimicry of non-essential metals and essential metals, when examining feather samples. This may be related to the homeostatic control of essential metals, since mimicry most is apparent in birds with diets deficient in essential metals such as Ca, Zn and Fe. The findings may indicate that the female common eiders sampled in Svalbard during the breeding season of 2011, exhibited sufficient levels of essential metals to withstand from metal mimicry.

References

AMAP, 1998. Arctic pollution issues 1998: Heavy metals. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, 373-453.

Appelquist, H., Drabæk, I. & Asbirk, S., 1985. Variation in mercury content of guillemot feathers over 150 years. Marine Pollution Bulletin, 16, 244-248.

Baird, C. & Cann, M. C., 2008. Environmental chemistry, New York. Freeman.

Bakken, V. & Strøm, H., 2004. Svalbard as a seabird breeding site. Vår Fuglefauna, 27, 11-17.

Ballatori, N., 2002. Transport of toxic metals by molecular mimicry. Environmental Health Perspectives, 110, 689-694.

Beckett, W. S., Nordberg, G. F. & Clarkson, T. W. 2007. Routes of exposure, dose, and metabolism of metals. In: Nordberg, G., Fowler, B. A., Nordberg, A. & Friberg, L. (eds.) Handbook on the toxicology of metals. Third ed. Amsterdam, Academic Press, 39-64.

Berg, T., Kallenborn, R. & Manø, S., 2004. Temporal trends in atmospheric heavy metal and organochlorine concentrations at Zeppelin, Svalbard. Arctic, Antarctic, and Alpine Research, 36, 284-291.

Beyer, W. N., Dalgarn, J., Dudding, S., French, J. B., Mateo, R., Miesner, J., Sileo, L. & Spann, J., 2004. Zinc and Lead Poisoning in Wild Birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). Archives of Environmental Contamination and Toxicology, 48, 108-117.

Bond, A. L. & Diamond, A. W., 2008. High within-individual variation in total mercury concentration in seabird feathers. Environmental Toxicology and Chemistry, 27, 2375-2377.

Bond, A. L. & Diamond, A. W., 2009. Mercury concentrations in seabird tissues from Machias Seal Island, New Brunswick, Canada. Science of the Total Environment, 407, 4340-4347.

Braune, B. M., 1987. Comparison of total mercury levels in relation to diet and molt for nine species of marine birds. Archives of Environmental Contamination and Toxicology, 16, 217-224.

Braune, B. M. & Gaskin, D. E., 1987. Mercury levels in bonaparte's gulls (*Larus Philadelphia*) during autumn molt in the Quoddy region, New Brunswick, Canada. Archives of Environmental Contamination and Toxicology, 16, 539-549.

Bridges, C. C. & Zalups, R. K., 2005. Molecular and ionic mimicry and the transport of toxic metals. Toxicology and Applied Pharmacology, 204, 274-308.

Brunström, B. & Halldin, K., 2000. Ecotoxicological risk assessment of environmental pollutants in the Arctic. Toxicology Letters, 112, 111-118.

Burger, J., 1994. Metals in avian feathers: bioindicators of environmental pollution. Reviews in Environmental Toxicology, 5, 203-311.

Burger, J. & Gochfeld, M., 1992. Trace element distribution in in growing feathers: Additional excretion in feather sheats. Archives of Environmental Contamination and Toxicology, 23, 105-108.

Burger, J. & Gochfeld, M., 1993. Heavy-metal and selenium levels in feathers of young egrets and herons from Hong-Kong and Szechuan, China. Archives of Environmental Contamination and Toxicology, 25, 322-327.

Burger, J. & Gochfeld, M., 1997. Risk, mercury levels, and birds: Relating adverse laboratory effects to field biomonitoring. Environmental Research, 75, 160-172.

Burger, J. & Gochfeld, M., 2000. Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. Science of the Total Environment, 257, 37-52.

Burger, J. & Gochfeld, M., 2009a. Comparison of arsenic, cadmium, chromium, lead, manganese, mercury and selenium in feathers in bald eagle (*Haliaeetus leucocephalus*), and comparison with common eider (*Somateria mollissima*), glaucous-winged gull (*Larus glaucescens*), pigeon guillemot (*Cepphus columba*), and tufted puffin (*Fratercula cirrhata*) from the Aleutian Chain of Alaska. Environmental Monitoring and Assessment, 152, 357-367.

Burger, J. & Gochfeld, M., 2009b. Mercury and other metals in feathers of common eider (*Somateria mollissima*) and tufted puffin (*Fratercula cirrhata*) from the Aleutian Chain of Alaska. Archives of Environmental Contamination and Toxicology, 56, 596-606.

Burger, J., Gochfeld, M., Jeitner, C., Snigaroff, D., Snigaroff, R., Stamm, T. & Volz, C., 2008. Assessment of metals in down feathers of female common eiders and their eggs from the Aleutians: arsenic, cadmium, chromium, lead, manganese, mercury, and selenium. Environmental Monitoring and Assessment, 143, 247-256.

Bustnes, J. O. & Tertiski, G. M. 2000. Common eider, *Somateria mollissima*. In: Anker-Nilssen, T., Bakken, V., Strøm, H., Golovkin, A. N., Bianki, V. V. & Tatarinkova, I. P. (eds.) The Status of marine birds breeding in the Barents Sea region. Tromsø, The Norwegian Polar Institute, 46-51.

Bustnes, J. O. & Erikstad, K. E., 1988. The diets of sympatric wintering populations of common eider *Somateria mollissima* and king eider *S. spectabilis* in Northern Norway. Ornis Fennica, 65, 163-167.

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

Clarkson, T. W. 1986. Effects - general principles underlying the toxic action of metals. In: Friberg, L., Nordberg, G. F., Vouk, V. B. & Kessler, E. (eds.) Handbook on the toxicology of metals. Volume II. Second ed. Amsterdam, Elsevier, 128-148.

Clarkson, T. W., 1993. Molecular and ionic mimicry of toxic metals. Annual Review of Pharmacology and Toxicology, 33, 545-571.

Coulson, J. C., 1984. The population-dynamics of the eider duck *Somateria mollissima* and evidence of extensive non-breeding by adult ducks. Ibis, 126, 525-543.

Cuypers, A., Vangronsveld, J. & Clijsters, H., 1999. The chemical behaviour of heavy metals plays a prominent role in the induction of oxidative stress. Free Radical Research, 31, 39-43.

Dacke, C. G., Arkle, S., Cook, D. J., Wormstone, I. M., Jones, S., Zaidi, M. & Bascal, Z. A., 1993. Medullary bone and avian calcium regulation. Journal of Experimental Biology, 184, 63-88.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R. & Eens, M., 2000. Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? Archives of Environmental Contamination and Toxicology, 39, 541-546.

Dauwe, T., Janssens, E., Bervoets, L., Blust, R. & Eens, M., 2004. Relationships between metal concentrations in great tit nestlings and their environment and food. Environmental Pollution, 131, 373-380.

Davidson, T., Ke, Q. & Costa, M. 2007. Selected molecular mechanisms of metal toxicity and carcenogenicity. In: Nordberg, G., Fowler, B. A., Nordberg, A. & Friberg, L. (eds.) Handbook on the toxicology of metals. Third ed. Amsterdam, Elsevier, 128-148.

Debacker, V., Jauniaux, T., Coignoul, F. & Bouquegneau, J. M., 2000. Heavy metals contamination and body condition of wintering guillemots (*Uria aalge*) at the Belgian Coast from 1993 to 1998. Environmental Research, 84, 310-317.

Elliott, J. E., Scheuhammer, A. M., Leighton, F. A. & Pearce, P. A., 1992. Heavy metal and metallothionein concentrations in Atlantic Canadian seabirds. Archives of Environmental Contamination and Toxicology, 22, 63-73.

Evers, D., Savoy, L., DeSorbo, C., Yates, D., Hanson, W., Taylor, K., Siegel, L., Cooley, J., Bank, M., Major, A., Munney, K., Mower, B., Vogel, H., Schoch, N., Pokras, M., Goodale, M. & Fair, J., 2008. Adverse effects from environmental mercury loads on breeding common loons. Ecotoxicology, 17, 69-81.

Evers, D. C., Kaplan, J. D., Meyer, M. W., Reaman, P. S., Braselton, W. E., Major, A., Burgess, N. & Scheuhammer, A. M., 1998. Geographic trend in mercury measured in common loon feathers and blood. Environmental Toxicology and Chemistry, 17, 173-183.

Fenstad, A., 2011. POP levels, DNA double-strand breaks and vitamin status during incubation fast in female common eiders (*Somateria mollissima*) in Svalbard. Master´s thesis in environmental toxicology, Norwegian University of Science and Technology.

Fimreite, N., Brun, E., Froslie, A., Frederichsen, P. & Gundersen, N., 1974. Mercury in eggs of Norwegian sea birds. Astarte, 7, 71-76.

Furness, R. W. 1993. Birds as monitors of pollutants. In: Furness, R. W. & Greenwood, J. J. D. (eds.) Birds as monitors of environmental change. London, Chapman & Hall, 86-143.

Furness, R. W. & Camphuysen, C. J., 1997. Seabirds as monitors of the marine environment. Ices Journal of Marine Science, 54, 726-737.

Gabrielsen, G. W., Mehlum, F., Karlsen, H. E., Andresen, Ø. & Parker, H., 1991. Energy cost during incubation and thermoregulation in the female common eider *Somateria mollissima*. Norsk Polarinstitutt Skrifter, 195, 51-62.

Garciá-Fernández, A. J., Sanchez-Garcia, J. A., Gomez-Zapata, M. & Luna, A., 1996. Distribution of cadmium in blood and tissues of wild birds. Archives of Environmental Contamination and Toxicology, 30, 252-258.

Geens, A., Dauwe, T., Bervoets, L., Blust, R. & Eens, M., 2010. Haematological status of wintering great tits (*Parus major*) along a metal pollution gradient. Science of the Total Environment, 408, 1174-1179.

Gellein, K., 2008. High resolution inductively coupled plasma mass spectrometry: Some applications in biomedicine. Doctorial thesis, Norwegian University of Science and Technology.

Goyer, R. A., 1997. Toxic and essential metal interactions. Annual Review of Nutrition, 17, 37-50.

Grand, J. B., Franson, J. C., Flint, P. L. & Petersen, M. R., 2002. Concentrations of trace elements in eggs and blood of spectacled and common eiders on the Yukon-Kuskokwim Delta, Alaska, USA. Environmental Toxicology and Chemistry, 21, 1673-1678.

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

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

Hargreaves, A. L., Whiteside, D. P. & Gilchrist, G., 2011. Concentrations of 17 elements, including mercury, in the tissues, food and abiotic environment of Arctic shorebirds. Science of the Total Environment, 409, 3757-3770.

Heinz, G. H., Pendleton, G. W., Krynitsky, A. J. & Gold, L. G., 1990. Selenium accumulation and elimination in mallards. Archives of Environmental Contamination and Toxicology, 19, 374-379.

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

Honda, K., Min, B. Y. & Tatsukawa, R., 1986. Distribution of heavy metals and their age-related changes in the eastern great white egret, *Egretta alba modesta*, in Korea. Archives of Environmental Contamination and Toxicology, 15, 185-197.

Högberg, J. & Alexander, J. 2007. Selenium. In: Nordberg, G., Fowler, B. A., Nordberg, A. & Friberg, L. (eds.) Handbook on the toxicology of metals. Third ed. Amsterdam, Elsevier, 783-807.

Jaspers, V., Dauwe, T., Pinxten, R., Bervoets, L., Blust, R. & Eens, M., 2004. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, *Parus major*. Journal of Environmental Monitoring, 6, 356-360.

Klaassen, C. D., Liu, J. & Choudhuri, S., 1999. Metallothionein: An intracellular protein to protect against cadmium toxicity. Annual Review of Pharmacology and Toxicology, 39, 267-294.

Koivula, M. J. & Eeva, T., 2010. Metal-related oxidative stress in birds. Environmental Pollution, 158, 2359-2370.

Korschgen, C. E., 1977. Breeding stress of female eiders in Maine. Journal of Wildlife Management, 41, 360-373.

Kovacs, K. M. & Lydersen, C., 2006. Svalbards fugler og pattedyr, Oslo. Norsk polarinstitutt.

Leonzio, C., Fossi, C. & Focardi, S., 1986. Heavy metals and selenium variation in a migratory bird wintering in a mercury-polluted lagoon. Bulletin of Environmental Contamination and Toxicology, 37, 219-225.

Lewis, S. A. & Furness, R. W., 1991. Mercury accumulation and excretion in laboratory reared black-headed gull *Larus ridibundus* chicks. Archives of Environmental Contamination and Toxicology, 21, 316-320.

Liu, J., Goyer, R. A. & Waalkes, M. P. 2008. Toxic effects of metals. In: Klaassen, C. D. & Casarett, L. J. (eds.) Casarett and Doull's Toxicology: the basic science of poisons. New York, McGraw-Hill Medical, 931-979.

Lorentsen, S.-H. & Røstad, O. W., 1990. Tellinger av mytende ærfugl, *Somateria mollissima*, langs Skagerrakkysten i 1987. Trondheim. Norsk institutt for naturforskning.

Malinga, M., Szefer, P. & Gabrielsen, G. W., 2010. Age, sex and spatial dependent variations in heavy metals levels in the glaucous gulls (*Larus hyperboreus*) from the Bjørnøya and Jan Mayen, Arctic. Environmental Monitoring and Assessment, 169, 407-416.

Manahan, S. E., 2010. Environmental chemistry. Boca Raton. Taylor & Francis Group.

Mehlum, F., Gjertz, I. & Brekke, A., 1990. The birds and mammals of Svalbard. Oslo. Norsk polarinstitutt.

Monteiro, L. R. & Furness, R. W., 1995. Seabirds as monitors of mercury in the marine environment. Water Air and Soil Pollution, 80, 851-870.

Osofsky, A., Jowett, P. L. H., Hosgood, G. & Tully, T. N., 2001. Determination of normal blood concentrations of lead, zinc, copper, and iron in hispaniolan amazon parrots (*Amazona ventralis*). Journal of Avian Medicine and Surgery, 15, 31-36.

Parker, H. & Holm, H., 1990. Patterns of nutrient and energy-expenditure in female common eiders nesting in the High Arctic. The American Ornithologists' Union, 107, 660-668.

Petering, H. G., 1978. Some observations on the interactions of zinc, copper, and iron metabolism in lead and cadmium toxicity. Environmental Health Perspectives, 25, 141-145.

Rattner, B. A., Golden, N. H., Toschik, P. C., McGowan, P. C. & Custer, T. W., 2008. Concentrations of metals in blood and feathers of nestling ospreys (*Pandion haliaetus*) in Chesapeake and Delaware Bays. Archives of Environmental Contamination and Toxicology, 54, 114-122.

Røv, N., Kroglund, R. T. & Bergstrøm, R. 1992. Bestandsstørrelse, utbredelse og underartstilhørighet hos ærfugl *Somateria molissima*. NINA oppdragsmelding 129. Trondheim: Norsk institutt for naturforskning, 1-18.

Saunes, H., 2011. Do mercury, cadmium and zinc cause oxidative stress in common eiders (*Somateria mollissima*) from Svalbard. Master`s thesis in environmental toxicology, Norwegian University of Science and Technology.

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

Scheuhammer, A. M., 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds : A review. Environmental Pollution, 46, 263-295.

Scheuhammer, A. M., Basu, N., Burgess, N. M., Elliott, J. E., Campbell, G. D., Wayland, M., Champoux, L. & Rodrigue, J., 2008. Relationships among mercury, selenium, and neurochemical parameters in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). Ecotoxicology, 17, 93-101.

Smith, R. M., 2004. Understanding mass spectra: A basic approach. New Jersey. John Wiley & Sons Inc.

Sodhi, G. S., 2005. Fundamental concepts of environmental chemistry. Harrow. Alpha Science International.

Steffen, A., Douglas, T., Amyot, M., Ariya, P., Aspmo, K., Berg, T., Bottenheim, J., Brooks, S., Cobbett, F., Dastoor, A., Dommergue, A., Ebinghaus, R., Ferrari, C., Gardfeldt, K., Goodsite, M. E., Lean, D., Poulain, A. J., Scherz, C., Skov, H., Sommar, J. & Temme, C., 2008. A synthesis of atmospheric mercury depletion event chemistry in the atmosphere and snow. Atmospheric Chemistry and Physics, 8, 1445-1482.

Sterner, O., 2010. Chemistry, health, and environment. Weinheim. Wiley-Blackwell.

Strøm, H. 2011. Common eider [Online]. http://www.npolar.no/en/species/common-eider.html: Norwegian Polar Institute. [Accessed 17.01. 2012].

Svensson, L., Grant, P. J., Mullarney, K., Zetterström, D. & Ree, V., 2004. Gyldendals store fugleguide: Europas og middelhavsområdets fugler i felt. Oslo. Gyldendal fakta.

Sørmo, E. G., Ciesielski, T. M., Øverjordet, I. B., Eggen, G. S. & Jenssen, B. M. 2010. Effekter av metaller på metallothionein i ørret fra Mjøsa og Losna. Trondheim: NTNU.

Thomas, R., 2004. Practical guide to ICP-MS, New York. Marcel Dekker.

Tietz, N. W., Burtis, C. A., Ashwood, E. R. & Bruns, D. E., 2008. Tietz fundamentals of clinical chemistry. St. Louis, Saunders Elsevier.

Waldeck, P., Hagen, J. I., Hanssen, S. A. & Andersson, M., 2011. Brood parasitism, female condition and clutch reduction in the common eider *Somateria mollisima*. Journal of Avian Biology, 42, 231-238.

Walker, C. H., 2006. Principles of ecotoxicology. Boca Raton, Taylor & Francis Group.

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

Wayland, M., Garcia-Fernandez, A. J., Neugebauer, E. & Gilchrist, H. G., 2001. Concentrations of cadmium, mercury and selenium in blood, liver and kidney of common eider ducks from the Canadian Arctic. Environmental Monitoring and Assessment, 71, 255-267.

Wayland, M., Gilchrist, H. G. & Neugebauer, E., 2005. Concentrations of cadmium, mercury and selenium in common eider ducks in the eastern Canadian Arctic: Influence of reproductive stage. Science of the Total Environment, 351–352, 323-332.

Wayland, M., Smits, J. J. E. G., Grant Gilchrist, H., Marchant, T. & Keating, J., 2003. Biomarker responses in nesting, common eiders in the Canadian Arctic in relation to tissue cadmium, mercury and selenium concentrations. Ecotoxicology, 12, 225-237.

Wenzel, C. & Gabrielsen, G. W., 1995. Trace element accumulation in three seabird species from Hornøya, Norway. Archives of Environmental Contamination and Toxicology, 29, 198-206.

Wilson, H. M., Flint, P. L. & Powell, A. N., 2007. Coupling contaminants with demography: Effects of lead and selenium in Pacific common eiders. Environmental Toxicology and Chemistry, 26, 1410-1417.

Wolfe, M. F., Schwarzbach, S. & Sulaiman, R. A., 1998. Effects of mercury on wildlife: A comprehensive review. Environmental Toxicology and Chemistry, 17, 146-160.

Appendices

Content

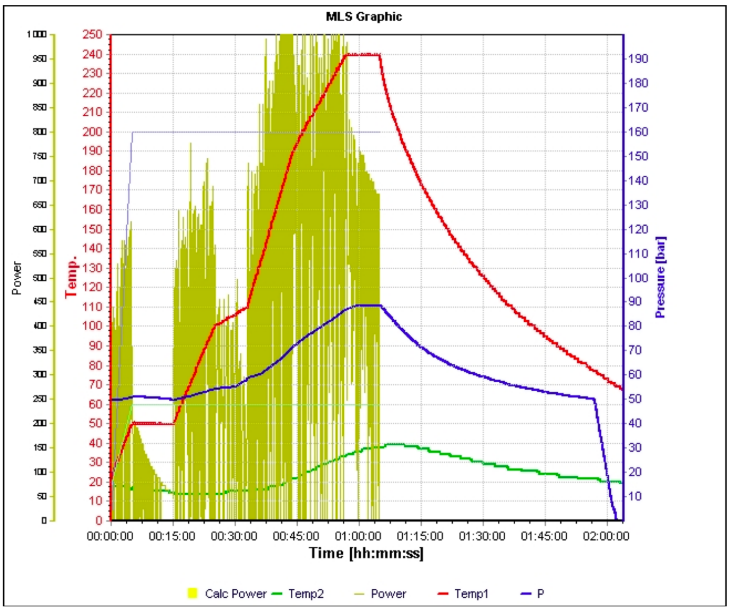
Appendix I: Course/procedure and temperature program UltraCLAVE…….. ii
Appendix II: Operational parameters……. iii
Appendix III: Trace element concentrations in common eider blood and feather…….. iv
Appendix IV: Relationships between blood and feather…….. v

Appendix I - Procedure and temperature program UltraCLAVE

MLS Microwave Report

Application: ultraCLAVE
Report 15.12.2011 15:20:05 **Operator:** Administrator
Filename: M:\A\2011\PROJECTS NOT FINNISHED\BIOLOGI\Anette Fenstad - PhD\590-141211-41-80-Anette-Fenstad-blod-1-37.dpr

MLS Milestone
www.milestonesrl.com



Parameter

```
Signature name  :
Signature date  :
Signature func. :
Operator        :Administrator
Date            :
Method filename
:Animal500mg-Vol-60ml.mpr
Create run      :Administrator
Microwave Power :pulse
Load pressure   :50,0 bar
Release temp.   :78,0 °C
Release pressure:10,0 bar/min
Cooling         :ON
Auto open       :OFF
Cooling on Temp.:80,0 °C
Ground load     :300ml MQ 2ml
H2SO4 30ml H2O2
Ventilation time:00:59:07
```

Remark:

Data HNO3: Grade Ultra Pure, Molarity 14.4, Density 1.40
approx. 500 mg blood is digested with 0.5ml conc. HNO3, diluted to a final volume of 12ml (12.2+-0.2g)

MW Program

Step	Time [hh:mm:ss]	Temp 1 [°C]	Temp 2 [°C]	Press [bar]	Engery [Watt]
1	00:05:00	50	60	160	1 000
2	00:10:00	50	60	160	1 000
3	00:10:00	100	60	160	1 000
4	00:08:00	110	60	160	1 000
5	00:11:00	190	60	160	1 000
6	00:05:00	210	60	160	1 000
7	00:08:00	240	60	160	1 000
8	00:08:00	240	60	160	1 000

Figure A1: Overview of procedure and temperature program from the high-pressure microwave system UltraCLAVE used for digestion of blood and feather samples of common eiders (*Somateria mollissima*), sampled in Kongsfjorden, Svalbard, June 2011.

Appendix II – Operational parameters

Table A1: Detection limits for metals analysed in feathers and blood of common eider (*Somateria mollissima*) by HR-ICP-MS. Resolution given in low (Lr), median (Mr) and high (Hr) and intern detection limit (IDL). Detection limits were calculated from IDL-25% values.

Element	Isotope	Resolution	IDI-25% (µg/l)	Blood Detection Limit (µg/kg)	Feather Detection Limit (µg/g)
Hg	202	Lr	0.0010	0.024	0.00080
Pb	208	Lr	0.0020	0.048	0.0016
As	75	Hr	0.025	0.60	0.020
Cd	114	Lr	0.0020	0.048	0.0016
Se	78	Hr	0.15	3.6	0.12
Mn	55	Mr	0.0060	0.14	0.0048
Zn	66	Mr	0.025	0.60	0.020
Cu	63	Mr	0.030	0.48	0.016
Ca	44	Mr	2.0	48	1.6
Fe	56	Mr	0.020	0.48	0.016

Table A2: Element concentrations in reference blood serum (SeronromTM Trace Elements Whole Blood L-1, Sero, values in µg/l), and tealeaves (GBW-07605, Landfang, China, Chem Scan, values in µg/g) in the ICP-MS analysis. Values are given in mean certified value and mean ± SD values for present work. Values in brackets denote proposed values.

Reference material / **metal**	**Certified value** **Mean / (proposed values)**	**Present work** **Mean ± SD**
Blood serum (SeronormTM) µg/l		
Hg	2.2	2.597 ± 0.250
Pb	27.6	27.8 ± 0.242
As	1.8	2.035 ± 0.122
Cd	0.74	0.688 ± 0.0183
Se	79.8	81.7 ± 1.83
Mn	10.6	11.2 ± 0.254
Cu	564	626 ± 17.1
Zn	5500	5463 ± 57.4
Ca	14200	13027 ± 265
Fe	432000	424798 ± 8891
Tea leaves (GBW-07605) µg/g		
Hg	(0.013)	0.0023 ± 0.0005
Pb	4.4	4.03 ± 0.374
As	0.28	0.212 ± 0.028
Cd	0.057	0.061 ± 0.006
Se	(0.072)	0.056 ± 0.006
Mn	1240	1097 ± 62.3
Cu	17.3	17.1 ± 1.66
Zn	26.3	24.5 ± 2.1
Ca	4300	4206 ± 410
Fe	264	218 ± 18

Table A3: Complete overview of metal concentrations in blood (µg/kg) and feather (µg/g) in female common eiders (Somateria mollissima) (n = 29) from Kongsfjorden, Svalbard, June 2011.

	Hg		Pb		As		Cd		Se	
Individual	**Blood µg/kg**	**Feather µg/g**	**Blood µg/kg**	**Feather µg/g**	**Blood µg/kg**	**Feather µg/g**	**Blood µg/kg**	**Feather µg/g**	**Blood µg/kg**	**Feather µg/g**
1	123	0.989	38.6	0.138	11.6	2.21	2.85	0.00714	2 603	1.87
2	119	1.61	55.9	0.156	9.64	0.475	4.90	0.00624	4 531	1.99
3	103	0.916	48.1	0.354	14.8	1.18	6.06	0.0101	2 679	1.57
4	179	1.25	19.9	0.277	26.8	0.531	5.50	0.0146	1 760	1.37
5	201	0.773	22.9	0.0930	7.45	0.307	3.04	0.00934	1 046	1.61
6	116	1.91	87.3	0.303	38.2	0.491	5.90	0.00784	8 413	2.56
7	126	0.868	34.5	0.163	23.4	2.25	3.97	0.00781	3 687	1.56
8	94.9	0.959	20.3	0.305	28.7	0.156	8.56	0.0154	1 107	1.32
9	75.1	0.614	44.6	0.274	31.2	0.412	12.1	0.00770	2 448	2.67
10	147	1.22	40.0	0.138	21.6	0.219	11.6	0.0167	4 173	1.24
11	88.6	0.919	31.6	0.134	19.6	0.606	3.30	0.00885	1 434	1.16
12	133	0.656	198	0.180	22.3	0.075	11.8	0.0125	4 837	2.70
13	101	0.594	54.1	0.116	18.9	0.175	3.07	0.00772	6 928	2.50
14	97.1	1.52	37.0	0.0952	18.1	0.288	4.59	0.0130	5 964	3.32
15	135	1.08	155	0.324	16.0	0.214	4.25	0.0120	3 893	2.37
16	97.3	1.26	62.3	0.238	22.6	1.02	7.09	0.0108	9 911	4.32
17	118	0.754	54.4	0.232	22.6	0.216	4.54	0.00946	10 511	3.02
18	122	0.462	38.0	0.166	25.3	0.260	2.22	0.00641	4 690	1.22
19	154	0.714	64.6	0.163	32.4	0.253	4.64	0.00703	3 153	1.15
20	62.0	0.660	25.2	0.164	14.5	6.07	2.37	0.00534	4 334	3.81
21	62.9	1.04	51.5	0.193	21.9	2.72	2.80	0.00756	4 879	2.51
22	89.4	0.862	23.5	0.0584	17.5	0.435	5.35	0.00530	2 594	1.31
23	118	0.603	60.9	0.127	26.4	0.357	4.59	0.0107	3 979	0.923
24	159	0.999	100	0.0644	33.7	1.41	6.28	0.00775	5 896	2.32
25	79.5	0.796	79.6	0.0907	13.4	4.00	2.74	0.00588	4 127	3.52
26	122	0.841	44.9	0.0623	25.5	0.865	3.13	0.00762	5 200	1.28
27	127	0.860	55.2	0.139	27.8	1.91	2.51	0.0116	3 319	2.88
28	127	1.15	36.9	0.0977	16.3	2.36	5.82	0.00670	4 502	4.48
29	123	1.22	46.9	0.144	21.6	0.299	8.09	0.00890	2 720	2.42

Table A3 Continued

Individual	Mn		Zn		Cu		Ca		Fe	
	Blood µg/kg	Feather µg/g	Blood µg/kg	Feather µg/g	Blood µg/kg	Feather µg/g	Blood µg/kg	Feather µg/g	Blood µg/kg	Feather µg/g
1	10.9	0.325	5 351	151	494	24.5	49 184	841	416 242	12.3
2	17.1	0.537	5 289	139	385	25.6	56 079	593	415 949	20.8
3	13.6	0.437	7 211	151	357	25.5	56 143	921	442 898	17.1
4	9.38	0.645	5 399	144	381	19.1	56 235	1 124	453 823	21.4
5	10.2	0.492	4 700	146	342	21.1	55 578	837	412 355	16.4
6	14.5	0.316	5 606	150	432	30.3	59 368	617	462 644	17.2
7	11.3	0.555	6 554	155	433	17.1	61 981	921	432 302	23.1
8	15.8	0.543	5 071	161	282	20.5	55 025	754	370 867	27.8
9	13.7	0.451	5 704	157	392	38.5	59 995	719	453 546	20.2
10	22.6	0.954	5 692	137	466	23.9	66 142	1 060	412 740	38.2
11	14.0	0.647	5 922	152	328	22.5	54 603	967	368 427	19.7
12	12.0	0.692	5 453	155	470	26.2	63 140	867	419 496	21.3
13	14.5	0.756	5 392	136	424	24.3	63 810	860	402 702	60.6
14	14.1	0.594	6 450	149	467	17.1	58 800	829	439 806	23.3
15	11.6	0.630	5 734	158	475	24.4	52 579	1 015	483 594	25.0
16	18.5	0.735	5 674	161	466	31.1	61 861	917	459 587	25.2
17	12.4	0.593	6 406	165	514	18.2	55 167	857	468 750	22.9
18	14.1	0.583	5 875	152	377	26.9	50 302	660	509 411	22.3
19	17.1	0.546	6 918	162	625	20.1	58 476	853	483 803	19.2
20	32.4	0.323	6 174	153	368	30.3	72 290	736	374 645	14.6
21	9.37	0.521	5 667	138	540	29.9	63 105	734	384 087	23.2
22	15.2	0.335	5 389	158	382	28.6	61 120	688	461 113	16.4
23	17.2	0.806	5 415	139	428	19.1	59 972	992	517 746	24.9
24	14.7	0.305	5 858	153	385	33.1	60 875	579	456 999	13.8
25	11.2	0.452	5 331	157	342	29.7	45 571	722	422 165	17.5
26	11.7	0.395	5 674	150	390	36.1	61 623	796	412 678	12.7
27	9.43	0.586	6 153	131	534	31.3	63 525	968	421 974	15.9
28	34.3	0.548	5 459	162	347	22.3	61 369	742	430 536	27.4
29	15.2	0.439	6 700	166	381	39.2	59 432	806	486 849	19.0

Table A4: Correlation table for all metals in blood and feather (n = 29) of female common eider (*Somateria mollissima*) from Kongsfjorden, Svalbard, June 2011. Metals in blood is indicated by B, while feather is indicated by F. Manganese in blood (MnB) and iron in feather (FeF) are represented with Spearman correlation, while rest with Pearson correlation. Significant correlations have $p < 0.05$ and borderline significant correlations have $p = 0.05$-0.09.

	CaF r p	**CaB** r p	**ZnF** r p	**ZnB** r p	**HgF** r p	**HgB** r p	**MnF** r p	**MnB** r p	**FeF** r p	**FeB** r p	**CuF** r p	**CuB** r p	**AsF** r p	**AsB** r p	**SeF** r p	**SeB** r p	**CdF** r p	**CdB** r p	**PbF** r p	**PbB** r p
CaF							0.66 0.000086		0.34 0.071		-0.43 0.021						0.67 0.000054			
CaB																				
ZnF							-0.33 0.078													
ZnB												0.35 0.06								
HgF																				
HgB															-0.32 0.088		0.34 0.070			
MnF	0.66 0.000086		-0.33 0.078						0.76 0.0000012		-0.44 0.017		-0.47 0.011				0.62 0.00033			
MnB																		0.40 0.030		
FeF	0.34 0.071						0.76 0.0000012				-.049 0.0073		-0.45 0.015				0.42 0.023			
FeB														0.33 0.080						
CuF	-0.043 0.021						-0.44 0.017		-0.49 0.0073								-0.33 0.077			
CuB				0.35 0.061												0.44 0.018				0.42 0.023
AsF							-0.465 0.0111		-0.449 0.0147						0.35 0.065		-0.49 0.0073	-0.47 0.0097		
AsB										0.3301 0.0803								0.33 0.084		
SeF						-0.32 0.088							0.35 0.065			0.51 0.0047				
SeB												0.437 0.0179			0.51 0.0047					0.55 0.0021
CdF	0.68 0.000054					0.34 0.070	0.62 0.00033		0.42 0.023		-0.33 0.077		-0.49 0.0073					0.47 0.011	0.39 0.036	
CdB								0.40 0.030					-0.47 0.0097	0.33 0.084			0.47 0.011			
PbF																	0.39 0.036			
PbB												0.42 0.023				0.55 0.0021				