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# Assessing the Diet of the Golden eagle (*Aquila chrysaetos*) and the Biomagnification of Metals by use of Stable isotope analysis and ICP-MS.

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**Abstract**

Feathers from the golden eagle and muscle tissue from the most important sources of prey (hare, sheep, grouse and reindeer) in the golden eagle diet were analysed for stable isotopes of carbon and nitrogen, and for the heavy metals lead (Pb), cadmium (Cd) and mercury (Hg), and the essential trace elements selenium (Se) and copper (Cu). The samples were obtained from two regions in Central-Norway (Nord-Trøndelag and Sør-Trøndelag), one coastal and one inland region. The Bayesian mixing model MixSIR was used to estimate the proportion of the different prey sources in the diet of the golden eagles. This proportion was used to calculate biomagnification of the selected metals. Regression analysis was used to investigate if there were significant correlations between the metal levels and the proportion of various prey in the diet of golden eagle.

The modelled diet of golden eagle varied depending on their age and the region they inhabited. Compared to other studies, metal levels were below harmful levels. Biomagnification were found for Pb and Hg. There were weak significant relationships between the proportion of sheep in the diet of golden eagle, and the levels of Cu and Hg, and the proportion of grouse and levels of Cu. Significant correlations between Se and Hg were found in the eagle feathers and also in the modelled diet of the golden eagle. However, the correlation was weak, and the antagonistic relation between Se and Hg found in many other studies was not evident here. This method is promising for evaluating the diet of the golden eagle. To my knowledge, no similar study has used this approach to assess biomagnification of metals.

**Sammendrag**

Fjær fra kongeørn og muskelvev fra de viktigste byttedyrartene i dietten hos kongeørn (sau, hare, rype og rein) ble analysert for stabile isotoper karbon og nitrogen, og for tungmetallene bly (Pb), kadmium (Cd), kvikksølv (Hg), samt de essensielle spormetallene selen (Se) og kobber (Cu). Fjær og vev ble hentet inn fra Nord- og Sør-Trøndelag, både fra kysten og fra innlandet. Softwarepakken MixSIR, som bruker bayesianks metode, ble brukt for å estimere andelen av de ulike byttedyrartene i dietten hos kongeørn. Denne andelen ble så brukt for å kalkulere biomagnifisering av de utvalgte metallene. Regresjonsanalyse ble brukt for å avdekke om det var sammenheng mellom metaller og andelen av bestemte byttedyr i dietten hos kongeørn. Korrelasjonsanalyse ble brukt for å finne korrelasjoner mellom Hg og Se.

Den modellerte dietten hos kongeørn varierte med alder og geografisk område. Nivåene av metaller var generelt lave, og under hva som er kjent som skadelige nivåer. Biomagnifisering ble funnet hos Pb og Hg. Det ble funnet svake sammenhenger mellom nivået av Cu og Hg og andelen sau, og mellom Cu og andelen skogsfugl i dietten hos kongeørn. Korrelasjoner ble funnet mellom Se og Hg i fjær og modellert diett. Sammenhengene var svake og kunne ikke påvise noe antagonistisk effekt av Se på Hg. Denne metoden er lovende for kartlegging av dietten hos kongeørn. Så lang jeg har oversikt, eksister det ikke tilsvarende studier som undersøker biomagnifisering med samme metode.

**Table of contest**

## 1. Introduction

Rachel Carson´s "Silent Spring" (1962) was the first book to raise public awareness about birds' vulnerability to pollution. Ever since, harmful effects of pesticides and other chemicals on birds and whole ecosystems has received much focus (Carson 1962, Furness and Greenwood 1993). Birds have been recognised as a well-functioning tool in biological monitoring of environmental contamination (Furness and Greenwood 1993). Raptors in particular are regarded as suitable biomonitors or bioindicators of environmental pollution, because they may express contamination risk for both ecosystem and human health (Burger and Gochfeld 2001, Eulaers et al. 2011). This is due to a very important feature; the position as top predator, and thus the possibility of biomagnification of contaminants (Castro et al. 2011, Furness and Greenwood 1993, Martinez et al. 2012, Movalli 2000).

There is an extensive literature on biomagnification in the aquatic food web available. On the other hand, terrestrial food web research on biomagnification is sparse. Although it may seem that the principle of magnification of contaminants would be the same in terrestrial and aquatic-linked food webs, there is a need to test this assumption and the effect of diet composition (Manosa et al. 2003, Palma et al. 2005). A species may express different pattern of biomagnification depending on the choice of prey (Palma et al. 2005). The reason for the literature gap from terrestrial food chains may be due to their shorter length compared to aquatic chains, which lessen the potential for biomagnification (Dietz et al. 2000, Palma et al. 2005).

Several methods are used to study biomagnification of contaminants. Some studies use a simple approach by classifying food chain length and trophic level on presence and ecology of species and vertical comparisons of contaminants between the trophic levels (e.g. Misztal-Szkudlinska et al. 2011, Rennie et al. 2011, Sørmo et al. 2006). In recent decades there has been an increased use of stable isotopes in biomagnification studies. One method is to assume an enrichment of δN at 3.4‰ for each trophic level, and comparing δN values directly to the contaminant concentration (e.g. Al-Reasi et al. 2007, Cheung and Wang 2008, Kidd et al. 1995, Murai et al. 2008). Another method calculates trophic level from δN and δC and a fractionation value, and relates the contaminant load directly to this level (Pethybridge et al. 2012). In this thesis, a new approach for calculating biomagnification is applied. By using the

stable isotope-mixing model MixSIR relative contributions of each prey is calculated and thus the contamination load in the diet of the top predator.

This method also provides valuable knowledge of the prey choice of the golden eagle. By using stable isotope techniques, fairly objective estimates of the relative contribution of the major food items can be obtained, without actually needing to find remains in the nest. This information is potentially important to the environmental management authorities, as it is highly relevant to the compensation scheme for the loss of livestock to protected predators in Norway.

### 1.1 Golden Eagle

The golden eagle (*Aquila chrysaetos*) is second largest bird of prey in Norway; only the white-tailed sea eagle (*Haileaeetus albicilla*) being larger. The habitat of the golden eagle is mainly inland forest and mountains, but it is also found along the coast, especially in north Norway. It is only absent from the lowland areas in the south-east (Gjershaug 1994). As other predators, it was previously regarded as a pest. From 1845 there was a bounty on the eagle paid by the state, but by 1932 this was transferred to the counties and local game and fish organisations. This led to a serious population decline. In 1968 the species was protected by law, and this caused a population growth in the successive years. Almost 40 years ago, Hagen (1952), estimated the Norwegian golden eagle population to consist of 344-523 territorial pairs, while in 2003 the population was estimated at 886-1190 pairs. After being categorised as near threatened on the red list in 2006, is was removed in the following red list published in 2010. The population was at this time categorised as least concern, with an estimated population over 2000 individuals (Jacobsen et al. 2010).

The diet of the golden eagle is diverse, but the size of prey is typically in the range of 0,5-4 kg. Principal food includes gallinaceous birds, hares (*Lepus timidus*) and ungulate calves, depending on what is available (Johnsen et al. 2007, Watson 1997). The golden eagles' role as a predator on reindeer (*Rangifer tarandus*) and sheep (*Ovis aries*) has been a controversial subject in the recent years, and a source of conflict between the husbandry industry and environmental management (Jacobsen et al. 2010, Johnsen et al. 2007, Nybakk et al. 1999).

Although the golden eagle is no longer listed as a threatened species, it may still be vulnerable to various forms of human activities. Today, the greatest concern is probably illegal persecution (Nygård et al. 2006), while other studies have shown that the golden eagle may be particularly sensitive to environmental pollution compared to other species (Gjershaug and Nygård 2003).

### 1.2 Presence of heavy metals in the golden eagle

In toxicology, there is a diffuse distinction between metals and non-metals, usually including metalloids (Cornelis and Nordberg 2007). Environmentally hazardous elements are loosely labelled "*heavy metals*" (Hodson 2004). It includes trace elements naturally present in low concentrations in organisms, and nonessential metals (Hg, Cd and Pb) which may be found in organic tissue due to anthropogenic exposure (Burger 1993). The origin of elevated heavy metal levels in the environment varies. Several studies examining different media, such as lakes, soil and moss, conclude that increased concentration of heavy metals found in Norway are mostly due to long-range atmospheric transport (Rognerud and Fjeld 1993, Steinnes et al. 1997, Steinnes et al. 2010). Over recent decades there has been a decline in levels in the concentrations of the most hazardous metals. This decrease makes local sources relatively more important. In areas characterized by extensive industrial activity, such as Mo i Rana and Odda, high levels of metal contamination are still measured (Steinnes et al. 2010). Although heavy metal contamination is present in the environment, it does not necessarily signify that it is toxic for living organisms. For exerting a toxic effect, the metal has to be bioavailable, bioaccumulate and potentially biomagnify (Gonzalez et al. 2008, Gorree et al. 1995).

Cadmium (Cd) is described as one of the most hazardous metals because of its persistence and toxicity (Battaglia et al. 2005). It may have teratogenic, carcinogenic and mutagenic effects. Several studies show that Cd can bioaccumulate, but there is contradictory evidence for biomagnification. The terrestrial food chain start with plants (Burger 2008). The bioavailability of Cd from soil into plants varies depending on physiochemical form and properties of both Cd and the soil (Efroymson et al. 2004). Furthermore, Cd can be accumulated by various types of vegetation, for example willow (*Salix*), chicory (*Cichorium*) and dandelion (*Taraxacum*) (Myklebust et al. 1993, Simon et al. 1996), and amplified further up the food chain into associated herbivores (such as bevers, *Castor fiber*) (Hillis and Parker 1993, Nolet et al. 1994). High levels of Cd are found in both rock ptarmigan (*Lagopus muta*)

and willow ptarmigan (*Lagopus lagopus*) (Myklebust et al. 1993, Pedersen et al. 2006, Wren et al. 1994b), the second species being important in the diet of golden eagle (Johnsen et al. 2007). Biomagnification depends on the toxicodynamics of Cd in the organism (Burger 2008). The two main organs for storage of Cd are liver and kidney, which may account for approximately 67-97% of the total body burden of Cd in birds (Garcia-Fernandez et al. 1996, Nam et al. 2005, Scheuhammer 1987, Wayland and Scheuhammer 2011). When absorbed in the kidney, Cd induces metallothionein (MT), a low-molecular weight, cysteine-rich metal-binding protein. Cd binds to MT, a complex that is fairly nontoxic. However, levels may increase to a threshold value, where MT no longer can protect cells against Cd toxicity (Wayland and Scheuhammer 2011). When bound to MT, Cd is retained in the tissues. This causes accumulation of Cd, leading to a long half-life in the body (10 to 20 years) (Nordberg et al. 2007), and potentially age-related bioaccumulation (Klaassen et al. 2009). Some studies have shown that Cd does biomagnify in a terrestrial food chain (Dietz et al. 2000). In the case of biomagnification, the levels of Cd may show a U-shaped curve, with high concentrations found in plants and herbivores, and in carnivores at the top of the food chain, while low levels are found in intermediate trophic level organisms (Burger 2008).

There are several studies examining lead (Pb) contamination in biota. Exposure may come from ingestion of contaminated soils near mines and smelters (Gallon et al. 2006, Leybourne et al. 2009), contact with lead based paint (Mielke et al. 2001), industrial waste (Franson and Pain 2011), and also from leaded gasoline (Burger and Gochfeld 1993, Omelchenko 2011). For many raptors, the predominant exposure that is of concern is by ingestion of ammunition or ammunition fragments of lead (e.g. Craig et al. 2009, Kelly et al. 2011, Mateo et al. 1999, Wayland et al. 1999, Wayland et al. 2003). For birds scavenging on game species, this may pose a severe risk (Franson and Pain 2011). In Norway, lead ammunition was prohibited from 2005. However, in 2009 it was still the largest source of anthropogenic induced lead in the Norwegian environment (Norge 2012). In Sweden, a high percentage of lethal lead poisoning is documented in sea eagles (45%) and golden eagles (27%) in a selection of carcasses brought in for analyses by the State Veterinary Institution (SVA). Ingestion of lead from carcasses and remains of ungulates shot with lead bullets are the main source (Axelsson 2009). Ingested lead shots dissolve in the acid environment of the stomach, and transfer into the blood stream (Battaglia et al. 2005). Uptake in relation to exposure depends on several factors, such as exposure time and uptake dynamics of the species (Wayland and Scheuhammer 2011). Lead may bioaccumulate to some extent in organisms on a high trophic

level (Burger and Gochfeld 2000). Several studies suggest that there is no biomagnification of lead for vertebrates (Rubio-Franchini and Rico-Martinez 2011, Szefer 1991, Ward et al. 1986). There rather seem to be a negative relationship between trophic level and lead levels (Sydeman and Jarman 1998). Lead poisoning and mortality is well documented in various birds (Burger and Gochfeld 2000, Mateo et al. 1999). Sub-lethal levels of lead poisoning have been shown to cause neurobehavioural, nephrotoxic, immunosuppressive and hematologic effects both in humans, animals and birds (Burger and Gochfeld 2000, Finkelstein et al. 2012, Rocke and Samuel 1991)

There is an extensive amount of research published on mercury (Hg) contamination. Effects are documented for decades in a number of organisms, both in the field and in laboratories (Appelquist et al. 1984, e.g. Burger and Gochfeld 1997, Furness et al. 1986, Hahn et al. 1993, e.g. Spronk and Hartog 1971). Publications as early as the 1960s review Hg levels in birds all the way back to the 1849. They reveal that Hg increased in the environment in the wake of industrialisation and use in agriculture, and rose to highly elevated levels in birds of prey (Berg et al. 1966). Many studies document the biomagnification capacity of Hg. However, bioavailability and degree of biomagnification depend upon the chemical form of (Hg). Organic Hg, methylmercury (MeHg), is regarded as much more toxic than inorganic mercury (Wolfe et al. 1998), as MeHg is lipid soluble, and biomagnifies in the food chain (Burger 1993). Inorganic Hg does not biomagnify to the same extent. Hg is known to have a wide variety of effects on biota. It is a neurotoxin (Wolfe et al. 1998) and affect behaviour and wing symmetry (Evers et al. 2008) and skew the sex ratio in bird populations (Bouland et al. 2012). Levels of Hg in Norway has decreased during the recent years, probably as a result of the prohibition of Hg in consumer products, decreased emissions from industry and lower levels of long range transport (Norge 2011, Steinnes et al. 2010).

Several metals, for example copper (Cu), manganese (Mn) and zinc (Zn), are naturally found in small amounts in organisms, and are necessary for health. However, in large quantities they may have a toxic effect (Komosa et al. 2009). Cu is such an essential element, important for maintenance for cardiovascular and haematopoetic systems (Carlton and Henderson 1963, Kaya et al. 2006), but excess levels may also be of harm. Toxicity can occur as haemorrhage in kidney, liver or intestine, and as proventricular, ventricular, nephritic or hepatic necrosis (Isanhart et al. 2011). Some studies show that birds associated with wetlands can bioaccumulate copper to a higher degree than terrestrial birds (Horai et al. 2007, Schummer et

al. 2011). Biomagnification of Cu has been proven (Gonzalez et al. 2008) but does not seem to biomagnify in most cases (Campbell et al. 2005, Cui et al. 2011, Jara-Marini et al. 2009). Presence of rich copper ores has resulted in the establishment of copper mining in parts of Norway. This causes both natural elevated levels of Cu in the environment, and excess levels due to runoff from mining activities (Klif 2012, NGU 2008).

Selenium (Se) is an essential trace element, but can also be toxic, depending on the dose (Burger 1993, Yang et al. 2008). Toxicity of Se depends on the chemical form ingested. Se is an oxidizing catalyst of for example glutathione, leading to oxidative stress and inducing apoptosis (Stewart et al. 1999). Toxic levels of Se are documented in aquatic birds, as a result of runoff from agricultural activities and other anthropogenic sources (Hoffman 2002). Elevated levels of Se compared to diet are found in lizards (*Lacertilia*) (Hopkins et al. 2005). Other than this there seems to be a lack of literature on biomagnification of Se in higher birds and animals.

In 1967, Parizek and Ostaldalova found that Se could mitigate the toxic effect of Hg in rats (Pařízek and Ošťádalová 1967). Since then, several studies have focused on Hg-Se interactions. Hg has a very high affinity for binding to Se, and Se can therefore sequester and reduce the bioavailability of Hg. Hg can also affect the activity of Se-dependent enzymes. Although the underlying mechanism is not clear, several possible pathways have been suggested (Berry and Ralston 2008, Khan and Wang 2009, Magos 1991, Raymond and Ralston 2004, Yang et al. 2008). Much research on metal contamination focuses on the molar ratio of Hg to Se. For fish it has been suggested that excess of Se provides protection from Hg toxicity (Burger et al. 2012). Correlations between Hg and Se are found in several fish-eating wildlife species, such as the great northern diver (*Gavia immer*) and bald eagle (*Haliaeetus leucocephalus*) (Scheuhammer et al. 2008, Scheuhammer et al. 1998). Goede and Wolterbeek (1994) speculated that high levels of Se might be accumulated in order to counteract the high levels of mercury in wading birds, however could not confirm this (Goede and Wolterbeek 1994, Norheim 1987). Increasing numbers of studies focusing on the Se/Hg ratio are becoming available. However, most of these focus on organisms connected to the aquatic environment, and there is an apparent lack of this kind of research in birds in a terrestrial environment.

### 1.3 Stable Isotope Analysis

Stable isotope analysis has become a powerful ecological tool during the last decades. It has the advantage of combining the use of trophic level, which is defined and characterized relatively easy, and the complex interactions of food web structures (Post 2002). The stable isotopes are also used to map biomagnification of contaminants in a food web (e.g. Bryan et al. 2012, Cui et al. 2011, Kim et al. 2012). The stable isotopes of carbon ($^{12}C$ and $^{13}C$) and nitrogen ($^{14}N$ and $^{15}N$) are most often used in dietary studies (Nilsen et al. 2012). The heavier isotope of nitrogen tends to end up progressively more in the diet of the consumer than the lighter, which results in an enrichment of the nitrogen-isotope ratio with every trophic level, a phenomenon known as trophic fractionation (DeNiro and Epstein 1981, Kelly 2000). This is because N balance is synonymous with protein balance, and gained from the diet (Gustine et al. 2011). As the composition of carbon isotopes usually does not alter noteworthy with trophic level, the ratios of C can be used to assess the dependency of an animal on the sources of primary producers, and their predators (Kelly 2000, Post 2002, Tieszen et al. 1983).

Estimating isotopic signatures in consumer tissues may be very complicated (Ben-David and Flaherty 2012), and should therefore be applied with caution (Hobson and Bond 2012). Knowledge about the incorporation rate (tissue turnover rate) in different tissues and species, and the discrimination factor between tissue and diet is necessary to determine the time period an isotopic signature reflects, and the degree to which it will differ from the signature of the food sources (Hobson and Clark 1992, Martinez del Rio and Carleton 2012).

Isotopic mixing models are useful tools to quantify contributions of multiple food sources in the diet of particular species. In recent year, Bayesian-mixing models such as MixSIR have become the predominant tool for analysis. This model estimates the proportional contribution of particular sources to a mixture, and has the advantage of also considering the uncertainties associated with sources, fractionation and isotopic signatures (Moore and Semmens 2008). MIXSir is considered a robust method towards unquantified errors. It has been shown to be consistently and accurate estimating the prey to predator diet (Semmens et al. 2009).

Feathers are tissues well suited for various type of biomonitoring for several reasons. They reflect the concentration of elements in the blood during the formation of the feather. Isotopic profiles are a result of the diet in that particular period (Hobson 1999 and Bearhop et al. 2002 i Resano et al, 2011). This is also true for elements such as heavy metals; however, these may

also be mobilized from storage in other tissues. Feathers are also easy to collect in a non-invasive manner, either as moulted feathers or from individuals located in the nest. Feathers consist of keratin, and are rich in sulfhydryl, which easily bind metals, in particular Hg (Bortolotti 2010, Burger 1993). These bindings are very stable, and may even be resistant against treatments used for preservation (Hogstad et al. 2003). This allows for use the of stored feathers for metal analysis, and even for investigation of time trends within particular metals (Appelquist et al. 1984, Berg et al. 1966).

### 1.4 Aim of study

The aim of this study was to investigate the diet of the golden eagle in Central Norway by use of stable isotope analysis of carbon and nitrogen in their feathers. I wanted to determine metal levels of the golden eagle and its major sources of prey, and discuss these in relation to harmful levels. Further, I wanted to assess whether some metals tend to biomagnify, and evaluate the model used for assessing biomagnification. I also wanted to investigate if this model could explain the levels of metals in the golden eagle.

## 2. Methods and materials

### 2.1 Samples

Feathers were collected from nests of golden eagles at known localities in Nord-Trøndelag in the summer of 2011 and 2012. Body feathers were collected directly from the nestlings' back. In addition, moulted feathers from the juveniles and adults were collected in and nearby the nest. The feathers were stored in polyethylene bags, and transported to Trondheim for storing at room temperature before analysis. Supplementary feather material was collected from storage in NINA archives. These feathers date from 1974-2012, and were stored in plastic bags, paper envelopes, or in binders. Total number of feather samples was n= 90.

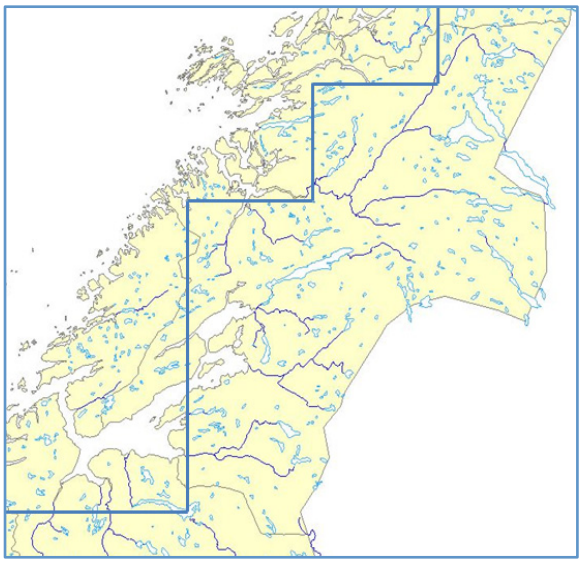

**Figure 1** Study area, and geographical division on eagles from coastal (left) areas and inland areas (right).

Muscle samples from willow ptarmigan (n=18) and western capercaillie (*Tetrao urogallus*) (n=4), were collected from local hunters during 2009-2011, and some from previous hunting seasons. Tissue from hare (n=22) was collected from stored meat from hunting season 2011-2012. Sheep (*Ovis aries*) samples (n=25) and reindeer muscle (n=21) were obtained directly from the reindeer owners during their autumn slaughter in 2011. The samples were stored at NINA at -20 °C prior to analysis.

### 2.2 Stable Isotope Analysis

Both feathers and muscle were prepared for stable isotope analysis (SIA) at the Norwegian Institute for Nature Research (NINA). Five pieces of feather of approximately 1x1 cm were clipped or cut out from one side along the shaft approximately even distances from base to tip. For small feathers, the whole vane on one side was used. Further on they were cut into as small pieces as possible, and transferred into glass tubes. Hair and tendons were removed from muscle tissue, before cutting and transferring into glass tubes. The procedure was from here on was the same for muscle and feather samples.

Fat is found within many other body tissues animals. It has shown to be significantly depleted in $^{13}C$ compared to diet, and may vary between tissues. Metabolisation of fat depend on environmental conditions, and thus the time period it reflects is not usually known (Tieszen et al. 1983). Lipids were therefore removed from all tissue by 1:1 chloroform:methanol treatment in two turns. First, samples were dried in an oven at 60°C for 24 hours. After drying, 100-300 ml of 1:1 chloroform:methanol were added, completely covering and soaking the samples. Samples were put in a sonicator for two minutes, and kept in a fume hood for 12-18 hours. Furthermore, the samples were centrifuged for ten minutes at 2000 rpm, and the supernatant discharged. The samples were again soaked in chloroform:methanol, and the entire procedure was repeated. After the second round of centrifuging and discharging of the supernatant, the samples were left for 12-18 hours in the fume cupboard loosely covered with aluminium foil, allowing the remaining chloroform:methanol to evaporate. The samples were then dried in an oven for 12-18 hours at 60°C, also here loosely covered with aluminium foil to prevent dust from getting in. After drying, caps were put on all tubes, and all samples packed and wrapped into boxes for transport to SeaLab (NTNU) for weighing.

Microplates (8x8) were used for storage and shipment. Mettler Toledo XP2U Ultra micro scale was used for the weighing. 0,5 mg samples of homogenized and dried material were weighed out into tin combustion cups for elemental analysis, and transferred onto the microplate. The precise weights were recorded in an excel-sheet.

The isotope analysis was carried out at Risø National Laboratory for Sustainable Energy at the Technical University Denmark (DTU), Copenhagen, Denmark. The total carbon and nitrogen contents and isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were measured in solid samples by Dumas combustion (1050 ºC) on an elemental analyser (CE 1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Acetanilide (Merck, Darmstadt, Germany) was used for elemental analyser mass calibration. For working standard for isotope ratio analysis pure gases of $CO_2$ and $N_2$ calibrated against certified reference materials of $^{13}C$-sucrose and $^{15}N$-$(NH_4)_2SO_4$, (IAEA, Vienna, Austria) respectively, were used. Performance of analysis (Qa/Qc) was assessed by the inclusion of reference samples of biological origin (Peach leaves (NIST 1547), National Institute of Standards and Technology, Gaithersburg, MD, USA).

All equipment was thoroughly washed with pure alcohol, and gloves changed between every sample.

Isotope values is noted as delta (δ), and by the equation

$$\delta X = ((R_{sample} / R_{standard}) - 1) \times 1000 \ (‰)$$

where X is $^{13}C$ or $^{15}N$, $R_{sample}$ is the ratio of heavy to light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample, and $R_{standard}$ is the ratio of heavy to light isotope in the standards (Tieszen et al. 1983).

**2.3 ICP-MS**

Preparation and analysis of metals for ICP-MS analysis was done in two turns; most of the feathers, hare and willow ptarmigan December 2011 till January 2012, and the remaining feathers (collected in 2012), sheep, reindeer, and various birds in September 2012. Three to five cm of the feather shaft was firstly scraped with a scalpel, and then cut out and transferred to vials for storage and transport. Between one to five grams of muscle tissue was cut out with a scalpel and transferred to vials for storage and transport. It was brought to NTNU for further preparation. At NTNU, a smaller portion, approximately 1,5g of muscle was cut out, and transferred to vials for freeze-drying. After freeze drying the samples were weighted (accurate to four significant digits) and transferred to PTFE-Teflo vials (18ml). Feathers were weighted (accurate to four significant digits) and transferred directly into vials. For samples prepared December-January 2011-2012, 2ml of 50% $HNO_3$ (nitric acid, Scan pure, equal to ultra pure grade, Chemscan, Elverum, Norway) was added to all samples, both muscle tissue and feathers. For samples prepared fall 2012, 6 ml and 2 ml 50% $HNO_3$ was added to the sample, for muscle and feathers, respectively. All samples were digested in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutrikirch, Germany). This system gradually increased temperature up to 240°C in one hour, followed by a cooling step that returned the temperature back to initial value within one hour. After the digestion, samples were diluted to a final volume of 24 ml for the December-January samples, and 30ml and 60 ml for feathers and muscle tissue respectively, for the September 2012 samples.

Thermo Finnigan model Element 2 instrument (Bremen, Germany) were used to preform High resolution Inductively Coupled Mass Spectrometry (HR-ICP-MS). Radio frequency

power was set to 1400W, and samples introduced using a SC-FAST flow injection analysis system (ESI, Elemental Scientific, Inc. Omaha, USA) with a peristaltic pump (1mL/min). The instrument had a PFA-ST nebulizer, spray chamber (PFA Barrel 35mm), demountable torch, quarts standard injector and AL sample skimmer and X skimmer cones. Nebulizer argon gas flow rate was adjusted to give a stable signal with maximum intensity for the nuclides $^{7}$Li $^{115}$In and $^{238}$U. Methane gas was used in the analysis to minimize interference from carbon and enhance sensitivity, especially for Se and As. Instrument was calibrated using 0.6 HNO3 solutions of matrix-matched multielement standards. Calibration curve consisting of five different concentrations was made from these standards. To check for instrument drift, one of these multi-element standards were analysed every tenth sample. Certified reference material Bovine Liver NIST 1577b (National Institute of Standards and Technology, Gaithersburd, MD) were used to verify the accuracy of the method. Results were in good agreement with certified values. Recovery of elements ranged from 86 – 161% (**appendix I**).

Method detection limit (MDL) (**appendix II**) was set to the highest value of either calculated instrument detection limit (IDL) or three times standard deviation of the blanks. Instrument detection limit of the different elements were calculated by subsequent analysis of solutions containing decreasing concentration of the element, and the concentration resulting in a relative standard deviation of approximately 25% were chosen as IDL with baseline corrections applied for these values. Elements with levels below limit of detection (LOD) were replaced with 1/2•LOD for that element. Low weight for some of the feather samples were taken into account, and own instrument detection limits calculated for these samples.

### 2.4 Statistics

The software modelling tool MixSIR was used to model the diet of golden eagle based on the isotope profiles of N and C of both the prey and the golden eagle, expressed as δN and δC values relative to standards, atmospheric nitrogen and PeeDee Belmnite for N and C respectively. MIXSir requires information about the mean and standard isotopic values from the δN and δC for the different food sources, δN and δC values for the individual consumers, a fractionation value, and standard deviation of the fractionation value. Fractionation values of C and N for the golden eagle are not yet established. The closest related species with known fractionation values is the peregrine falcon (*Falco peregrinus*) with a fractionation value of +2,7 for N and +2,1 for C (Hobson and Clark 1992). As these values seem

reasonable, they were applied as fractionation value in the model. Western capercaillie and willow ptarmigan, two closely related species in the order of *galliformes* belonging to the group grouse, were grouped for analysis. Outlying values considered to have been caused by analytical errors were removed.

To estimate the biomagnification factor (BMF), I first calculated the contribution from each prey group by multiplying the median level of a metal in that group by its relative contribution (as biomass) in the diet, and then summing over all prey groups to find the total contribution. I then divided the metal level in each feather by the total contribution from prey. The overall magnification level was calculated as the mean value of all feathers for each metal.

IBM SPSS Statistics 20 for Mac, (SPSS Inc., Chicago, IL), was used for statistical analysis in this study. P-values less than 0.05 were considered statistically significant. Group comparisons between different species, and different groups of golden eagle were performed by the Kolmogorov-Smirnov Z test (reported by Z-value). Bonferroni's correction was applied to pairwise comparisons to control the overall Type I error rate (Field 2009). Regression analysis was preformed to investigate the relationship between proportion of prey and metal levels (reported by R-value). Correlation between levels of Se and Hg in feathers and diet, and between actual levels in feather and modelled levels in diet were calculated using Spearman's rho (reported by R-value).

## 3 Results

### 3.1 Stable Isotope Analysis

The isotopic profiles of the different species or group of species are shown in Figure 2. A distinct pattern is evident between the golden eagle and the different prey species. Clustering of groups reflects contribution in terms of biomass to the golden eagle diet.

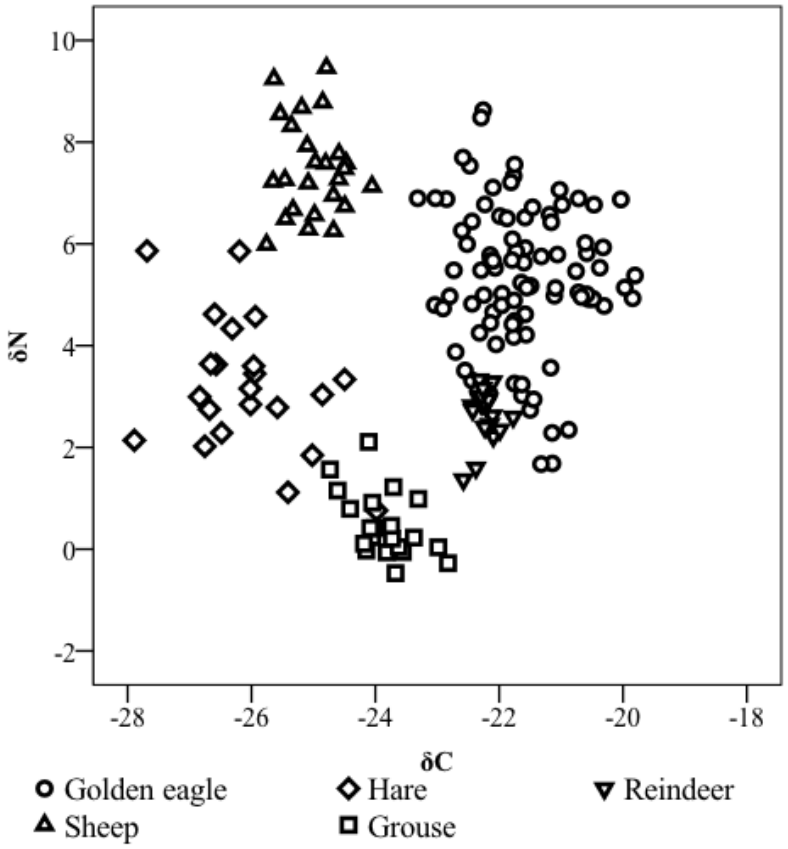


**Figure 2** Isotope profiles of δN and δC in golden eagle, sheep, hare, grouse and reindeer.

Isotopic profiles were significantly different if one or both of the isotopic values differed significantly between groups. All species differed significantly with respect to isotopic profile, (Tables 1 and 2). Sheep showed the highest δN values, followed by golden eagle, hare, reindeer and grouse. Golden eagle had the highest δC values, followed by reindeer, grouse, sheep and hare.

**Table 1** Mean ± SD of δN. Pairwise comparisons of δN between different species. Z-values marked with* are significantly different. (Groups were significantly different when P < 0.05)

| **δN - Pairwise comparison** | | | | | | |
|---|---|---|---|---|---|---|
| **Kolmogorov-Smirnov test** | **N** | **Mean±SD** | **Sheep** | **Hare** | **Grouse** | **Reindeer** |
| **Golden eagle** | 90 | 5.30±1.46 | 3,047* | 2.748* | 3.920* | 3.576* |
| **Sheep** | 25 | 7.48±0.94 | | 3.421* | 3.421* | 3.378* |
| **Hare** | 22 | 3.21±1.31 | | | 2.714* | 1.490 |
| **Grouse** | 22 | 0.61±0.88 | | | | 2.831* |
| **Reindeer** | 21 | 2.67±0.51 | | | | |

**Table 2** Mean ± SD of δC. Pairwise comparison of δC between different species. Z-values marked with* are significantly different. (Groups were significantly different when P < 0.05)

| **δC - Pairwise comparison** | | | | | | |
|---|---|---|---|---|---|---|
| **Kolmogorov-Smirnov test** | N | **Mean±SD** | **Sheep** | **Hare** | **Grouse** | **Reindeer** |
| **Golden eagle** | 90 | -21.63±0.80 | 4.423* | 4.205* | 3.780* | 2.417* |
| **Sheep** | 25 | -25.00±0.44 | | 2.488* | 2.973 | 3.378* |
| **Hare** | 22 | -26.09±0.94 | | | 3.015* | 3.279* |
| **Grouse** | 22 | -23.77±0.57 | | | | 3.129* |
| **Reindeer** | 21 | -22.22±0.17 | | | | |

Table 3 shows the mean isotopic values in the different golden eagles at different age and regions. Inland adults had significantly higher δN values than inland juveniles (Z = 2.343, P < 0.001) There were no significant differences between other groups of golden eagle in δN or δC.

**Table 3** Mean ± SD of δN and δC of golden eagles at different age and location

| | **Costal adult** | | **Costal juvenlie** | | **Inland adult** | | **Inland juvenile** | |
|---|---|---|---|---|---|---|---|---|
| | N | Mean | N | Mean | N | Mean | N | Mean |
| δN | 4 | 6.96±0.44 | 7 | 5.04±2.03 | 66 | 5.60±1.11 | 13 | 3.41±1.28 |
| δC | 4 | -22.49±0.80 | 7 | -22.31±0.36 | 66 | -21,49±0.80 | 13 | -21,74±0.60 |

### 3.1.1 Relative proportion of sources of prey

The relative contribution of different food sources (prey species) in the golden eagle estimated by MixSIR is shown in Figure 3. Diets are evaluated based on age and geographical differences. MixSIR calculates the most likely median values of proportions of sources of prey. Thus, Figure 3 represents mean of the median values of prey in the diet of the golden eagle. The complete diet is set to one. This is not necessary true, as there are other potential prey species. However, studies have shown that the four species in this study are the dominant among available prey (Hagen 1952, Johnsen et al. 2007, Lunde 1985).

No significant difference between adult and juveniles eagles on the coast was detected. This is likely to be due to a low number of samples for both juveniles and adults on the coast (N=7 and N=4, respectively). There were significant age differences inland. The proportion of grouse was significantly higher in juveniles ($Z = 2.397$, $P < 0.001$), and the proportion of reindeer was highest in adults ($Z = 2.193$, $P < 0.001$). Between coastal and inland adults there was a significant higher proportion of sheep in adults on the coast ($Z = 1.648$, $P = 0.038$). There were no significant differences between the diets of juveniles from the inland compared to those from the coast.

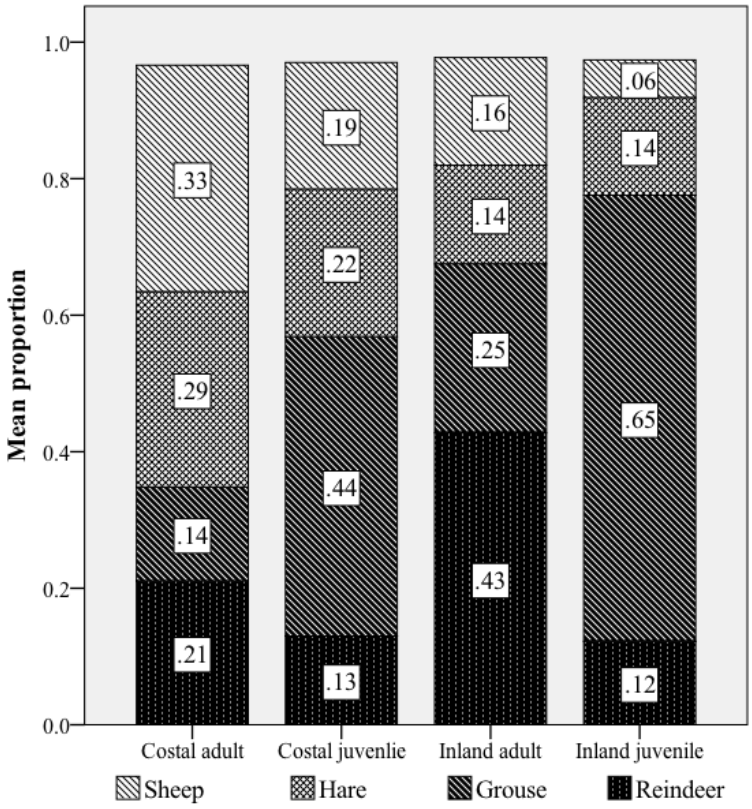


**Figure 3** Mean relative proportion of prey in the diet of golden eagles in different regions and at different age

### 3.2 Metal levels in golden eagle

The mean, median and standard deviation metal levels in the golden eagle are shown in Table 4. Cd was below detection limit of the instrument. There were no significant difference between metal levels in juveniles and adults on the coast. In the inland group, Pb, Cu and Hg were significantly higher in adult than in juvenile golden eagles ($Z=1.722$, $P = 0.02$, $Z =$

1.924, P = 0.004, and Z = 1.62, P = 0.04, respectively). There were no significant differences with respect to location, neither in adults nor in juveniles.

**Table 4** Mean (median) ± SD metal levels (dw) given in ppm for Se, Cd, and Pb golden eagle at different regions and age. N.D.=non detectable.

| Location | Age | N | Se | Cd | Pb | Cu | Hg |
|---|---|---|---|---|---|---|---|
| Costal | Adult | 4 | 2.35(0.60)±3.61 | N.D. | 0.34(0.16)±0.54 | 29.40(8.44)±42.36 | 1.83(0.67)±2.76 |
| | Juvenile | 7 | 0.84(0.79)±0.42 | N.D. | 0.09(0.01)±0.23 | 7.88(7.45)±5.45 | 0.18(0.03)±0.24 |
| Inland | Adult | 65 | 0.73(0.67)±0.39 | N.D. | 0.25(0.15)±0.46 | 10.43(10.25)±2.62 | 0.25(0.05)±0.33 |
| | Juvenile | 13 | 0.73(0.66)±0.17 | N.D. | 0.08(0.04)±0.12 | 7.24(6.52)±2.99 | 0.11(0.01)±0.22 |

### 3.3 Metal levels in prey

Tables 5 and 6 shows metal levels found in all prey groups. Median values were used to calculate biomagnification, as this was a better indicator of the central tendency than mean, due to outliers.

**Table 5** Mean (median) ± SD metal levels (dw) given in ppm for Se, Cd, and Pb in hare, sheep, grouse and reindeer. * Values calculated from values above 50% of detection limit.

| Species | Se | | Cd | | Pb | |
|---|---|---|---|---|---|---|
| | N | | N | | N | |
| Hare | 18 | 0.272(0.272)±0.115 | 12* | 0.13(0.010)±0.007 | 18 | 0.202(0.063)±0.328 |
| Sheep | 21 | 0.224(0.214)±0.066 | | N.D. | 16* | 0.010(0.004)±0.014 |
| Grouse | 20 | 0.485(0.436)±0.290 | 12 | 0.062(0.031)±0.071 | 18 | 0.044(0.021)±0.067 |
| Reindeer | 18 | 0.521(0.527)±0.109 | 14* | 0.010(0.008)±0.006 | 10 | 0.011(0.010)±0.005 |

Se levels were highest in reindeer, followed by grouse, hare and sheep. Cd values were highest in grouse, and lower in hare and reindeer. Highest Pb values were found in hare, followed by grouse, reindeer and sheep. Cu values were highest in hare, followed by grouse reindeer and sheep. Hg values were highest in reindeer, followed by sheep, and equally low in hare and grouse.

**Table 6** Mean (median) ± standard deviation metal levels (dw) given in ppm for Cu and Hg in hare, sheep, grouse and reindeer. * Values calculated from values above 50% of detection limit.

| Species | Cu | | Hg | |
|---|---|---|---|---|
| | N | | N | |
| Hare | 18 | 9.250(9.011)±3.206 | 15* | 0.004(0.004)±0.001 |
| Sheep | 23 | 2.379(2.404)±0.406 | 12 | 0.012(0.008)±0.010 |
| Grouse | 20 | 7.891(6.858)±4.694 | 9* | 0.004(0.004)±0.002 |
| Reindeer | 18 | 6.994(6.911)±1.698 | 19 | 0.029(0.031)±0.589 |

### 3.4 Biomagnification of metals from selected prey to the golden eagle

Biomagnification of metals were calculated based on estimated proportion of prey in diet and median metal levels in these different prey species, compared to the levels in feathers. The BMF are listed in Table 7.

**Table 7** Mean (median) BMF of Se, Pb, Cu and Hg in golden eagle

| N | Se | Pb | Cu | Hg |
|---|---|---|---|---|
| 89 | 3.11(1.74) ± 2.38 | 11.59(6.48)±17.99 | 1.74(1.49)±1.93 | 24.33(3.99)±51.63 |

There was some biomagnification of both Se and Cu. The highest mean BMF was found for Hg, of 24.33. However, the standard deviation is also high (51.63). The median BMF display a much lower biomagnification of Hg. Similarly, Pb showed a high BMF, accompanied by a high standard deviation. The median Pb BMF was lower than the mean, and was even higher than the median BMF of Hg, but the latter the highest BMF of all metals.

### 3.6 Regression analysis between metal levels and prey consumed

Regression analysis revealed weak relationships between certain metal levels in the golden eagle and the relative amount of the different prey types consumed, as estimated from MixSIR. There was a significant relationship between the levels of Cu in the golden eagle and the proportion of sheep consumed ($R=0.252$, $P = 0.017$), and the proportion of grouse consumed ($R=0.22$, $P=0.032$). There was also a significant relationship between the levels of Hg in the golden eagles and the proportion of sheep in their diet($R=0.004$, $P=0.003$)

### 3.6 Relationship between Se and Hg

Figures 4 shows the relationship between the analysed levels of Se and Hg in feathers of the golden eagle. There was no correlation between these two metals in the feathers (R = -0.38, P = 0.727). However the correlation between the modelled levels of Se and Hg in the diet was significant (R= 0.578, P <0.001) (Figure 5).

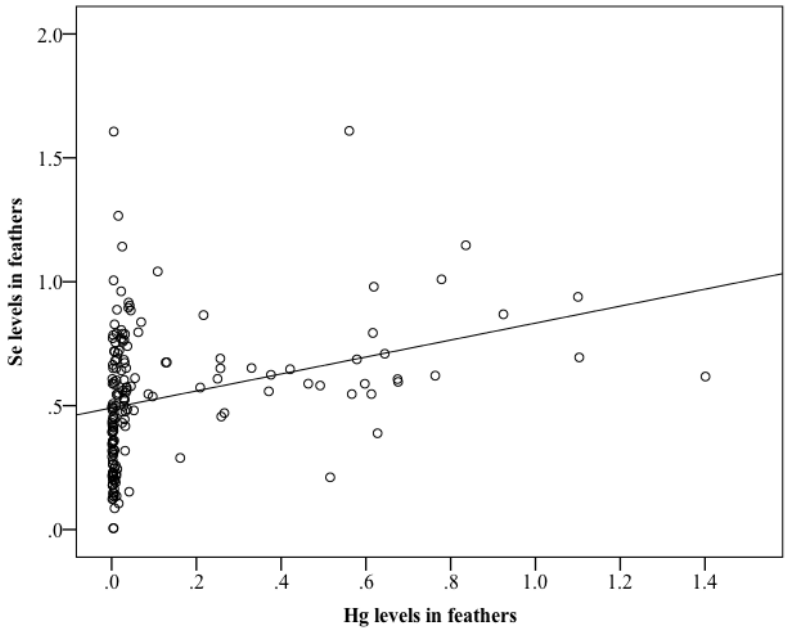


**Figure 4** The relationship between Se and Hg levels in feathers of golden eagle

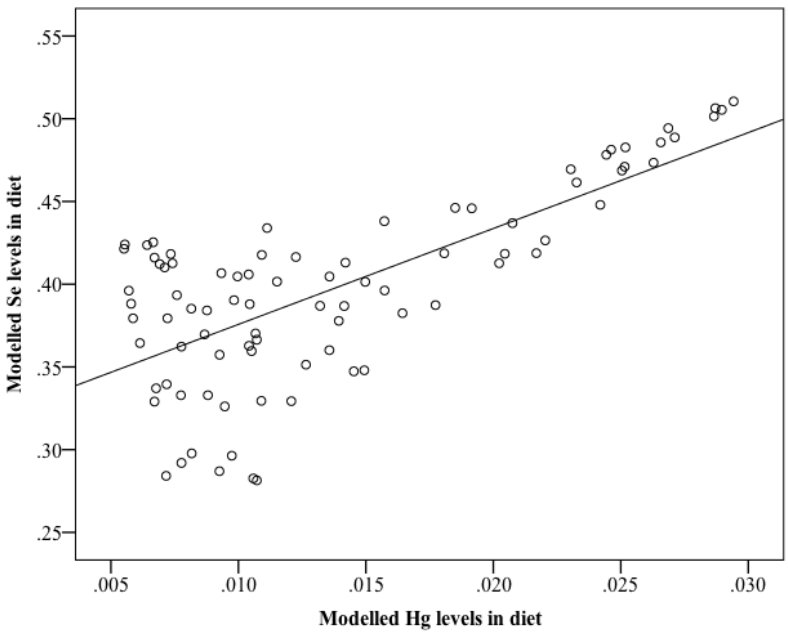


**Figure 5** The relationship between Se and Hg levels in feathers of golden eagle

### 3.7 Correlations between modelled and actual metal values

The relationship between the modelled levels of metal levels modelled and the actual levels in the feathers are shown in Figure 6-9.

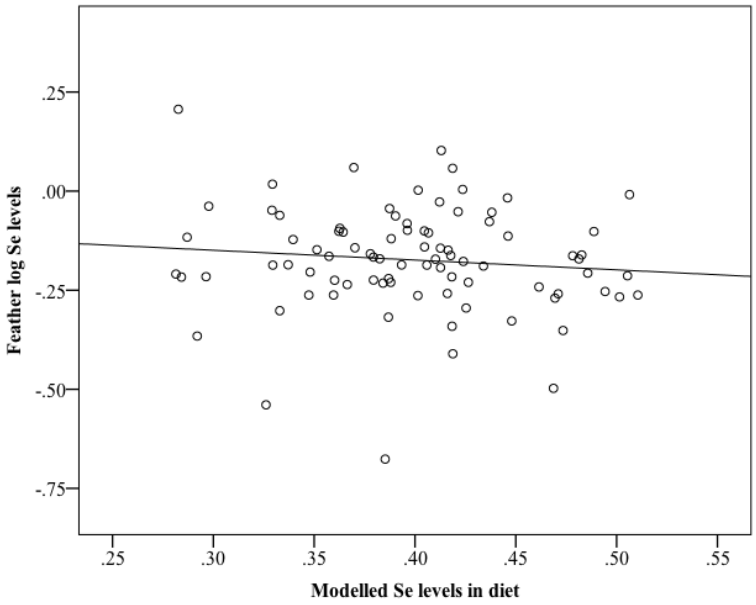


**Figure 6** Relationship between actual and modelled Se values in golden eagle feathers

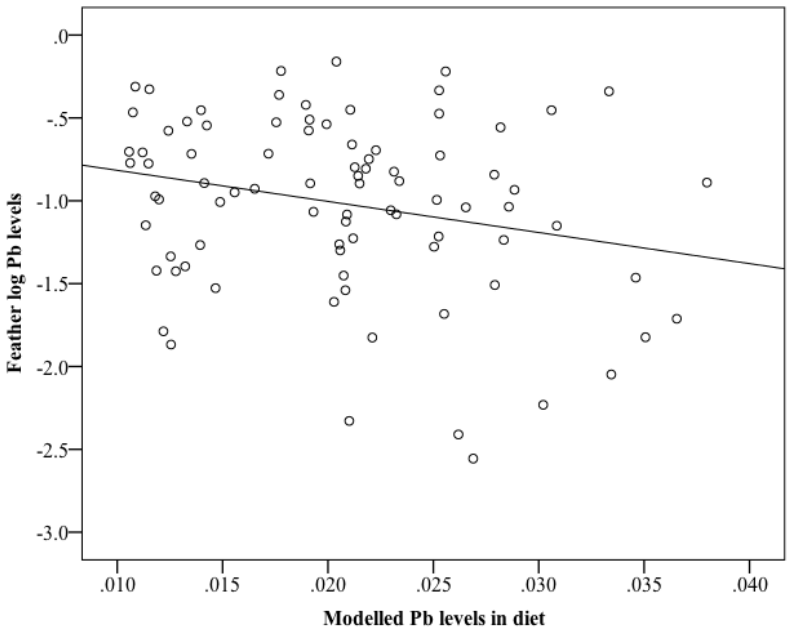


**Figure 7** Relationship between actual and modelled Pb values in golden eagle feathers

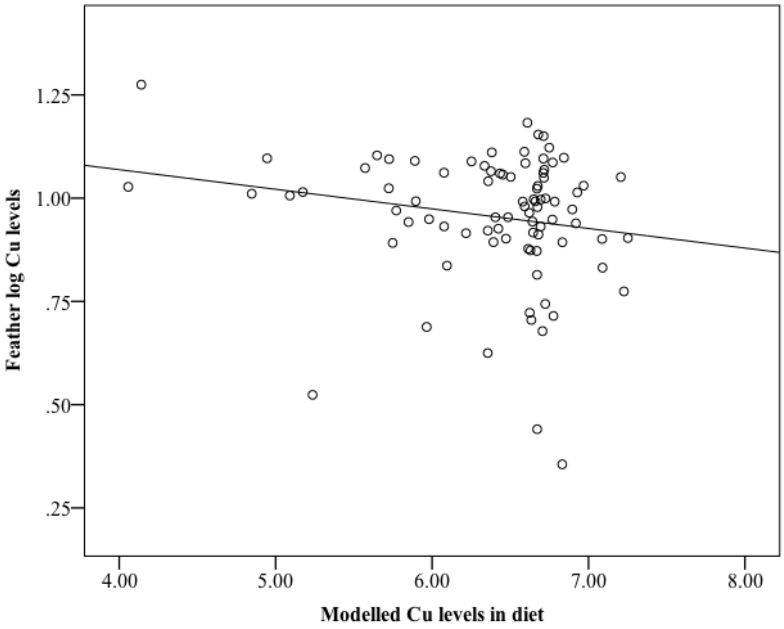


**Figure 8** Relationship between actual and modelled Cu values in golden eagle feathers

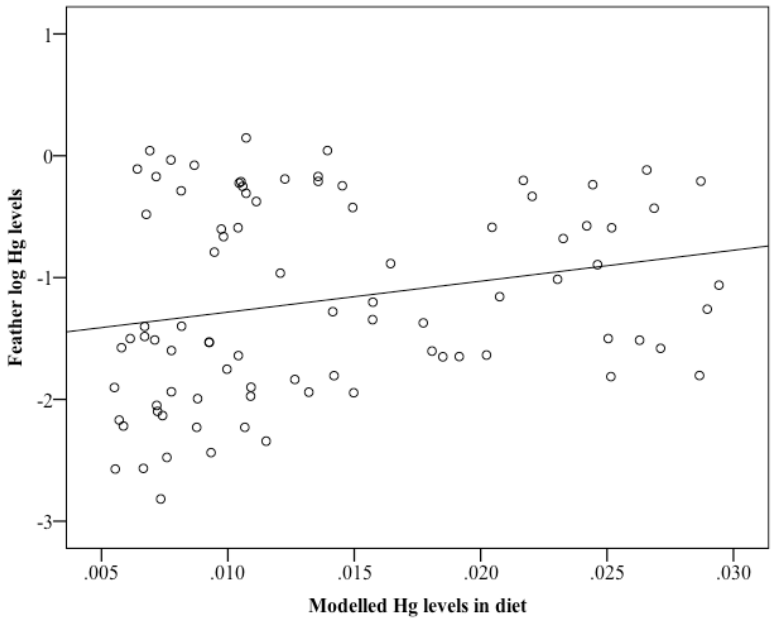


**Figure 9** Relationship between actual and modelled Hg values in golden eagle feathers

A significant negative correlation was found between modelled and actual levels of Pb in the diet and feather of the golden eagle ($R = -0.220$, $P = 0.042$). For Hg there was a significant positive correlation between modelled and actual levels ($R = 0.259$, $P = 0.016$). For Cu and Se no significant correlation was revealed.

## 4 Discussion

### 4.1 Stable Isotope profiles

Figure 2 illustrates the distinct isotope profiles of different species, with Tables 1 and 2 providing information about the isotope values. High δN values in the golden eagle are consistent with the fact that the heavier isotope of nitrogen is enriched in every trophic level (Kelly 2000). Sheep display very high δN, even higher than the golden eagle. δN values tend to vary between different locations, and on type of breed of sheep (Piasentier et al. 2003). The high levels of δN in sheep from this study can be explained by grazing on fertilised land, and/or by consumption of concentrate feed. Application of fertiliser is known to increase the $^{15}N$ level of nitrogen in soil and plant (Kreitler and Browning 1983). Dairy products from cows grazing in mountains areas have been shown to have lower δN values than products from cows in arid and fertilised regions (Piasentier et al. 2003). The same situation may be the case for the sheep in this study. Another explanation of the elevated δN levels in sheep may be the use of concentrate feed containing high levels of animals proteins during winter when sheep are kept indoors (Delegado and Garcia 2001, as quoted in Piasentier et al. 2003). Hare δN values are higher than found in other species of hare (2.1 for snowshoe hare, *Lepus americanus* (Urton and Hobson 2005) and 2.51 for plateau hare *Lepus oiostolus* (Yi et al. 2006)). The hares in the present study also had a much higher standard deviation than reported for the other species herein. For species in the family Leporidae microbial protein synthesis in the caeum is a crucial source of high quality protein (Belenguer et al. 2008). Intake of these food pellets faeces may vary depending on age, quantity and nutritional feed ingested (Halls 2008) and may therefore be a source of high variance. The reindeer in the present study showed a lower δN and higher δC than found in muscle in reindeer in Finland, fed with concentrate during the winter (Halley et al. 2010). The diet of reindeer consists mainly of graminoids and lichens, which has low protein content and thus a much lower δN compared to meat (Halley et al. 2010, Parker et al. 2005). The δN in reindeer may vary depending on several factors, such as sex, reproductive stage and seasonality (Halley et al. 2010, Parker et al. 2005). Grouse shows an even lower δN value, indicating an even lower trophic level.

The distinct grouping of prey species (illustrated in Figure 1), is beneficial for the diet modelling as it diminishes uncertainties and therefore increases accuracy (Ben-David and Flaherty 2012).

Table 3 describes the isotopic profiles of the juvenile and adult golden eagles at from the two regions (coastal and inland). The only significantly different profile was between inland juveniles and inland adults, where coastal juveniles had significantly lower values of δN than adults from the same region. This difference is difficult to explain, but can be due to the fact that juvenile grow their feathers during a short period in the spring, while the adults' feather may be formed during the whole summer season, allowing for a wider range of prey species.

### 4.2 Selection of prey

A survey done in 1985 investigated the diet of golden eagle in Nord-Østerdalen, south of our study area. The study concluded that hare and reindeer constituted the highest proportion of biomass in the diet of golden eagle, at 27.6% and 26.4%, respectively (Lunde 1985). Hagen (1952) also found hare to be the most important source of prey for the golden eagle. A report from 2003 found hare and different grouse species to be the most important sources of prey during time of nesting, but emphasised that the golden eagle could feed on a wide range of potential prey (Gjershaug and Nygård 2003). Of a total of 15 nest localities visited during fieldwork in 2011 and 2012 (whereas six nests were empty), I found hare remains and carcasses were found in six. By MixSIR modelling (Figure 3), the proportion of hare was estimated to be from 0.29-0.14, depending on age and region of the bird. This corresponds to what previously found, or is somewhat lower. Both Lunde (1985) and Gjershaug and Nygård (2003) pointed out the importance of grouse in the diet of the golden eagle. In the study from Nord-Østerdalen, grouse made up 24.7% of the diet. I found the proportion of grouse to be age dependent at the inland region, with juveniles having a higher proportion (0.65) of grouse in the diet than the adult golden eagles (0.25). During fieldwork I found remains and feathers of grouse were found in six nests. Sheep and reindeer are common prey of the golden eagle in Norway (Gjershaug and Nygård 2003, Johnsen et al. 2007). Lunde (1985) found that sheep constituted a minor part (6,4%) of the diet. This corresponds well with our finding in the inland eagles. A study in a coastal region of Norway revealed that that sheep and goat constituted 6,6% of the diet, based on number of prey items, but did not estimate the biomass contribution (Gjershaug 1981). This is consistent with my results showing that the proportion of sheep was highest the coast. However, I found no sheep remains in the nests, but this could be a chance effect due to a low number of nest visits. The proportion of reindeer was significantly higher in the diet of adult golden eagles than of juveniles from the inland.

Reindeer remains were present in and around five nests visited in my study. The amount of reindeer modelled by MixSIR was age dependent in inland golden eagles. In the diet of adults, reindeer constituted a proportion of 0.43, higher than what Lunde (1985) reported in his study. I found no explanation on the significant difference in proportions of reindeer and grouse between juveniles and adults, or why the proportion of sheep was higher in adults on the coast. However, sample size for coastal golden eagle was very low, thus this difference should be treated with caution.

### 4.3 Metal levels

Toxicological research in animals and birds tends to focus on liver and kidney, both organs important in the detoxification process (e.g. Baars et al. 1986, Larter and Nagy 2000, Lutz and Slamecka 1997, Pedersen et al. 2006, Sivertsen et al. 1995, Vikoren et al. 2011, Wren et al. 1994a). Some studies that have investigated levels of metals in feathers and muscle tissue are listed in **appendix III** and **IV**, respectively.

Both lead and selenium levels in feathers of golden eagles in this study can be regarded as low, especially compared to levels found in other species of various birds of prey around the world (Battaglia et al. 2005, Boncompagni et al. 2003, Burger 1995, Burger and Eichhorst 2007, Burger and Gochfeld 1993, Burger and Gochfeld 2009, Burger et al. 1994, Burger et al. 1992, Connell et al. 2002, Custer et al. 2008, Dauwe et al. 2003, Denneman and Douben 1993, Lounsbury-Billie et al. 2008, Movalli 2000). Cu values in osprey (*Pandion haliaetus*) from the present study area were considered as very high in 2005 and 2007 (Kroglund et al. 2007, Reitan 2009), compared to levels found in other birds (Connell et al. 2002, Custer et al. 2008, Dauwe et al. 2003, Lounsbury-Billie et al. 2008). However, even higher Cu values were found in various seabirds in Siberia (Kim et al. 1996). Hence, levels of Cu in my study were in the mid range compared to other studies. Levels of Cd in golden eagle were below the detection limit of the instrument.

Levels of Pb in hare were higher than the levels found in muscle in mountain hare (*Lepus timidus*) in Northern Finland (Venalainen et al. 1996) and in arctic hare (*Lepus arcticus*) in Nunavut, Canada (Pedersen and Lierhagen 2006). Pb levels in sheep were also lower than found in other studies (Jankovska et al. 2012, Liu 2003, Rudy 2009, Swaileh et al. 2009). Pb in reindeer was lower than found in forest reindeer (*Rangifer tarandus fennica*) in Russia

(Medvedev 1999), and levels were lower than what found in rock ptarmigan and willow ptarmigan in Quebec (Rodrigue et al. 2005).

Much of the research on metal levels in various tissues only discusses the levels found, and compares the values with those found in other studies rather than clarifying whether levels are of any harm. In order to tell which levels are toxic, there is a need for knowledge about what levels are harmful. Burger and Gochfeld (1997) indicated that mercury levels from 5 to 40 ppm in feathers were related to adverse effects. For lead, adverse effects occur at 4 ppm in feathers (Burger and Gochfeld 2002, Custer and Hohman 1994). For other metals, there was a lack of literature on harmful levels in feathers. However, levels in feathers are very often correlated with levels found in other tissues (Burger 1993). For Cd 40 ppm in liver can be set as a threshold concentration for adverse effects. However for some birds it may be as low as 10 ppm, wet weight (as in Burger 2008, Burger and Gochfeld 2002, Eisler 1985, Furness 1996). Selenium levels of 15 ppm in liver have shown to reduce growth in ducklings, 29 ppm cause histopathological lesions, and 5 ppm diminish immune function in adults of mallards (*Anas platyrhynchos*) (Hoffman 2002). A study in 1995 revealed very high levels of copper in reindeer in Norway, at the extreme 230 ppm in the liver. However, no sign of copper poisoning was observed. Very high levels of copper are also observed in various wild birds without any signs of toxicity (Kim et al. 1996).

Based on previous studies, there is no reason to believe that levels of metals in golden eagle or its prey represent any hazard, as I found no values approaching levels known to be toxic for any of the metals analysed for.

### 4.4 Effect of prey choice in relation to metal biomagnification

High BMFs were found of Pb and Hg. BMF of Pb was higher in coastal eagles than in inland, and higher in adults than in juveniles. No significant relationship was found between the levels of Pb and proportion of any prey. The levels of Hg showed a weak relationship with the proportion of sheep in the diet. The proportion of sheep was highest in the adult golden eagles from the coast, followed by juveniles on the coast. Levels of Hg were also highest in the coastal adults, but inland adults had higher levels than the juvenile golden eagles from the coast, despite a lower proportion of sheep in the diet. A significant relationship was found between Cu and the proportion of sheep and the proportion of grouse in the diet. Levels of Cu

were also higher in the adults both on the coast and inland, and neither the proportion of sheep or grouse displayed the same pattern. In my study, there were no clear and direct relationship between estimated prey proportion and metal levels. However, as previously mentioned, only four selected prey species were accounted for in this study. Some unknown prey species might have contributed an unknown amount. This consideration is valid for all metals.

The levels of metals may be influenced by many factors, such as age-related magnification, and vicinity marine or aquatic ecosystems (such as in coastal golden eagles). Prey not accounted for may introduce some noise in the results, especially if they are of marine origin. This is especially true for mercury (Monteiro and Furness 1995). Some pollutants have the ability to accumulate in tissues by age. It can be stored, and later mobilized to the blood and sequestered into feathers (Burger 1995). Age differences have previously been found for Pb, Cu and Hg in glacous-winged gull (*Larus glaucescens*) in the Aleutians (Burger et al. 2009), and in grebes (*Podicipediformes*) in Northern Minnesota, Geographical factors may also explain levels of metals, as the feathers of juvenile golden eagles represent the exposure from the hunting grounds near the site (Burger and Eichhorst 2007). Adults may range farther away when they are not raising chicks, raising the possibility of access to other types of prey, which can contribute to differences in the metal load.

Our model shows that there were biomagnificaition of Pb and Hg, especially for adult golden eagle on the coast. Hg is the only metal that consistently has shown to biomagnify. For other metals there are contradictory evidences about biomagnification in the terrestrial food web (Bryan and Langston 1992, Gray 2002, Palma et al. 2005). Although elevated levels of lead are found in birds (Bannon et al. 2011, Burger and Gochfeld 1991), studies focusing specifically on dietary inputs have not found Pb to biomagnify in birds (Ramos et al. 2013).

The median of biomagnification of Hg was far lower than the mean, and there was a very high standard deviation. Pb also had a lower median than mean, and a high standard deviation. This high variation tells us that there were there were large differences in biomagnification between individual golden eagles.

### 4.5 Relationship between Se and Hg

As mentioned in the introduction, several studies have proven the mitigating effect of Se on high levels of Hg (Cabanero et al. 2005, Scheuhammer et al. 1998). No correlation was found between levels of Se and Hg in feathers of the golden eagle, but a positive correlation was found in the modelled concentration of Se and Hg in diet. However, as levels of Hg were very low, there is no reason to draw conclusions on the antagonistic effect of Se on Hg in golden eagle at the low levels of Hg in the study.

### 4.6 Methodological considerations

#### 4.6.1 Limitations of dietary estimates using MixSIR

MixSIR calculates the estimated proportion of different sources to the material of which a given tissue (in this case feathers) is composed. It does not estimate how many individuals of each prey type are consumed by the predator, but rather the biomass. Therefore, one must be careful in trying to estimate how many of each prey species are consumed by any eagle population, as this will depend on the weight of each animal, which vary considerable between young and fully grown individuals. The prey may also be found dead or have been killed by other predator, and later scavenged by the eagles (Gjershaug and Nygård 2003). Another feature of this mixing model is that it is very sensitive to changes in the assumed fractionation values. From Figure 1, the lowest and highest possible fractionation were determined. The highest possible fractionation values were estimated to be 4 ppt for C and 2,5 for N, where the lowest possible fractionation values were estimated to be 2 ppt for C and 0 for N. The results from the modelling with these fractionation values are shown in Figure 10 and 11.

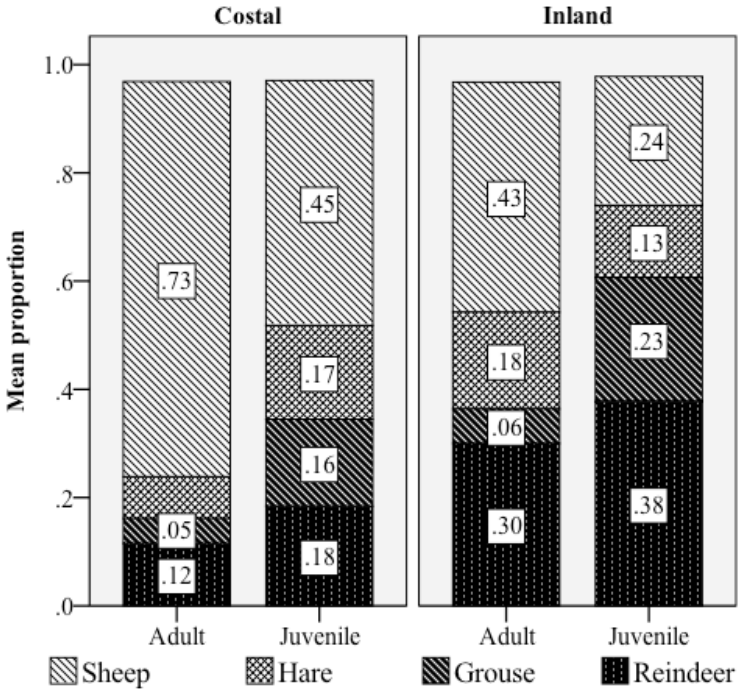


**Figure 10** Relative proportion of prey in the diet of golden in different regions and at different age, with minimum fractionation values

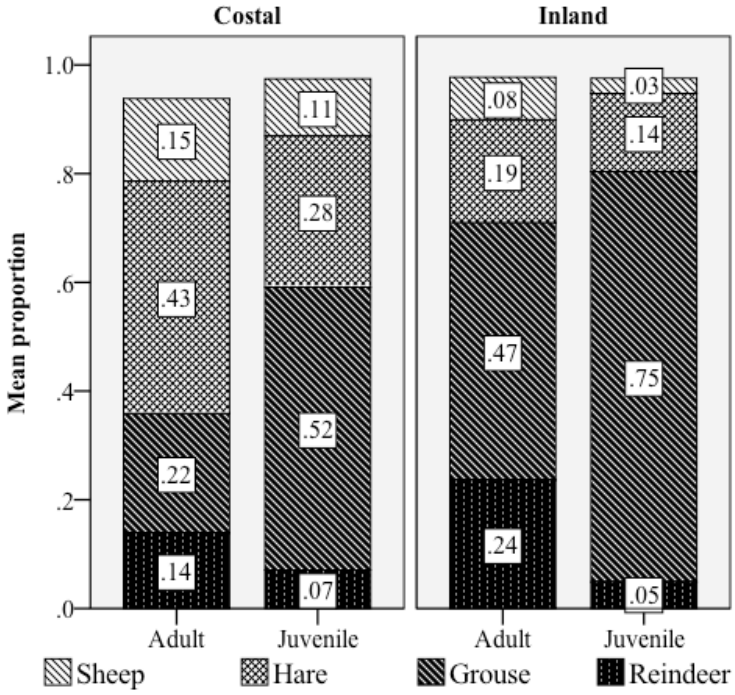


**Figure 11** Relative proportion of prey in the diet of golden in different regions and at different age, with maximum fractionation values

These two figures show clearly different estimations of the dietary proportion of the prey species. With fractionation values of 2 for C and 0 for N the model output displayed a majority of sheep in the diet, especially for adults on the coast. This pattern tended to decrease inland and for younger birds. This output also estimates a small proportion of sheep, and also not a great deal of hare. When the highest fractionation values were applied the proportion of sheep goes down, decreasing inland and with lower age. The proportion of reindeer also goes down. However, the estimated proportion of grouse increases. The fractionation values presented in the results above were selected from an already established fractionation value from peregrine falcon. As far as I am aware, no fractionation values for a species more closely related to the golden eagle than the peregrine falcon has yet been established. For this reason, the peregrine values were the best currently available for this species. In addition, this value corresponded well with fractionation values estimated from the δN:δC scattergram.

### 4.6.2 Use of different tissue

From the golden eagle, feathers were used for both SIA and ICP-MS. For all prey, muscle samples collected form hunters and slaughterhouses.

For SIA use of different tissue does not represent a problem, as the aim is to investigate what the golden eagles tissue, including feathers, is made from. Golden eagles mainly consume muscle tissue from the prey. The important factor for this analysis is the digestive fractionation discussed above. Feathers become inert after completion of growth. The isotopic profile is a result of the fractionation when there is metabolic activity in the tissue. For example, turnover rate is typically much higher in more metabolically active tissues such as liver and fat than in bone (Tieszen et al. 1983). Factors such as age, seasonality and starvation, are all factors that may influence the fractionation value (Halley et al. 2010, Tieszen et al. 1983).

Different tissue have different uptake rate of metals, and this may lead to incorrect interpretation of the results (Evers et al. 2005). As previously mentioned, the feathers reflect the levels of metals in the period of growth. When the feather is fully grown, the blood supply is cut off. Studies investigating intravariability in metal levels between tissues have found feathers to attain the highest levels of metals (Denneman and Douben 1993, Lucia et al. 2010, Tsipoura et al. 2011). This makes sense as birds sequester metals in feathers, and get rid of

metals by moulting (Burger 1993). This produces intravariability also between feathers. Some studies have concluded that feathers are unsuitable as monitors for metal contamination due to this variation. (Deben et al. 2012, Denneman and Douben 1993). Moulting can create particular contaminant patterns in the bird feathers because of unequal sequestering of metals during the moulting cycle (Dauwe et al. 2003, Furness et al. 1986). The moulting period of the golden eagle begins in March or April, and continues through September and October, but it does not complete one moulting cycle per season (Bloom 2001). Therefore, it is very difficult to assign a particular contour feather of an adult eagle to be grown at a specific time of the year. As all feathers of juveniles are grown at the same time, the variability between their feathers will be minimal.

Muscle seems to sequester low levels of metals, compared to other tissues. Pedersen et al. (2006) revealed somewhat low levels of Cd in muscle, but high levels in liver and very high levels in kidney in arctic hare. Medvedev (1999) documented that levels of various metals in muscles were lower than other tissue. Also Lucia et al. (2010) found that Hg was much lower in muscle than in other tissues, such as feathers, kidney and liver in various types of aquatic birds. Borgå et al (2006) concluded that all levels of metals were generally lower in muscle than liver Although there is intravariability in metal levels between different tissues, many studies states correlation between levels in muscle, kidney, liver and feather (Dauwe et al. 2002, Lucia et al. 2010). For comparative purposes there is need to know if metals vary or if they are constant between tissues. An overview of various metals and their distribution in different tissue in birds, is listed in Table 8 (Burger 1993).

**Table 8**: correlation of metal levels between various tissues (Burger, 1993).

| Metal | Feather | Liver | Kidney | Muscle | Number of studies |
|---|---|---|---|---|---|
| Selenium | 1 | 1,2 ± 0,0 | 2,2 ±0,0 | 0,4 ±0,0 | 1 |
| Cadmium | 1 | 2,75 ± 0,63 | 8,67 ± 1,95 | 0,53 ±0,12 | 10 |
| Lead | 1 | 0.42 ± 0.10 | 0.33 ± 0,07 | 0,14 ±0,05 | 11 |
| Copper | 1 | 11,3 ± 5,02 | 0,50 ± 0,09 | 0,83 ±0,26 | 11 |
| Mercury | 1 | 0,48 ± 0,11 | 0,31 ±0,06 | 0,10 ±0,03 | 21 |

It shows that different metal ratios vary between metals with respect to tissue. An equivalent overview of these ratios in the mammal prey in my study is not found. One could, if these ratios are representative for the golden eagle, calculate the corresponding levels in sensitive organs such as their liver and kidney.

My study illustrates that it is difficult to find strong relationships between metal levels in prey and those in the feather of an avian predator, the golden eagle. This is probably caused by metabolic regulations of the essential elements, but to a lesser degree of the non-essential elements such as Pb and Hg. The finding that Cd was below detection limits in the golden eagle feathers was surprising, indicating a strong binding effect of metallothionein in the liver (Habeebu et al. 2000).

### 4.6.3 Assumptions of prey

There are a number of assumptions regarding both food sources and the toxicodynamics in study. Western capercaillie and willow ptarmigan was grouped together due to similar isotopic values, and that they were considered to be the main contribution from the order of *galliformes* in the diet of golden eagle. However, their species difference may contribute differently to the contamination load in the golden eagles. Studies have shown that there is a significant difference in cadmium levels in rock ptarmigan and willow ptarmigan, two closely related species. This difference was explained by the difference in the diet (Rodrigue et al. 2007). Other species of grouse may show distinct isotopic profiles, but this was not taken into account in the present study. This may therefore be a source of error. Another source of error is the location of the various prey and golden eagles. Both golden eagle and prey are grouped into either coastal or inland. For some groups of prey, there were only samples from one area, either coastal (sheep) or inland (grouse and reindeers). For some species, this has a natural explanation. Reindeer are mainly located inland, and grouse mostly in inland forest and mountain areas. However, hare can be found in both regions. Thus, geographical differences in isotope and metal values between the prey species may exist, and not accounted for here.

Although the golden eagle in most studies in Scandinavia is found to consume primarily grouse, hares and ungulates, it may feed on a wide variety of prey species. In this study, there are only four groups of potential prey are used in the model, these four making up the most of biomass consumed from the golden eagle. Other sources of prey cannot be ruled out. Prey observed in the nest of golden eagle are *Mustelidae, Turdidae*, other kind of birds, fox, snakes and also fish, and others. Although these constitute a minor part of the biomass consumed, excluding these species may lead to misinterpretation of the results (Lunde 1985).

### 4.7 Correlation between actual levels in feathers and modelled levels in the diet

Figures 6-9 illustrate how the modelled levels of metals in the diet correspond with the actual values found in golden eagle. As shown on Figure 7, the levels of Pb remain rather unaffected by the modelled values of Pb, but stayed at levels which on the average were six times higher. This indicates some type of physiological regulation. For Hg there was a significant positive relationship (Figure 9), as expected in case of biomagnification. For Cu and Se, the method is evaluated as not feasible, as they will be regulated as most other essential elements. Cd seems to behave completely different from other metals, as they were below detection limits in the feathers, but not in the prey.

## Conclusion

By using SIA and ICP-MS, I was able to detect biomagnification of mercury from prey to golden eagle, which was expected. However, for Se, Cd, Pb and Cu estimating the metal levels in golden eagle feathers based on the modelled contribution of metals from of each source of prey was not successful.

There are apparently too many factors not accounted for in the current study, such as spurious prey, local variations, age and moult effects, local bedrock effects, etc. However, it confirmed the pattern consistent with the literature stating that only Hg biomagnify. Another drawback of this method is use of different tissues. Feathers may be good indicators of metal contamination as metals can be sequestered in feathers. Antagonism between Hg and Se is documented in many studies. Correlation between Hg and Se were found in both modelled diet values and real values. However, both concentrations of Hg and also Se are below levels considered harmful, and hence no conclusion on protective effect can be drawn.

The stable isotope analyses showed results on the food choice of the golden eagle which seemed realistic, and well in accordance with that which had been found in other studies in the field. It is therefore considered a helpful, non-destructive and innovative tool for studying food choice and food intake, which is applicable also to other species.

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# APPENDIX

## Appendix I - Accuracy of the method

**Table A1** Measured levels in reference material (Bovine Liver), with standard deviation, standard reference values (Bovine liver 1677b) and recovery

| | Average values | Standard deviation | Certified value (µg/g) | Estimated uncertainty (µg/l) | Recovery (%) |
|---|---|---|---|---|---|
| Se 82 | 0,749 | 0,0182 | 2,43 | 0,0258 | 103 |
| Cd 111 | 0,509 | 0,0193 | 3,80 | 0,0273 | 102 |
| Pb 208 | 0,11 | 0,000162 | 0,147 | 0,0002291 | 85,6 |
| Cu 63 | 150 | 2,71 | 1,81 | 3,84 | 93,9 |
| Hg 202 | 0,004844 | 0,000165 | 3,40 | 0,000233 | 161 |

## Appendix II - Detection limits (DL) for ICP-MS

**Table A2** Detection limits for the selected chemicals

| | Detection limit | Detection limit for samples (feather) below 0,01 mg |
|---|---|---|
| Se 82 | 0,115 | 0,15 |
| Cd 111 | 0,00913 | |
| Pb 208 | 0,00542 | 0,006 |
| Cu 65 | 0,0421 | 0,06 |
| Hg 202 | 0,00422 | |

## Appendix III

**Table A3** Metal levels various birds from other studies

| Species | Location | Se | Cd | Pb | Cu | Hg | Source |
|---|---|---|---|---|---|---|---|
| **Golden eagle adult** | **Coastal Trøndelag** | | **2,35** | | **0,34** | **29,4** | **1,83** | **Current study** |
| **Golden eagle juvenile** | | | **0,84** | | **0,09** | **7,88** | **0,18** | |
| **Golden eagle adult** | **Inland Trøndelag** | | **0,73** | | **0,25** | **10,43** | **0,25** | |
| **Golden eagle juvenile** | | | **0,73** | | **0,08** | **7,24** | **0,11** | |
| Little egret *(Egretta garzetta)* | Haleji Lake - Pakistan | | 1,71 | | | | 0,21 | Boncompagni, E, et al 2003 |
| | Karachi-Pakistan | | 3,83 | | | | 0,89 | |
| | Taunsa - Pakistan | | 9,02 | | | | 0,97 | |
| Cattle egret *(Bubulcus ibis)* | Taunsa - Pakistan | | 7,74 | | | | 0,41 | |
| Intermediate egret (*Mesophoyx intermedia*) | Haleji Lake - Pakistan | | 1,15 | | | | 0,16 | |
| Eared grebe (*Podiceps nigricollis*) | Northen Minnesota | | 1,557 | 0,339 | 1,69 | | 17,119 | Burger, J, Eichhorst, B, 2007 |
| | | | 1,267 | 0,009 | 1,192 | | 1,421 | |
| Pie-billed grebe (*Podilymbus podiceps*) | | | 1,333 | 0,029 | 2,667 | | 6,785 | |
| | | | 1,941 | 0,016 | 3,622 | | 1,881 | |
| Red-necked grebe (*Podiceps grisegena*) | | | 2,2 | 0,093 | 1,096 | | 4,948 | |
| | | | 2,259 | 0,036 | 2,089 | | 0,956 | |
| Western grebe (*Aechmophorus occidentalis*) | | | 0,792 | 18 | 4,481 | | 2,519 | |
| Pond heron (*Ardeola grayii*) | Szechuan - China | | 1 | 0,18 | 4,2 | | 2,4 | Burger, J, Gochfeld, M, 1993 |
| Black-crowned night heron (*Nycticorax nycticorax*) | | | 2 | 0,22 | 5,6 | | 2,3 | |
| Black-crowned night heron | Hong Kong | | 2,8 | 0,14 | 9,1 | | 0,84 | |
| Cattle egret | | | 1,2 | 0,43 | 4,6 | | 1,3 | |
| Little egret | | | 1,7 | 0,048 | 4,4 | | 2,2 | |
| Great egret (*Ardea alba*) | | | 1,3 | 0,072 | 1,5 | | 0,27 | |
| Great egret | | | 1,8 | 0,12 | 4,8 | | 1,5 | |
| Cattle egret | New York - USA | | 1,317 | 0,09 | 1,163 | | 0,596 | Burger, J, et al 1992 |
| | Cairo, Egypt | | 0,331 | 0,077 | 9,664 | | 0,331 | |
| | Aswan, Egypt | | 1,041 | 0,08 | 0,234 | | 3,484 | |
| | Pea Patch, Delaware | | 1,56 | 0,085 | 1,089 | | 1,637 | |
| | Humacao, Puerto Rico | | 1,273 | 0,425 | 1,606 | | 0,286 | |
| Common tern (*Sterna hirundo*) | Bird Islands, Massachusettes USA | | | 0,104 | 1,15 | | 3 | Burger, J, et al 1994 |
| Black-legged kittiwake (*Rissa tridactyla*) | Shoup Bay, Alaska | | 2,42 | 0,0328 | 0,707 | | 2,91 | Burger, J, et al 2008, b |
| Black oystercatcher (*Haematopus bachmani*) | Shoup Bay, Alaska | | 8 | 0,0913 | 1,25 | | 1,24 | |
| Night heron (*Nycticorax nycticorax*) | Mai Po Village - Hong Kong | | | 0,06 | 2,6 | 6,9 | 0,3 | Connell, D, W et al 2002 |
| | A Chau - Hong Kong | | | 0,04 | 0,7 | 6 | 1,7 | |
| Little egret | Pak Nai - Hong Kong | | | 0,1 | 4,4 | 13 | 0,8 | |
| | Mai Po Village - Hong Kong | | | 0,07 | 2,7 | 13 | 0,6 | |
| | Au Tai - Hong Kong | | | 0,05 | 1,5 | 12,2 | 4,1 | |
| | Tai Po Market - Hong Kong | | | 0,06 | 2 | 11,8 | 0,5 | |
| | Penfold Park - Hong Kong | | | 0,03 | 1 | 5,9 | 0,3 | |
| | A Chau - Hong Kong | | | 0,03 | 0,8 | 8 | 0,7 | |

| Species | Location | Se | Cd | Pb | Cu | Hg | Source |
|---|---|---|---|---|---|---|---|
| Black-crowned night-heron | Baltimore Harbor | 2,18 | 0,016 | 0,32 | 6,05 | 0,805 | Custer, T, W, et al 2008 |
| | Holland Island | 2,11 | 0,012 | 0,11 | 7,9 | 0,81 | |
| | Pea Patch Island - Delaware | 2,16 | 0,029 | 0,41 | 6,63 | 1,25 | |
| | Agassiz Minnesota | 2,1 | 0,15 | | 6,21 | 1,69 | |
| Sparrowhawk (*Accipiter nisus*) | Flanders, Belgium | | 0,09 | 2,61 | 3,16 | 1,1 | Dauwe, T, et al 2003 |
| | | | 0,06 | 2,51 | 3,07 | 1,19 | |
| | | | 0,07 | 3,21 | 4,45 | 1 | |
| | | | 0,15 | 3,95 | 4,39 | 0,86 | |
| | | | 0,33 | 4,73 | 4,63 | 0,88 | |
| | | | 0,25 | 4,6 | 4,84 | 0,57 | |
| | | | 0,17 | 5,5 | 5,31 | 0,38 | |
| | | | 0,16 | 4,95 | 5,76 | 0,22 | |
| | | | 0,15 | 4,69 | 6,18 | 0,22 | |
| | | | 0,25 | 6,03 | 6,61 | 0,15 | |
| Little owl (*Athene noctua*) | | | 0,05 | 3,99 | 8,98 | 0,32 | |
| | | | 0,06 | 3,88 | 9,13 | 0,31 | |
| | | | 0,07 | 4,43 | 8,85 | 0,36 | |
| | | | 0,09 | 4,01 | 7,33 | 0,25 | |
| | | | 0,09 | 4,67 | 6,92 | 0,27 | |
| | | | 0,1 | 4,89 | 6,84 | 0,16 | |
| | | | 0,11 | 4,78 | 6,65 | 0,16 | |
| | | | 0,1 | 4,75 | 6,73 | 0,17 | |
| | | | 0,1 | 4,83 | 6,43 | 0,12 | |
| | | | 0,15 | 6,09 | 7,43 | 0,15 | |
| Barn owl (*Tyto alba*) | | | 0,07 | 4,5 | 6,09 | 0,86 | |
| | | | 0,06 | 4,7 | 5,91 | 0,9 | |
| | | | 0,07 | 5,4 | 5,55 | 0,83 | |
| | | | 0,09 | 7,2 | 0,606 | 0,77 | |
| | | | 0,07 | 7,6 | 5,75 | 0,81 | |
| | | | 0,09 | 9,9 | 5,99 | 0,83 | |
| Osprey (*Pandion haliaetus*) | Florida Bay Western | | 0,07 | 0,293 | 9,07 | 16,5 | Lounsbury-Billie M, J,, et al 2008 |
| | Florida Bay Central | | 0,139 | 1,06 | 7,61 | 18,7 | |
| | Florida Bay Eastern | | 0,026 | 0,102 | 10,1 | 2,74 | |
| Barn owl (*tyto alba guttatus*) | Netherlands | | 1* | 40* | 22* | | Denneman and Douben, 1993 |
| | | | 1,2* | 19* | 33* | | |
| Herring gull (*Larus argentatus*) | Long Island, USA - down | 1,72 | 1,2 | 1,58 | | 1,105 | Burger, 1995 |
| | Long Island, USA - fledging | 1,43 | 1,15 | 1,95 | | 1,799 | |
| | Long Island, USA - adult | 0,906 | 0,369 | 4,1 | | 3,807 | |
| Common buzzard (*Buteo buteo*) | Galicia, Spain | | | | | 1,94** | Martinez et al,,2012 |
| Northern Gooshawk *(Accipiter gentilis*) | | | | | | 0,467** | |
| Common buzzard (Buteo buteo) | Northern Italy | | | 1,48** | | 0,06** | Battaglia et al, 2005 |
| Bald eagle (*Haliaeetus leucocephalus*) | Aleutian Chain of Alaska | 2,55 | 0,253 | 4,57 | | 4,91 | Burger and Gochfeld, 2009 |
| Laggar falcon (*Falco biarmicus jugger*) | Pakistan | | 0,1 | 1,56 | | 3,09 | Movalli, 2000 |

## Appendix IV

**Table A3** Metal levels various prey species from other studies

| Species | Location | Se | Cd | Pb | Cu | Hg | Source |
|---|---|---|---|---|---|---|---|
| **Hare(*Lepus timidus*)** | **Trøndelag, Norway** | **0,27** | **0,01** | **0,20** | **45901,00** | **0,00** | **Current study** |
| Arctic hare (*Lepus arcticus*) | Nunavut, Canada | | 0,01 | 0,01 | 11,00 | 0,00 | Pedersen and Lierhagen, 2006 |
| | | | 0,08 | 0,01 | 10,10 | 0,00 | |
| Mountain hare (*Lepus timidus*) | Northern Finland | | 0,02 | 0,20 | 6,57 | | Venäläinen et al, 1996 |
| Arctic hare (*Lepus arcticus*) | Baffin Island | | | 9,10 | 16,00 | | Mallory et al, (2004) |
| **Sheep (*Oves aries*)** | **Trøndelag, Norway** | **0,21** | **N,D,** | **0,01** | **2,38** | **0,01** | **Current study** |
| Sheep (*Oves aries*) | Poland | | 0,01 | 0,05 | | 0,00 | Rudy, 2008 |
| Sheep (*Oves aries*) | Western Bohemia, Czech Republic | | | 0,17 | 2,86 | | Jankovska et al,, 2012 |
| | | | | 0,18 | 3,96 | | |
| | | | | 0,69 | 4,51 | | |
| Sheep (*Oves aries)* | Baiyin of Gansu, China | | 0,62 | 1,85 | 8 | | Liu, 2003 |
| | | | 0,17 | 0,86 | 5,9 | | |
| Sheep (*Oves aries*) | West Bank, Palestinian Authority | | 0,45 | 0,25 | 2,78 | | Swaileh et al, (2009) |
| **Grouse** | **Trøndelag, Norway** | **0,49** | **0,06** | **0,4** | **7,91** | **0.00** | **Current study** |
| Willow ptarmigan (*lagopus lagopus)* | Qubec, Canada | | | 2,02 | | | Rodrigue et al, 2004 |
| Rock ptarmigan (*Lagoous mutus*) | | | | 1,34 | | | |
| **Reindeer(*Rangifer tarandus*)** | **Inland Nord-Trøndelag** | **0,49** | **0,062** | **0,007** | **6,2** | **0,0274** | **Current study** |
| Wild forrest reindeer (*Rangifer tarandus fennica*) | Karelian, Russia | | 0,58 | 2,14 | 1,63 | | Medvedev, 1997 |