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Comparison of the Composition of Chemical Elements in the Liver of Ringed Seal (*Phoca hispida*) from three different populations

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Abstract

The ringed seal (*Phoca hispida*) is a circumpolar Arctic species, with several subspecies and –populations. One subspecies, *Phoca hispida botnica*, is found in the Baltic Sea. Large ecological differences between these regions may cause variations of trophic positioning and chemical composition in tissues among the inhabiting ringed seal populations. The Baltic Sea is one of the worlds' most polluted seas, and the metal pollution in this area may be problematic for Baltic ringed seals. Liver samples from juvenile ringed seals (1-3 years) from three distinct populations, in northwest Greenland, East Greenland and the Baltic Sea were collected during 2008-09. Concentrations of 42 chemical elements, including As, Cd, Hg, Pb and Se, were determined using HR-ICP-MS. Stable isotope analysis (IRMS) of $\delta^{13}C$ and $\delta^{15}N$ were conducted in order to reveal population differences in feeding preferences (pelagic versus benthic species) and trophic positioning, respectively. Significant differences for $\delta^{13}C$ and $\delta^{15}N$ were found between all three populations, and the Greenland populations showed benthic preferences and higher trophic positioning relative to the Baltic Sea. The chemical element analysis revealed spatial differences between the Baltic Sea population and either one or both of the Greenland populations for several elements. However, none of the elements of concern in the Baltic Sea were present at significantly higher concentrations in the Baltic Sea population compared to the two Greenland populations.

The lower trophic positioning and preferred pelagic foraging of Baltic ringed seals contribute to protect them from impacts of their polluted habitat.

Abstrakt

Ringselen (*Phoca hispida*) finnes rundt hele Arktis og består av flere underarter og underpopulasjoner. En slik under art er å finne i Østersjøen (*Phoca hispida botnica*). Stor økologiske forskjeller mellom regionene ringselen hører til i kan forårsake store variasjoner mellom ulike populasjoner. Ulike trofiske nivå og komposisjonen av grunnstoff i ulike vevstyper kan være slike variasjoner. Ettersom Østersjøen er en av verdens mest forurensede sjøer kan den høye tilstedeværelsen av potensielt farlige metaller være et problem for den iboende ringsel populasjonen. Prøver av lever fra juvenile ringsel (1-3 år) ble høstet i tre forskjellige populasjoner mellom 2008-09. Disse populasjonene holdt til ved kysten av nordvest Grønland og øst-Grønland, og i Bottenviken nord i Østersjøen. Konsentrasjonene av 42 grunnstoff ble analysert ved bruk av HR-ICP-MS. Analyse av stabile isotoper av C og N ved bruk av IRMS ble også gjennomført. Ratio av disse stabile isotopene, $\delta^{13}C$ og $\delta^{15}N$, ble benyttet for å belyse henholdsvis matpreferanser (bentiske eller pelagiske arter) og trofisk posisjonering. Signifikante forskjeller ble funnet mellom alle tre populasjonene for de to stabile isotopene. Ringselpopulasjonene fra Grønland viste en sterkere preferanse for bentiske arter samt høyere trofisk posisjonering sammenliknet med Østersjøpopulasjonen. Signifikante populasjonsforskjeller ble funnet for flere grunnstoff mellom Østersjøpopulasjonen og en eller begge Grønlandspopulasjonene. Ingen av de potensielt farlige metallene i Østersjøen viste signifikant høyere konsentrasjoner i seler fra dette området, sammenliknet med selene fra Grønland.

Den lavere trofiske posisjoneringen og preferansen for pelagiske arter kan bidra til å beskytte ringsel i Østersjøen fra påvirkningene av deres forurensede habitat.

List of chemical elements

Ag	Silver	**N**	Nitrogen
Al	Aluminium	**Na**	Sodium
As	Arsenic	**Nb**	Niobium
Au	Gold	**Nd**	Neodynum
B	Boron	**Ni**	Nickel
Ba	Barium	**P**	Phosphorous
C	Carbon	**Pb**	Lead
Ca	Calcium	**Pr**	Praseodymium
Cd	Cadmium	**Rb**	Rubidium
Ce	Cerium	**S**	Sulphur
Co	Cobalt	**Sb**	Antimony
Cr	Chromium	**Se**	Selenium
Cs	Caesium	**Sm**	Samarium
Cu	Copper	**Sn**	Tin
Fe	Iron	**Sr**	Strontium
Ga	Gallium	**Th**	Thorium
Hf	Hafnium	**Ti**	Titanium
Hg	Mercury	**Tl**	Thallium
K	Potassium	**U**	Uranium
La	Lanthanum	**V**	Vanadium
Mg	Magnesium	**W**	Tungsten
Mn	Manganese	**Y**	Yttrium
Mo	Molybdenum	**Zn**	Zinc

Abbreviations

BS	Baltic Sea
DDT	Dichlorodiphenyltrichloroethane
dw	Dry weight
EG	East Greenland
GI tract	Gastrointestinal tract
HCB	Hexachlorobenzene
HR-ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
IDL	Instrument Detection Limit
IRMS	Isotope Ratio Mass Spectrometry
MDL	Method detection limits
MT	Metallothionein
P. h.	*Phoca hispida*
PC	Principal Component
PCA	Principal Component Analysis
PCB	Polychlorinated biphenyls
POPs	Persistent Organic Pollutants
REE	Rare Earth Elements
rpm	Revolutions per minute
TBT	Tributyltin
Tukey HSD	Tukey Honestly Significant Difference
WG	West Greenland
$\delta^{13}C$	per mill ratio of stable carbon isotopes ($^{13}C/^{12}C$)
$\delta^{15}N$	per mill ratio of stable nitrogen isotopes ($^{15}N/^{14}N$)

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1. Introduction

1.1 Ringed seal (*Phoca hispida*)

The ringed seal is a small ice dwelling seal in the genus *Phoca* in the family *Phocidae*, with a circumpolar distribution. It is probably the most abundant ice seal in the northern hemisphere. There are five known subspecies or ringed seal: *P. hispida hispida* in the Arctic Ocean, Canadian Arctic, Greenland Waters and Bering Sea, *P. hispida ochotensis* in the Ochotsk Sea, *P. hispida ladogensis* in Lake Ladoga in Russia, *P. hispida saimensis* in Lake Saimaa in Finland, and *P. hispida botnica* in the Baltic Sea (Perrin et al., 2008; Reeves, 1998). Genetic tests have revealed genetic flow from *P. h. hispida* to *P. h. botnica* equal to one seal per year, causing less genetic differentiation between these two subspecies than would be expected if the Baltic Sea population was truly isolated (Palo et al., 2001).

Ringed seals are carnivores feeding on a wide range of fish and crustaceans. Individual seals rarely feed on more than 10-15 species of prey, and are likely to favour two to four of these (Reeves, 1998). Seasonal variations in foraging preferences are also observed (Labansen et al., 2011; Reeves, 1998; Siegstad et al., 1998), as well as the preference for relative small prey, rarely larger than 20 cm (Reeves, 1998; Siegstad et al., 1998). The Baltic Sea ringed seals are considered to be top predators, while ringed seals in the Arctic are prey for polar bears (*Ursus maritimus*), killer whales (*Orcinus orca*), and possibly also walruses (*Obobenus rosmarus*) (Reeves, 1998). Ringed seals are an important species for the Inuit people, as they are a source for food, fuel and clothing material (Reeves, 1998; Teilmann and Kapel, 1998). Long lifespans and high trophic level in combination with long biological half-life of elimination of pollutants are qualities that can cause high accumulation of persistent organic pollutants (POPs) in ringed seals. Seals can thus serve as indicators of pollution in their habitat. Temporal changes in pollution can be studied within populations, and the wide distribution of the ringed seal can be used for comparison of local pollution between Arctic regions (Das et al., 2003). Absorption of pollutants can occur through skin, the lungs, over the placenta, and through milk when lactating. However, the major route of exposure for ringed seals is through ingestion of food. Heavy metals that are acquired through food will distribute unevenly between organs. The liver is a major detoxifying organ and receives a large range of toxic and harmful substances. Many heavy metals, such as Hg, are known to accumulate to higher concentrations in the liver than in any other organ, while Cd reaches highest levels in the kidneys where it binds to specific proteins (Wagemann et al., 1996).

1.2 The Baltic Sea

The Baltic Sea is bordered by nine countries and has a catchment area that stretches into another five adjacent countries. More than 85 million people live within this area, and contribute to the pollution of this once clean sea. The Baltic Sea is one of the largest brackish waters in the world, and the inhabiting species are of both marine and freshwater origin (HELCOM, 2010b; Neumann and Schernewski, 2005). The salinity in the Baltic Sea varies, with levels as low as 3-4 ‰ in the Bothnian Bay, increasing towards the southern Baltic Sea and west to the Danish Strait. The composition of marine and freshwater species changes within the Baltic Sea depending on salinity, temperature and other abiotic factors which create different ecosystems within the sea (Kuparinen et al., 1996). Comparatively, in the Arctic salinities near 35 ‰ are common (Barrie et al., 1992). Climate change may threaten species in the Baltic Sea that already live at the edge of their physiological tolerance limits by altering the salinity and/or the temperature in the sea (Schiedek et al., 2007). Increased sea and air temperatures also alter the formation rate, size and duration of the winter ice, which is vital for ringed seal during the pupping and nursing season.

Pollution of the Baltic Sea followed the industrial revolution, and industrial as well as raw sewage, agricultural run-offs, shipping and atmospheric depositions have caused the Baltic Sea to become a severely polluted sea (Rheinheimer, 1998). The water in the Baltic Sea has a long retention time, close to 30 years, resulting in slow rates of dilution of pollutants and a general accumulation of contaminants (HELCOM, 2010b).

Organochlorines, heavy metals and nutrients are three major pollution problems in the Baltic Sea. Recent observations indicate reduced emissions of certain pollutants, such as dioxins and furans, in the neighbouring countries. These reductions are reflected by decreased atmospheric concentrations (Gusev et al., 2007). Some dioxin congeners have expressed a decreasing trend in guillemot eggs as well, but most congeners are at stable or even growing levels (Wiberg and Josefsson, 2009). Organochlorines such as PCB and HCB have decreased in guillemot eggs and herring in the Baltic Sea, but this trend has not been obvious in sediments (Wiberg and Josefsson, 2009).

Inputs of heavy metals and nutrients (P and N) to the sea occur through transportation with riverine waters and as atmospheric deposition (Gusev, 2010; HELCOM PLC-Group, 2010). In the period from 1990 to 2009 atmospheric deposition of Cd, Hg and Pb decreased (Gusev, 2010), and total N-deposition was 19 % lower in 2009 than in 1995 (Bartnicki, 2011).

Decrease of waterborne heavy metal input from certain countries has also been observed (HELCOM PLC-Group, 2010), while riverine nutrient loads vary locally, resulting in an insignificant decrease for the entire Baltic Sea (HELCOM, 2011).

The population of ringed seals in the Baltic Sea has undergone severe changes over the last century. The population count in the early 1900s has been estimated between 100,000 and 250,000 ringed seals (Härkönen et al., 1998; Reeves, 1998). Ringed seals were popular game in Sweden and Finland as well as Estonia. The population was heavily overexploited, and severely reduced by the 1960s. Though the hunting subsided gradually, the population continued to diminish dramatically, and although hunting was banned in the 1980s the ringed seal population increased only at a very slow rate. In 1996 the population was estimated to consist of 5,510 ± 42 % (Härkönen et al., 1998), and annual population increase has been estimated to 4.5 % (Harding and Härkönen, 1999). High levels of PCBs and DDTs are believed to have caused physiological changes in the uterus of female seals, causing them to be sterile for life (Helle et al., 1976b). In the 1970s, 27 % of females of reproductive age were estimated to be pregnant in the Baltic Sea, compared to 80-90 % in more pristine areas such as the Arctic (Helle et al., 1976a). Consequently, organochlorines are believed to have contributed significantly to the decline of both ringed and grey seals, as well as limiting the recovery rate of these species (Rheinheimer, 1998). Recent studies indicate that the fertility among female ringed seals is rising, and only 8 % of examined females were sterile between 1991-2009 (Kunnasranta et al., 2010). Using the annual increase and estimated population size proposed by Harding and Härkönen (1999) and Härkönen et al. (1998) respectively, calculations indicate that the ringed seal population may consist of approximately 11,000 individuals today.

1.3 Greenland Arctic

The Arctic environment is inhabited by very few humans, and there are few anthropogenic pollution sources. Greenland is populated by approximately 56,700 people living primarily of fishing and hunting, and to some extent mining and minor industries (Statistics Greenland, 2012). Despite the lack of local emissions, pollutants such as various chlorinated compounds and heavy metals have been found at surprisingly high levels in Arctic animals, as well as the human populations. Both ocean currents and long range atmospheric transportation are known to carry a wide range of contaminants to the Arctic from industrialized areas elsewhere (Barrie et al., 1992). Arctic rivers may also contribute to the transportation of contaminants to the Arctic, though their contribution is often considered relatively low (Barrie et al., 1992).

For many metals, erosion of coastal shelves is a more important annual contributor (AMAP, 2005).

Transportation through the atmosphere is the fastest route to the Arctic, and pollution in the Northern Hemisphere will reach the Arctic within days or weeks depending on distance and weather conditions (Shevchenko et al., 2003). Particles as well as volatile and semi-volatile compounds may be transported through air. Particles are usually transported in a single step of movement, while volatile compounds may travel in intervals (AMAP, 2005; Barrie et al., 1992). Analysis of ice cores in the Arctic have revealed temporal changes in atmospheric loads of heavy metals (Cu, Zn, Cd and Pb), which increased between the 18th century and 1960s due to increased anthropogenic emissions (Candelone et al., 1995). During the last decades decreasing trends have been observed. The natural ocean currents entering the Arctic Ocean through the Atlantic Ocean and Bering Strait, also carry particles and dissolved compounds from industrialized areas, and though this is a slower process it is still a significant contributor (Barrie et al., 1992).

There are ocean currents along both the east and west coast of Greenland. Large volumes of water leave the Arctic Ocean through the Fram strait, either by the East Greenland Current that flows along the surface, or modified (cooled) Atlantic water and bottom water that flow lower down in the water column (Barrie et al., 1992). The West Greenland current is a continuation of the East Greenland Current when it has passed Cape Farewell, and follows the west coast of Greenland northwards to Baffin Bay (Reverdin et al., 2003).

The Arctic ecosystem have been described as having low biodiversity and high population densities of a few species, and relatively short food webs (Barrie et al., 1992). Marine Arctic species inhabit three interacting realms: the sea ice, pelagic and benthic communities. Primary production occurs mainly in the sea ice and pelagic communities, while benthic species generally rely on organic inputs from the water column, consequently playing a major role in the carbon cycle in the Arctic (Gradinger et al., 2010). Greenland waters have generally higher benthic biomass than many other areas in the Arctic (Grebmeier and Barry, 1991).

1.4 Metal toxicity

Metals differ from other environmental pollutants in being elements, and thus often ubiquitously distributed in the environment. Due to their natural presence, some metals have become essential elements for organisms and are important in a long range of biological processes. However, essential elements are only required within a specific range, and depleted

or exceeded concentrations relative to this range can cause harm in an organism. The adaptation to and dependence on certain elements, can be exploited by other non-essential and possibly toxic metals. By mimicking essential elements, other metals may gain access to an organism or a specific tissue where it may cause adverse effects. Different metals and various speciations of the same metal often behave differently within an organism, and can cause a wide range of harmful effects (Florea and Busselberg, 2006).

Though metals are not produced or destroyed, humans have learned to extract and concentrate them, and change their chemical forms and speciation. Human impact has thus caused a shift in the environmental distribution of many metals by removing them from stable ores, and releasing them into the world's atmosphere, waters or soils. The existence and properties of many metals have been known for hundreds of years, but the industrial revolution markedly increased both the rate of detection of new metals, and the environmental concentrations of many "new" and "old" metals (Klaassen, 2008). These increases in the environment are a cause of concern as the elevated levels may result in organisms obtaining harmful concentrations, perhaps leading to population declines and further ecosystem impacts.

The Baltic Sea has been referred to as the most polluted sea in the world, a statement based on its long history of chemical exposure from the highly populated countries surrounding it (HELCOM, 2010a). Metals have made up a fair portion of this chemical exposure, and their possible effects on wildlife are worrisome.

1.5 Stable isotopes

The relationship between different isotopes of N ($^{15}N/^{14}N$) and C ($^{13}C/^{12}C$) have been used for estimations of trophic position and foraging preferences in marine as well as terrestrial animals for the last thirty years. Preferences for ^{14}N in biological excretion processes cause the heavier ^{15}N isotope to be retained, and thus accumulate in organisms. The ratio between heavier and lighter N isotopes also increase with trophic levels, causing animals higher up in the trophic pyramid to have higher $\delta^{15}N$ than their prey and lower segments of the pyramid (Newsome et al., 2010; Post, 2002). The ^{13}C isotope experience a similar phenomenon of trophic increase, but as phytoplankton and other primary producers have a natural affinity to the ^{12}C-isotope, the ^{13}C-isotope is naturally depleted in the ecosystem, causing a negative ratio compared to standards (Newsome et al., 2010; Post, 2002).

The ratio between N-isotopes is determined in order to estimate the trophic level, while ^{13}C is used to estimate the foraging preferences of the analysed organism. The levels of $\delta^{13}C$ at the

bottom of a food chain vary between different ecosystems (Kelly, 2000), generally being higher in high production areas such as shores and upwelling zones, and lower in areas of less production such as open water areas, and highly stratified waters. In addition, there are also large differences between producers due to size and genetics, and macroscopic marine plants such as kelp and sea grass (near shore/benthic species) are known to have higher $\delta^{13}C$ than phytoplankton (Kelly, 2000; Newsome et al., 2010). Lack of CO_2 dissolved in the water due to low turbulence also enhances the uptake of ^{13}C, an incidence that commonly occur for periphyton at the bottom of benthic ecosystems. The stable isotope-signature is maintained in higher trophic levels of the ecosystem, and can thus be used to indicate individual foraging preferences of predators that may feed of both pelagic (low $\delta^{13}C$) and benthic (higher $\delta^{13}C$) species (France, 1995; Newsome et al., 2010; Post, 2002). However, the potentially large differences between ecosystems challenge the comparison of different populations without knowledge of the baseline levels of the stable isotopes in the respective ecosystems, as observed variations may reflect the variations in baseline levels and not foraging preferences of the studied organism (Newsome et al., 2010; Post, 2002).

The increase of $\delta^{13}C$ for each trophic level have estimated to be 1 ‰ by Deniro and Epstein (1978), though others have found a range from 0.5-3 ‰ depending on tissue (Newsome et al., 2010). Nitrogen ratios are estimated to increase between 2-5 ‰ per trophic level generally (Hobson et al., 1996; Newsome et al., 2010), but pinnipeds have expressed a more narrow range of 3-5 ‰ (Newsome et al., 2010).

The composition of isotopes may vary between tissues and organs within an animal due to variations in amino acid and lipid composition. In addition, the metabolic rates of a specific tissue affect the isotopic turnover rates in this tissue. Higher metabolic rates increase isotopic turnover and cause the tissue to reflect stable isotope ratios mirroring those in resent diet. Thus, organs with high metabolic rates, such as blood serum and liver, mirror the stable isotope ratios from diet of the last few days to weeks. Comparatively, lower metabolic turnover cause muscle tissue to reflect ratios of diet from the last few months. Collagen and bone structures reflect the diet during an entire lifespan (Newsome et al., 2010). This enables the investigation of diet history, but also complicates comparisons of different tissues. Other factors that influence stable isotope ratios are body conditions, seasonal variation in diet, lactation and starvation (Newsome et al., 2010).

1.6 Aim of study

The aim of this study is to investigate geographical differences and correlations between heavy metals and other trace elements between two ringed seal populations from Greenland waters and the Baltic ringed seal population, and compare selected chemical elements to stable isotope ratios. It is hypothesized that distinct ringed seal populations will express different hepatic concentrations of chemical elements, and that observed differences can be explained by diet composition. Concentrations of chemical elements are also expected to reflect pollution levels in the respective populations' habitats. It is hypothesized that seals from the relatively polluted Baltic Sea will accumulate high concentrations of chemical elements of concern in this area.

To our knowledge this is the first time trace element levels and stable isotope ratios in ringed seal populations from the Baltic Sea and coastal waters of Greenland are compared.

2 Material and method

2.1 Samples

Liver samples were obtained from ringed seals (*Phoca hispida*) between April 2008 and October 2009. The animals were shot at three different locations; the northern Baltic Sea (n = 20); Ittoqqortoormiit in East Greenland (n = 10); and Qaanaaq in West Greenland (n = 10). All samples were freeze dried before analysis. Biological data for the samples can be seen in Appendix A.

2.2 Analysis

2.2.1 Chemical element analysis

All samples were digested in a high pressure microwave system (Milestone UltraClave, EMLS, Leutkirch, Germany) prior to element analysis by a High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS). Approximately 0.5 g of mechanically crushed sample was weighed on a 4 digit balance scale and transferred to a PTFE-Teflon vial (18mL). 3 mL of ultrapure water, and 3 mL concentrated nitric acid (HNO_3, Scan Pure, Chemscan, Elverum, Norway) were added. The samples were digested during a temperature program that gradually increased up to 240 °C over the course of one hour, and then returned to the original temperature during the next hour. The digested samples were then diluted to a final acid concentration of 0.6 M, and transferred to polypropylene vials (11 mL).

High resolution Inductively Coupled Mass Spectrometry analyses were performed using a Thermo Finnigan model Element 2 instrument (Bremen, Germany). The radio frequency power was set to 1400 W. Samples were introduced using a SC-FAST flow injection analysis system (ESI, Elemental Scientific, Inc. Omaha, USA) with a peristaltic pump (1 mL/min). The instrument was equipped with a PFA-ST nebulizer, spray chamber (PFA Barrel 35 mm), demountable torch, quarts standard injector and Al sample skimmer and X skimmer cones. The 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 to provide enhanced sensitivity, especially for Se and As. The instrument was calibrated using 0.6 HNO_3 solutions of matrix-matched multi-element standards. A calibration curve consisting of 5 different concentrations was made from these standards. To check for instrument drift one of these multi-element standards were analysed for every ten samples. The accuracy of the method was verified by analysing the certified reference material Scallop GBW10024 (Appendix B).

Method detection limits (MDL) were determined by comparing the instrument detection limits (IDL) to 3 times standard deviation of the blank samples. The highest value of the two was chosen for MDL (Appendix C). The calculations of IDL were done by analysing solutions of decreasing concentrations of each chemical element. The concentration resulting in a relative standard deviation of approximately 25 % (n = 3 scans) were set as the IDL, applying baseline corrections for these values.

Results that were below the determined detection limit were replaced with half the value of this detection limit, and this was done for some samples for Ag, B, Ba, Cr, Ga, Hg, La, Nb, Nd, Pr, Sm, Sn, Th, Ti, Tl, U, W and Y. Hafnium was excluded from statistical analysis as more than 50 % of the samples were below the detection limit for this element, while Au was excluded due to an unstable baseline.

2.2.2 Stable isotope analysis

The preparations of samples for stable isotope analysis involved homogenization of the samples and lipid removal. As the samples were from liver tissue, there may have been some slight heterogeneity of the isotope distribution within each sample. By powdering the sample, homogeneity is gained and the risk of different results between replicates is reduced (Newsome et al., 2010).

Lipids are known to have a lower ratio of $^{13}C/^{12}C$ compared to the diet of the animals, and also vary between tissues and species. They can thus cause large differences between samples, and disguise significant differences or produce erroneous results. For these reasons lipids were removed in order to obtain a better estimation of the level of depleted/increased relationship of stable isotopes compared to standards.

The samples were homogenized into fine powder using a mixing mill (MM 400, Retch, Haan, Germany). Lipids were removed by soaking samples in approximately 3 mL of a 2:1 (volume:volume) solution of chloroform and methanol, and vortexed for 30 seconds. The mixture was left to settle for at least 30 minutes before centrifugation at 1500 rpm for 5 minutes. The supernatant was then removed and the remaining pellet submerged in the solution once more. These steps were repeated until the supernatant was colourless and no differences were observed between repetitions. The remaining pellet was then dried at 60 °C for 24 hours.

The stable isotope analysis was performed at the Institute of Energy Technology, at Kjeller, Norway, using Isotope Ratio Mass Spectrometry (IRMS). Approximately 1.0 mg of sample was weighed into 8 x 5 mm tin capsules and combusted in the presence of O_2 and Cr_2O_3 at 1700 °C in a Eurovector element analyser. Reduction of NO_x to N_2 was done in a Cu oven at 650 °C. Water was removed in a chemical trap of $Mg(ClO_4)_2$ before separation of N_2 and CO_2 on a 2 m Poraplot Q gas-chromatography (GC) column. N_2 and CO_2 were directly injected on-line to a Nu Instruments Horizon, Isotope Ratio Mass Spectrometer (IRMS) (Wrexham, North Wales, UK) for determination of $\delta^{13}C$ and $\delta^{15}N$. Isotope values are reported in delta notation (δ), and calculated according to the following equation:

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

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 heavy to light isotope ratio in the standards. Accuracy and precision of analysis were measured by internal standard (IFE trout) and international standards - USGS-24 (carbon; from the International Atomic Agency [IAEA]) calibrated against PeeDee Belemnite, Vienna (VPDB) for ^{13}C, and IAEA-N-1 and IAEA-N-2 ($[(NH_4)_2SO_4]$ – ammonium sulphate) calibrated against atmospheric N_2 for ^{15}N. Repeated analysis of the internal standard indicated analytical errors of 0.06 ‰ and 0.12 ‰ for carbon and nitrogen, respectively.

2.3 Statistical analysis

For the statistical analysis seals were grouped according to geographical location, resulting in three distinct populations; the Baltic Sea (n = 8), East Greenland (n = 10), and West Greenland (n = 10). In order to obtain a similar age group between the populations, adult seals (four years or older) and seals of unknown age were omitted. In addition, five yearlings were removed from the data set, after testing for significant differences between yearlings and juveniles (1-3 years) using the Student's t-test. These modifications of the data set reduced the sample size in the populations from the Baltic Sea (n = 7) and West Greenland (n = 6). All samples were log transformed prior to analysis in order to approximate normal distribution.

One way analysis of variance (ANOVA) was used to investigate possible differences in mean concentrations of chemical elements between the populations. As the ANOVA is a stable statistical procedure it can resist, to a certain degree, the impacts of violations of normal distribution and/or homogeneity (Mcguinness, 2002). The ANOVA model was supplemented with Tukey honestly significant difference (HSD) test in order to reveal differences between

populations. Tukey HSD is a conservative method when sample sizes are unequal, which limits the possibility of type I errors. The Student's t-test was applied when analysing possible differences in mean chemical element concentrations between sexes and age groups, overall and within populations. The Student's t-test determines the significance of the differences between two means based on the standard errors.

In order to examine the relationships between chemical elements, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), Hg:Se molar ratio and age, principal component analysis (PCA) and the Pearson correlation were applied.

Biological data sets often consist of variables that are inter-correlated, incomplete, and to a certain degree noisy. The PCA extracts the most important information and patterns from the data set, excludes much of the noise, and simplifies the data set while retaining the maximum level of variation (Abdi and Williams, 2010; Wold et al., 1987). The simplified information is expressed as a set of new variables called principal components, of which the first contain the highest level of variance and the second the highest possible level of variance under the constraint of being orthogonal to the first principal component (Abdi and Williams, 2010). Two plots are created, one of the observations or samples (score plot), and another of the variables (loading plot). Samples that group or are closely associated in the score plot can be expected to show similar variable compositions. Variables that cluster or are near each other in the loading plot are likely to correlate positively. Possible outliers can be examined using the Hotellings T2 with a 95 % confidence interval, which forms an ellipse in the score plot.

The ANOVA, Student's t-test, and Pearson's correlation were executed using SPSS (version 19 for Windows, SPSS Inc., Chicago, IL, USA). PCA analysis was performed using SIMCA P+ (Version 12, Umetrics, Umeå, Sweden). The significance level was set to $p < 0.05$ for all analysis. For comparison between wet weight (ww) and dry weight (dw) results, dry weight results were converted to wet weight using the factor of 0.3 as suggested by (Yang and Miyazaki, 2003).

3 Results

In total, concentrations of 68 chemical elements were analysed in liver samples from 23 animals from three different populations. Of these elements, 42 were detected to a sufficient degree and thus treated statistically. The mean concentrations with standard deviation, median, and ranges (min-max) of each element within the different populations are listed in Appendix D and E. All results are reported in $\mu g \cdot g^{-1}$ dry weight (dw), or $ng \cdot g^{-1}$ (dw) when marked with asterisk. In addition, the results of the ratios of stable isotopes of C ($^{13}C/^{12}C$) and N ($^{15}N/^{14}N$) in the same liver samples are presented in Appendix F along with age and Hg:Se molar ratio.

3.1 Stable isotopes

The stable isotopes of $\delta^{13}C$ ranged from -22.6 ‰ found in an animal from the Baltic Sea, to -17.9 ‰ in an animal from the West Greenland area, and averaged at -20.5 ‰. The lowest level of $\delta^{15}N$ was 12.9 ‰, found in a seal from the Baltic Sea, and the highest level from a seal from West Greenland reaching 17.1 ‰, and with a total average of 14.6 ‰.

There was a significant correlation (Fig. 1) between the two stable isotopes (r=0.839, R^2=0.704, p<0.001). Removing a possible outlier from the West Greenland population improved the correlation further (r=0.938, R^2=0.879, p<0.001).

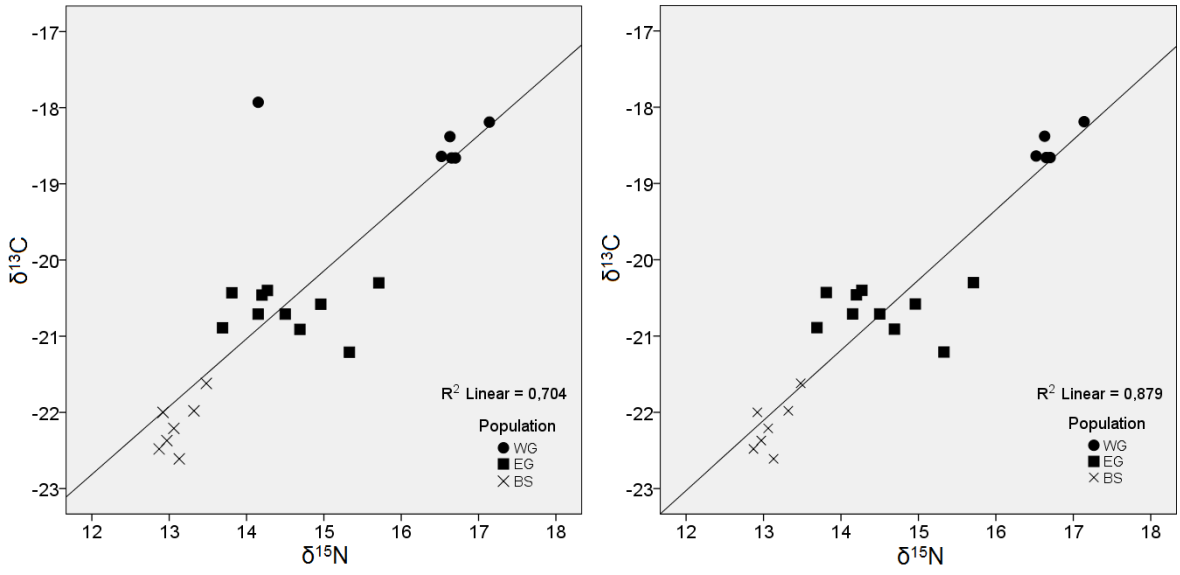

Figure 1: Relationship between $\delta^{15}N$ and $\delta^{13}C$ in ringed seals from the three populations: Baltic Sea (BS, n=7), East Greenland (EG, n=10) and West Greenland (WG, n=6). The regression line and R^2 factor are presented. The figure to the left correspond to all samples, while the figure to the right has had one outlier removed.

Significant differences were observed between the three populations for both $\delta^{15}N$ ($F_{2,22}$=34.7, p<0.001) and $\delta^{13}C$ ($F_{2,22}$=239, p<0.001). Seals from the Baltic Sea had significantly lower levels of $\delta^{13}C$ compared to both East Greenland and West Greenland (Tukey HSD; p<0.001).

The East Greenland population also had significantly lower levels than the West Greenland population (Tukey HSD; $p<0.001$). The same trend was observed for $\delta^{15}N$ as the seals from the Baltic Sea had significantly lower levels than both East Greenland and West Greenland (Tukey HSD; $p<0.001$) seals, and the East Greenland seals had significantly lower $\delta^{15}N$ levels compared to West Greenland seals (Tukey HSD; $p<0.001$). There were no significant differences in stable isotope ratios between males and females.

3.2 Chemical elements

3.2.1 Spatial variation

ANOVA revealed significant spatial differences for 24 elements between the populations, either between two of the populations ($X \neq Y$) or between two similar population and the third ($X=Y \neq Z$) (Table 1). Arsenic was the only element with significantly different mean concentrations between all three populations ($X \neq Y \neq Z$), similarly to the previously mentioned stable isotopes. The level of significance ranged from Na ($F_{2,22}=3.87$, $p=0.038$) to As ($F_{2,22}=190$, $p<0.001$) (Table 2).

The concentrations of As in the seals from the Baltic Sea were significantly lower than concentrations in seals from both East Greenland (Tukey HSD; $p<0.001$) and West Greenland (Tukey HSD; $p<0.001$). The concentrations in East Greenland seals were significantly higher than in the West Greenland seals (Tukey HSD; $p<0.001$).

Strong significant differences were seen between populations for 13 chemical elements: As, Cd, Cr, K, Mg, Mo, P, S, Sb, Sn, Tl, U, and V ($p<0.001$). The concentrations of Cd, Sb, Cr, Mo and Cs, were all significantly lower in the Baltic Sea population compared to the two Greenland populations, though not between the Greenland populations (Table 2). Thallium, however, had higher mean concentration in the Baltic Sea seal compared to seals from either of the two Greenland populations.

Table 1: Significant differences, and the respective p-values (significance set at $p<0.05 = p<5.00\cdot10^{-2}$) found between populations by using the Tukey's test. The populations are designated the following abbreviations: Baltic Sea = BS, East Greenland = EG, West Greenland = WG.

Variable	Tukey	p-value	Variable	Tukey	p-value
$\delta^{13}C$	BS ≠ EG	$1.06\cdot10^{-8}$	**P**	BS ≠ EG	$3.23\cdot10^{-2}$
	BS ≠ WG	$5.20\cdot10^{-9}$		EG ≠ WG	$6.05\cdot10^{-4}$
	EG ≠ WG	$5.24\cdot10^{-9}$	**S**	BS ≠ WG	$3.20\cdot10^{-6}$
$\delta^{15}N$	BS ≠ EG	$6.80\cdot10^{-4}$		EG ≠ WG	$1.13\cdot10^{-6}$
	BS ≠ WG	$1.86\cdot10^{-7}$	**Ti**	BS ≠ WG	$7.85\cdot10^{-3}$
	EG ≠ WG	$3.67\cdot10^{-4}$		EG ≠ WG	$2.50\cdot10^{-2}$
Ca	BS ≠ EG	$6.75\cdot10^{-3}$	**V**	BS ≠ EG	$2.49\cdot10^{-5}$
	EG ≠ WG	$6.84\cdot10^{-3}$		EG ≠ WG	$5.19\cdot10^{-3}$
Cd	BS ≠ EG	$6.80\cdot10^{-9}$	**Cr**	BS ≠ EG	$6.80\cdot10^{-6}$
	BS ≠ WG	$2.54\cdot10^{-8}$		BS ≠ WG	$7.79\cdot10^{-4}$
Mo	BS ≠ EG	$3.77\cdot10^{-4}$	**Cu**	BS ≠ WG	$2.81\cdot10^{-2}$
	BS ≠ WG	$1.55\cdot10^{-4}$	**Rb**	BS ≠ EG	$1.80\cdot10^{-3}$
Sn	BS ≠ WG	$1.16\cdot10^{-5}$		EG ≠ WG	$2.89\cdot10^{-3}$
	EG ≠ WG	$4.36\cdot10^{-5}$	**Sr**	BS ≠ WG	$2.56\cdot10^{-2}$
Cs	BS ≠ EG	$8.55\cdot10^{-3}$	**Ag**	EG ≠ WG	$6.89\cdot10^{-3}$
	BS ≠ WG	$4.98\cdot10^{-2}$	**Sb**	BS ≠ EG	$1.79\cdot10^{-6}$
Tl	BS ≠ EG	$1.10\cdot10^{-6}$		BS ≠ WG	$3.61\cdot10^{-6}$
	BS ≠ WG	$3.72\cdot10^{-6}$	**La**	BS ≠ EG	$1.97\cdot10^{-2}$
Th	BS ≠ EG	$3.59\cdot10^{-2}$		EG ≠ WG	$2.00\cdot10^{-2}$
	EG ≠ WG	$4.77\cdot10^{-3}$	**K**	BS ≠ EG	$1.63\cdot10^{-5}$
U	BS ≠ EG	$2.19\cdot10^{-5}$		EG ≠ WG	$5.26\cdot10^{-7}$
	EG ≠ WG	$1.63\cdot10^{-3}$	**Fe**	BS ≠ WG	$1.39\cdot10^{-2}$
As	BS ≠ EG	$5.20\cdot10^{-9}$	**Na**	BS ≠ EG	$4.73\cdot10^{-2}$
	BS ≠ WG	$1.64\cdot10^{-8}$	**Mg**	EG ≠ WG	$4.70\cdot10^{-4}$
	EG ≠ WG	$3.89\cdot10^{-7}$	**Al**	BS ≠ EG	$4.73\cdot10^{-3}$

3.2.2 Sample variations

Aluminium, B, Ba, Co, Cr, Nb, Ni, Pb, Sb, Th, Ti, and W showed higher standard deviations than mean concentrations within the Baltic Sea population, indicating a wide range of sample concentrations. One single sample from the Baltic population is responsible for the high concentrations and standard deviations of Ba, Co, Cr, Nb, Ni, Pb, and Ti, while another sample had high concentrations of Sb. Multiple Baltic samples were responsible for the observed variations in Al, B, Th and W.

Table 2: The results of analysis of variances in mean element concentrations between the three populations of ringed seals (*Phoca hispida*). Bold F-values indicate significant models, while asterisk denotes the degrees of freedom applied.

	$F_{2,22}$-value	p-value		$F_{2,22}$-value	p-value
Trace Elements					
Ag	**6.03**	<0.010	Ti	**6.39**	<0.010
Al	**7.12**	<0.010	Tl*	**37.1***	<0.001
As	**190**	<0.001	U*	**19.9***	<0.001
B	3.43	0.0523	V	**18.4**	<0.001
Ba	0.485	0.623	W*	0.563*	0.579
Cd*	**83.6***	<0.001	**Macro Elements**		
Co	0.717	0.500			
Cr	**21.9**	<0.001	Ca	**8.56**	<0.010
Cs*	**6.28***	<0.010	Fe	**4.96**	<0.050
Cu	**4.54**	<0.050	K	**35.8**	<0.001
Ga	0.150	0.862	Mg	**10.7**	<0.001
Hg*	1.27*	0.306	Na	**3.87**	<0.050
Mn	0.023	0.977	P	**10.7**	<0.001
Mo	**15.9**	<0.001	S	**32.4**	<0.001
Nb	3.19	0.0628	Zn	2.66	0.0942
Ni	0.459	0.639			
Pb*	1.39*	0.276	**Rare Earth Elements**		
Rb	**11.1**	<0.001	Ce*	1.76*	0.201
Sb	**32.2**	<0.001	La	**6.33**	<0.010
Se	1.08	0.367	Nd*	0.720*	0.500
Sn*	**24.8***	<0.001	Pr*	1.48*	0.253
Sr	**4.28**	<0.050	Sm*	0.409*	0.670
Th	**7.47**	<0.010	Y*	0.851*	0.443

* $F_{2,18}$-values

For the East Greenland population the standard deviation was higher than the mean for Ba, caused by one sample with high concentration, and for Cr, due to large variance between samples. For the West Greenland population, Cr, Nb and Ni showed the similar trends of high standard deviations, Cr and Ni influenced by one high concentrated sample, while Nb by a generally large variance between samples.

3.2.3 Hg:Se molar ratio

The molar ratio between Hg and Se (Hg:Se) ranged from 0.189 – 0.91, indicating that all the animals had a molar excess of Se in their liver. There were no significant differences between

populations or sex for the Hg:Se, but there was an overall positive correlation between Hg and Se (r=0.805, p<0.001) (Fig 2).

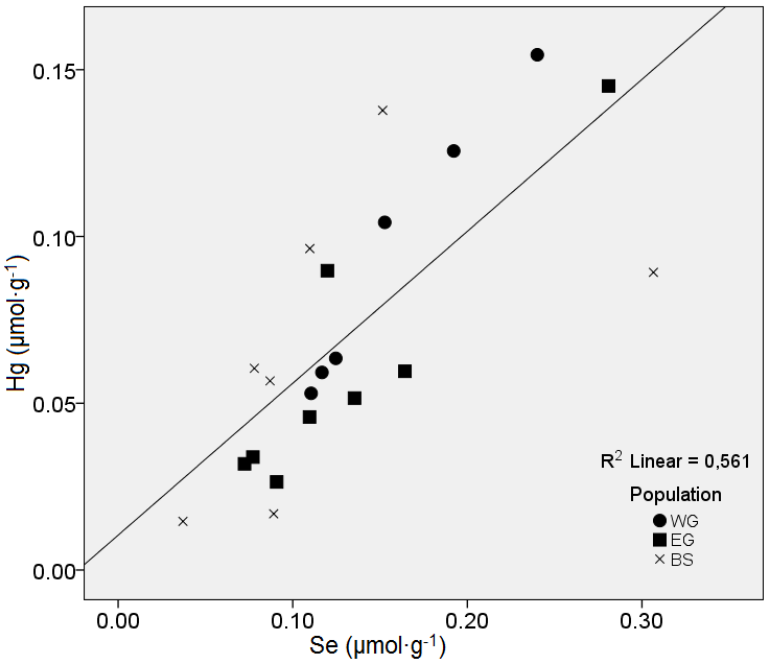

Figure 2: the regression line of the molar ratio between Hg and Se using the pooled results from the three populations; West Greenland (WG, n=6), East Greenland (EG, n=8), and Baltic Sea (BS, n=7).

Hg:Se correlated positively with B, W, Hg, Mg, P, and most of the rare earth elements (p<0.05).

3.2.4 Correlations between chemical elements and stable isotopes

Detailed results from correlation analysis are presented in appendix G.

Several significant correlations were discovered between chemical elements, the strongest included U-Cd-Cr-As-Sb, W-Co-Pb, Mo-Cd, P-Mg, and Se-Hg (p<0.001). All rare earth elements (REE) correlated positively (p<0.01), and except for La-Sm and La-Y, they correlated very strongly (r=0.770, p<0.001). The REE were also correlated with Th (p<0.05). Correlations were also found between the stable isotopes and some chemical elements, the strongest being δ^{13}C-δ^{15}N-S, δ^{13}C-δ^{15}N-Sb, δ^{13}C-δ^{15}N-Sn, δ^{13}C-δ^{15}N-Tl and δ^{13}C-Cd-Mo (p<0.001), while weaker correlations were found for δ^{13}C-δ^{15}N-Sr, δ^{13}C-δ^{15}N-Fe, δ^{13}C-Cu, δ^{13}C-Ti, δ^{15}N-Cd, δ^{15}N-Cr and δ^{15}N-Sr (p<0.01).

3.2.1 Sex differences

There were few indications of differences between the sexes. For the overall sample collection (9 females and 13 males) Mn was the only element with significant difference (t = 2.313, p = 0.031) having higher mean concentrations in females (10.8 ± 1.72 µg·g^{-1}) than in

males (9.06 ± 1.70 µg·g^{-1}). In the population from the Baltic Sea (3 females and 4 males) the only significant sex difference was for V (t = 2.887, p = 0.0278) with higher concentrations in females (0.300 ± 0.105 µg·g^{-1}) than in males (0.148 ± 0.0474 µg·g^{-1}). In the East Greenland population (4 females and 5 males, one missing value) the only difference between sexes was for Al (t = -3.293, p = 0.0132) with higher levels in males (0.394 ± 0.114 µg·g^{-1}) than in females (0.195 ± 0.0376 µg·g^{-1}). The West Greenland population consisted of only two females and four males, limiting the possibilities for statistical comparison. However, when comparing the sexes using only the two females, differences were observed for B (t = 6.701, p = 0.00258), Se (t = 4.448, p = 0.0113), Cd (t = 3.294, p = 0.0301), Hg (t = 3.589, p = 0.0230) and Tl (t = 3.614, p = 0.0225), all of which showed higher concentrations in females than males (0.146 ± 0.0119 versus 0.0742 ± 0.0125 µg·g^{-1} for B; 17.1 ± 2.67 versus 9.96 ± 1.47 µg·g^{-1} for Se; 40.7 ± 4.29 versus 18.3 ± 8.73 µg·g^{-1} for Cd; 28.1 ± 4.09 versus 14.0 ± 4.66 for Hg; and 0.00287 ± 0.000592 versus 0.00143 ± 0.000407 µg·g^{-1} for Tl). Due to low sample size these slight differences have not been discussed further.

3.3 Principal Component Analysis

PCA reveals patterns in the element compositions between and within the three populations of ringed seals.

The analysis resulted in a model (R^2X=0.76, Q^2=0.0275) with five significant principal components (eigenvalues > 1). These five components described 24.2, 23.1, 13.2, 8.87 and 6.73 % of the variability, respectively. All samples were within the Hotellings T2 range seen in the score plot (Fig. 3), though two Baltic samples appear to be borderline cases.

The loading of PCA (Fig. 4) show the aggregation of the stable isotopes and chemical elements such as Cd, Cr, Cu, Mo and U to the left along the PC 1. Bivariate analysis generally confirms correlations between these elements stable isotopes. REE and some of the macro molecules (K, Mg, P and Ca) are oriented along the PC 2, as are Hg:Se and Pb. Age was situated near centrum of the plot along with Mn, indicating that these variables were less important for the model.

The three populations are clearly separated in the score plot primarily by the PC 1, indicating the importance of stable isotopes, and chemical elements such as Cd and Tl.

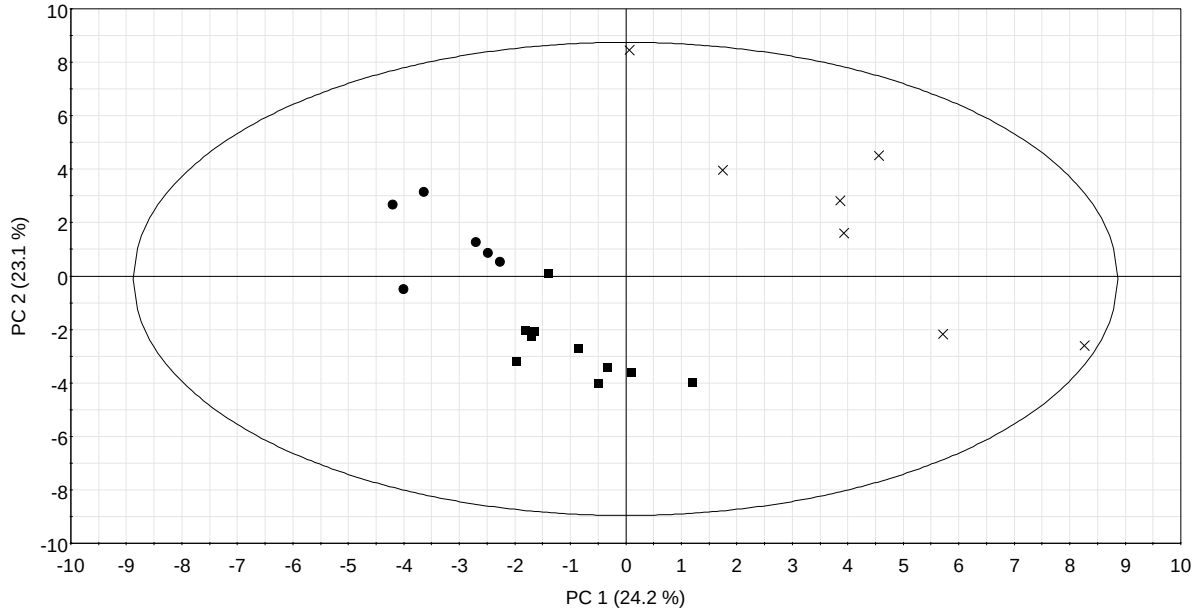

Figure 3: Score plot of ringed seals from the Baltic Sea (×), East Greenland (■) and West Greenland (●). Principal components 1 and 2 are used.

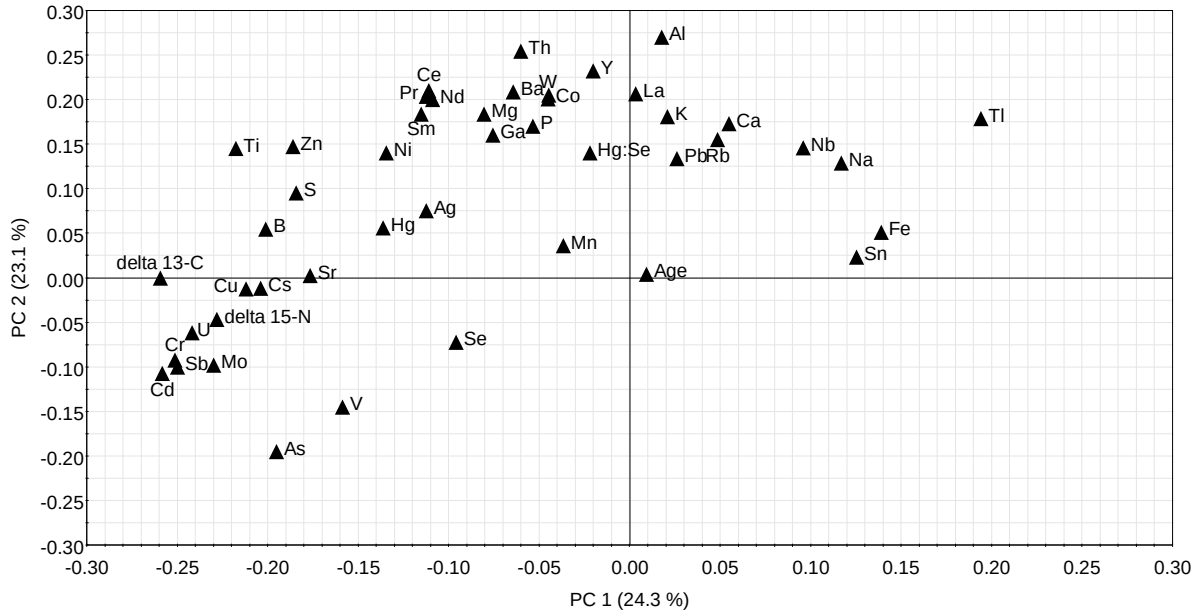

Figure 4: PCA loading plot for principal components 1 and 2. Includes all chemical elements, age and stable isotopes, as well as molar ratio of Hg:Se from liver samples of ringed seals (*Phoca hispida*).

4 Discussion

4.1 Stable isotopes

The ratios of stable isotopes of both N and C were significantly different between all the three populations, and showed the same pattern with lower ratios in the Baltic Sea, intermediate ratios in the East Greenland, and highest in the West Greenland.

The seals from the Baltic Sea had $\delta^{13}C$ ranging from -22.6 ‰ to -21.6 ‰ and $\delta^{15}N$ from 12.9 ‰ to 13.5 ‰. These ranges were similar to findings in other studies on hepatic tissue from Baltic ringed seals. Sinisalo et al. (2008) found $\delta^{13}C$ ranged from -24.2 ‰ to -21.7 ‰, and $\delta^{15}N$ from 12.7 ‰ to 13.9 ‰, while Routti et al. (2005) found a mean $\delta^{15}N$ of 13±1.1 ‰ for ten seals. Both studies used adult seals, and the similar $\delta^{15}N$ levels found in juvenile animals indicate that ringed seals in the Baltic Sea maintain the same trophic level during their lifespan.

The $\delta^{15}N$ found in liver from seals from West Greenland in this study are similar to those found in muscle tissue from ringed seals from the Barrow strait by Atwell et al. (1998) (16.4 ‰), while Hobson et al. (2002) found $\delta^{15}N$ of 17.0±0.1 ‰ and $\delta^{13}C$ levels of -19.4±0.1 ‰ in muscle tissue from seals caught near Qaanaaq. These results for $\delta^{15}N$ are generally similar or slightly higher than those found in liver tissue in this study (16.3±1.07). The $\delta^{13}C$ in this study (-18.4±0.303) are slightly higher than the levels reported in ringed seal muscles by (Hobson et al., 2002).

Relatively few studies have been conducted on stable isotopes in ringed seals from the East Greenland area, and none that examine hepatic tissue have been found. McKinney et al. (2011) reported in their supporting information stable isotope ratios of both N and C in ringed seal muscles from east Greenland, though specific catch sites are not reported. Mean $\delta^{15}N$ was similar to that found in this study, while $\delta^{13}C$ was slightly lower (McKinney et al., 2011). Falk-Petersen et al. (2009) found stable isotope levels of N in muscle tissue from two other seal species, hooded seal (*Cystophora cristata*) and harp seal (*Phoca groenlandica*), in the east Greenland area and Jan Mayen. The ratio found for hooded seals appear similar to those found in ringed seal in this study, while $\delta^{15}N$ in harp seals were slightly lower, though the presentation is graphical rather than numeral. Falk-Petersen et al. (2009) calculated hooded seals to occupy a trophic position of 3.9, and harp seals a position of 3.5. When applying the $\delta^{15}N$ from East Greenland seals in this study to the same model, ringed seals appear to occupy a trophic level intermediate of these two seal species (3.8). Aubail et al. (2010) investigated

teeth from ringed seals in the Ittoqqortoormiit area and found $\delta^{15}N$ similar to that in the present study for the same area, whereas the $\delta^{13}C$ were not within the same range. As previously mentioned, biochemical compositions varies between tissues as well as species (Newsome et al., 2010), and therefore likely affect the stable isotope ratios as well. Thus, and the preceding comparisons between tissues and species are uncertain.

Assuming similar baseline levels for all three populations, the significant variations in $\delta^{15}N$ between the three populations in this study show different trophic positioning in different regions. The enrichment of $\delta^{15}N$ is approximately 3-5 ‰ per trophic level in marine environments (Newsome et al., 2010). This reveals that the three ringed seal populations lie within the same trophic level, even though they are significantly different from each other. Assuming similar baseline levels of stable isotopes for all three populations, the observed $\delta^{15}N$ variations indicate differences in prey preference among the populations. This is further supported by the observed variations in $\delta^{13}C$, as this indicate that seals in the Baltic Sea feed on more pelagic species compared to the two Greenland populations.

As the heavier carbon isotope is slightly enriched for each trophic step, the feeding on prey of higher trophic levels could also partly explain the observed higher $\delta^{13}C$ in the Greenland populations compared to the Baltic Sea population. Nevertheless, $\delta^{13}C$ is only enriched by approximately 1 ‰ per trophic level (Newsome et al., 2010), and as the $\delta^{13}C$ vary by roughly 2 ‰ between each population (Baltic Sea - East Greenland - West Greenland). Thus, additional factors such as the preferred foraging of benthic or pelagic prey are needed to explain the observed variation of $\delta^{13}C$ between the three populations.

4.1.1 Diet

Ringed seals in the Baltic Sea have been reported to feed on both pelagic species such as herring (*Clupea harengus*), whitefish (*Coregonus lavaretus lavaretus*) and vendance (*Coregonus albula*), and benthic species such as ruffe (*Gymnocephalus cernuus*) and fourhorn sculpin (*Myoxocephalus quadricornis*) (Routti et al., 2005; Sinisalo et al., 2008; Sinisalo et al., 2006; Valtonen et al., 2010). Routti et al. (2005) reports that the Baltic ringed seal diet consist mostly of European smelt (*Osmerus eperlanus*), three-spined sickleback (*Gasterosteus aculeatus*), eelpout (*Zoarces viviparus*) and sand goby (*Pomatoschistus minutus*). Of these four species only sand goby is a truly benthic species, the others being pelagic or seasonally pelagic (Sinisalo et al., 2006). Bergman (2007) reported from Söderberg (1975) twelve species of fish and eel, and additional species of molluscs and crustaceans, according to their

per cent occurrence in captured ringed seals. Based on studies of otolith abundance and mollusc/crustacean residues in the gastrointestinal tract, the prey that occurred in most seals were herring and the crustaceans opossum shrimp (*Mysis relicta*) and *Saduria entomon*. Of these, *Saduria entomon* is found in the littoral zone, opossum shrimp in both benthic and pelagic areas, and herring in pelagic areas. A $\delta^{15}N$ of 9.93 ‰ in herring reported by Nfon make the difference to ringed seal in this study and herring 3.17 ‰, which is within the estimated trophic transfer factor of 3-5 ‰.

The diet of ringed seals in Greenland waters were studied by Siegstad et al. (1998). It consists of gadoids such as polar cod and Arctic cod, redfish and capelin, benthic crustaceans, and cephalopods. Regional differences between populations were also observed, and seals from the Avenersuaq area in northwest Greenland were found to forage largely on hyperiid amphipods and polar cod. Seals caught from the King Oscars Fjord area in East Greenland also foraged on Arctic and polar cod, as well as amphipods, but had a larger contribution of Cottidae than the samples from Avenersuaq. Cephalopods were also a minor food source in both populations. The presence of benthic Cottidae in the diet of seals in the East Greenland population may explain the lower $\delta^{13}C$ found for this population compared to the West Greenland population. In addition, according to (Siegstad et al., 1998) the ratios of cod and amphipods vary between the Avenersuaq and King Oscars Fjord areas. In the Northwest the mass of the two cod species are reported to contribute to 86.8 % of the diet and amphipods (*Parathemisto spp*) to 7.8 %, while in the East the cods contribute to 65 %, *Parathemisto* with 4.2 % and Cottidae with 22.4 % of the mass of the diet. These differences in foraging preference may also affect the $\delta^{15}N$ as these preys may occupy different trophic levels themselves. Holst et al. (2001) suspected that Arctic cod may have lower $\delta^{13}C$ values than polar cod. This may explain the difference seen in $\delta^{13}C$ between the East and West Greenland population as described by (Siegstad et al., 1998), since polar cod was reported to be dominant prey in the Avenersuaq population (86.8 %) in Northwest Greenland, while Arctic cod dominated the diet in the King Oscars Fjord population (60.0 %) in East Greenland.

The diet of seals along the northeast coast of Greenland has been further investigated by Labansen et al. (2011). These authors confirmed polar and Arctic cod and *P. libellula* as key prey species in the ringed seal diet, and found occurrence of Cottidae in seal stomach similar to the finding by Siegstad et al. (1998). Some differences between age groups were observed for seals from Ittoqqortoormiit, where juveniles had more residue of invertebrates than adult seals (Labansen et al., 2011).

The domination of polar cod in the diet of ringed seal in the Northwest areas of Greenland as described by Siegstad et al. (1998), was confirmed by Holst et al. (2001). It was reported that polar cod represent 91.8 % of the biomass in the diet of immature ringed seals, and 78.6 % in adult seals, with arctic cod being the second largest biomass contributor (Holst et al., 2001). Juvenile animals were found to feed more on amphipods than adults.

4.2 Chemical elements

4.2.1 Populations differences

Arsenic, Cd and Tl expressed the strongest population differences of all analysed elements ($F>37$, $p<0.001$). The PCA loading plot (Fig. 4) also indicates that these elements contributed to the separations of the three populations observed in the PCA score plot (Fig. 3). Other chemical elements that showed spatial differences were most macro elements (K, S, Mg, P, Ca, Fe and Na), Sb, Sn, and Cu.

Arsenic

Among the chemical elements analysed, As was the only element with significantly different levels between all three populations, with low values in the Baltic Sea population, intermediate in seals from the West Greenland, and highest in the East Greenland population.

Little data were available on As concentrations in the Baltic Sea ringed seals for this study. Concentrations found in blubber samples of juvenile ringed seals from the same region were far higher than those found in this study (Frank et al., 1992). This difference is probably caused by distribution differences between tissues, rather than drastic environmental reductions. The concentrations of As in herring from the Baltic Sea (Larsen and Francesconi, 2003) were higher than As in ringed seals in this study from the same area, as were As levels in livers from Baltic harbour porpoise (Kuenstl et al., 2009).

No data of As levels in ringed seals from East Greenland have been found for direct comparison. However, the As levels found in the East Greenland population in this study are somewhat higher than previous findings in harp seals and hooded seals from the same area (Brunborg et al., 2006; Julshamn and Grahl-Nielsen, 2000), and higher than levels found in ringed seals in other studies (Becker et al., 1997; Kubota et al., 2001). The As in seals from West Greenland in this study are lower than previous findings in ringed seals from the North Water Polynya (Campbell et al., 2005) and similar to levels found in Pangnirtung, Canada (Ebisuda et al., 2002).

Marine mammals that forage on crustaceans and cephalopods in addition to fish showed higher hepatic levels of arsenic than species foraging on fish alone (Kubota et al., 2001). The absence of cephalopods in the Baltic Sea may thus be a factor for the low As concentrations observed in seals from this area.

The higher As levels in the East Greenland population compared to the West Greenland population may reflect the dietary pattern of this population, as cephalopods and crustaceans are stronger contributions in the diet of seals from East Greenland (Siegstad et al., 1998). Marine fish species are known to have higher levels of As than freshwater species, and Larsen and Francesconi (2003) found a correlation between salinity and As levels in flounder, cod and herring. Though the underlying cause of this relationship between salinity and As is not known, it may explain the low levels of As found in the Baltic Sea population.

Similar to many metals, arsenic is known to be transported to the Arctic through atmospheric air movements, and can be found in gaseous form in air when methylated, and otherwise associated with aerosols (Barrie et al., 1992). Such transportation could perhaps add As to the Arctic environment, but as As is generally enriched in the marine environment (Reimann et al., 2009) the terrestrial Arctic environment might suffer more from the effects of transported As. In nature, this potentially toxic metalloid usually occur in trivalent or pentavalent forms (Klaassen, 2008). These speciations are also found in a wide variety of both inorganic and organic compounds and the toxicity of these compounds vary. In marine organisms As is commonly found as arsenobetaine, one of the least toxic As compounds (Ebisuda et al., 2002; Klaassen, 2008). For this reason, marine organisms are generally assumed not to suffer from As toxicity despite having relatively high concentrations of total As (Ebisuda et al., 2002).

Cadmium

The Cd concentrations found in the Greenland and Baltic populations in this study are similar to previous findings in the same areas (Campbell et al., 2005; Fant et al., 2001; Frank et al., 1992; Nyman et al., 2002; Perttila et al., 1986; Riget et al., 2005). Cadmium concentrations were significantly higher in the Greenland populations than in the Baltic Sea population. Though there were no significant differences between the two Greenland populations, the East Greenland population show slightly higher mean concentrations than the West Greenland population. This is in agreement with some previous findings for hepatic tissue from ringed seals from the same areas (Dietz et al., 1998b; Riget et al., 2000).

Several studies have revealed spatial patterns of Cd in biota, including ringed seals, within the Arctic, and many have found relatively high concentrations of Cd in the Greenland or east Canadian areas (Dietz et al., 1996; Muir et al., 1992; Riget et al., 2005; Wagemann et al., 1996). Such spatial patterns are attributed to several factors such as geological conditions and sediment run-offs, diet composition, and trophic level (AMAP, 2005). Pohl et al. (1993) proposed that Cd originating from Russian riverine water were transported by the East Greenland current. Such transportation was further proposed to contribute to observed elevated sediment and aquatic Cd concentrations observed in East Greenland (Pohl et al., 1993). However, Loring and Asmund (1996) found no such spatial differences along the coasts of Greenland in a later study. The lack of any clear spatial pattern of Cd has resulted in diet preferences being proposed as a main factor for those patterns that have been observed in marine mammals along the Greenland coast. The presence of hyperiid amphipods (*Parathemisto libellula*) in the diet may contribute to higher Cd levels as these amphipods are known to accumulate Cd and thus introduce higher Cd levels to the food web (Macdonald and Sprague, 1988). Cephalopods have also been observed to accumulate Cd to greater concentrations than those found in fish (Hamanaka et al., 1982), and presence of cephalopods in the diet of ringed seals in Greenland waters may also contribute to elevated Cd levels. Thus, the absence of *P. libellula* and cephalopods in the Baltic Sea can partially explain the relatively low Cd levels in this ringed seal population. The pattern of high Cd levels in animals from Greenland and significantly lower levels in animals from the Baltic Sea has previously been observed in harbour porpoise (*Phocoena phocoena*) (Szefer et al., 2002).

Accumulation of Cd occurs to a higher degree in renal tissue than hepatic tissue as investigated in this study. Kidneys are more prone to damage caused by elevated Cd levels, and ringed seals and other marine mammals are known to accumulate levels of Cd that would cause damage in many terrestrial animals, including human beings. However, high Cd levels have not been linked to renal damages or skeletal alterations in ringed seals (Sonne-Hansen et al., 2002), and it has been hypothesized that this species have evolved a high tolerance to Cd as a response to naturally high exposures (Dietz et al., 1998a). Cadmium binds to metallothionein (MT), and can also induce further synthesis of this high affinity metal-binding protein (Klaassen, 2008). This biologic response provides limited protection from the harmful effects of Cd. As another possible adaptation, high Cd levels have been found in ringed seal bile which can be associated with higher levels of Cd excretion (Dietz et al., 1998b).

Thallium

Thallium was the only chemical element with significantly higher concentrations in the Baltic Sea population than in the Greenland populations, though the concentrations were low, within the $ng \cdot g^{-1}$ range. Concentrations of Tl is generally low in nature, although it is a widely spread element. It is most often found in sulphur ores of copper, zinc and lead, as well as in coal (DelValls et al., 1999; Peter and Viraraghavan, 2005). The main sources of anthropogenic emissions of Tl are related to mining of these metals and associated industry emissions, as well as burning of coal (Peter and Viraraghavan, 2005). Levels of Tl have been known be high in rivers draining smelter areas, as well as in wastewaters (Peter and Viraraghavan, 2005; Zitko et al., 1975). It also occurs in fairly large concentrations in crookesite, a selenide mineral that is found in e.g. Sweden (DelValls et al., 1999). There are few studies on Tl, despite its high toxicity to mammals. Thallium is easily absorbed through skin and by the GI tract, and has been reported as more acutely toxic to mammals than Hg, Cd, Pb and Cu (Peter and Viraraghavan, 2005). Runoffs from Swedish and Finnish mining industries and the low turnover rate of water in the Baltic Sea, may contribute to the higher Th levels seen in Seals from the Baltic Sea compared to seals in Greenland populations. Few studies have been done on thallium and its toxicity in general, but the concentrations found in this study are similar to levels previously found in Caspian seals (Anan et al., 2002). Thallium also expressed a strong negative correlation with the stable isotopes, both $\delta^{13}C$ ($r= -0.724$, $p<0.001$) and $\delta^{15}N$ ($r= -0.697$, $p<0.001$), indicating that diet is an important factor for the acquired Tl levels.

Macro elements, antimony and tin

Significant spatial differences were also found for all macro elements except Zn. Of these, K, Mg, P, Na and Ca all showed low concentrations in the East Greenland population, and excluding Na they are all positively correlated ($r=0.536$, $p<0.01$). These elements play crucial roles for organisms, their concentrations are high and closely regulated (Hill et al., 2008). Thus, these elements could be expected to have similar concentrations in individuals of the same species. The observed differences are difficult to explain. Except for Na which correlates negatively with $\delta^{15}N$, neither of the mentioned macro elements correlates with any of the stable isotopes. This may indicate that the observed spatial differences are not related to diet, but could also reflect their close regulation.

The opposite may be true for S, Sb, and Sn. Spatial differences were also observed for these chemical elements, and they each strongly correlated with both $\delta^{13}C$ and $\delta^{15}N$ (but not each other). This relationship with the stable isotopes indicates that diet is a possible factor

contributing to the acquired levels of these elements. Sulphur and Sb show positive correlations with the stable isotopes, while Sn is negatively correlated with the stable isotopes. Antimony (Sb) and As belong to the same periodic group and share some chemo-toxicological similarities (Gebel, 1997; Klaassen, 2008), and these two elements also show a positive correlation in this study ($r=0.760$, $p<0.001$), which may reflect similar routes of absorption and behaviour.

For Sn, the spatial differences and negative correlation with the isotopes can be explained by higher Sn pollution in seals food in the Baltic Sea compared to the Greenland Arctic. Common Sn sources are organotin compounds such as tributyltin (TBT), which among other uses, have been used in antifouling paint on ships. Discharge from such paints are largely held responsible for elevated TBT levels in harbours and shipping lanes, even in remote areas such as Northwest Greenland (Strand and Asmund, 2003) and Antarctica (Negri et al., 2004). Because TBT associates with suspended matter and sediments, benthic species are more prone to exposure. Adverse effects such as the induction of imposex, the development of male sex characteristics, have been seen in benthic species such as prosobranch gastropods. Even low concentrations of TBT can cause imposex in these animals, and their sensitivity to this toxicant is severe. Strand et al. (2006) observed this effect in gastropods in waters where TBT concentrations were below the analytical detection limits. The development of imposex can reduce the fecundity of a gastropod population, and presently TBT is found in such concentrations in benthic species in the Baltic Sea that it is recognized as an environmental risk (HELCOM, 2010a).

Few studies have been conducted on the effects of TBT on marine mammals, but reduced immune system has been linked to high TBT levels (Kannan et al., 1998). Harbour porpoises have been the most used marine mammal for TBT studies in the Baltic Sea, but some individuals from different seals species have also been analysed. Tin levels in ringed seals from the Baltic Sea population in this study were intermediate previous findings in a single ringed seal and two grey seals (*Halichoerus grypus*) (Ciesielski et al., 2004) and harbour seals (*Phoca vitulina*) (Strand and Jacobsen, 2005). While there is evidence of bioaccumulation and biomagnification of TBT in harbour porpoise (Strand et al., 2005), biomagnification has not been found for harbour seals (Strand and Jacobsen, 2005).

Copper

Significant differences between Cu concentrations in the Baltic Sea population and the West Greenland population were revealed in this study ($F_{2,22}$ = 4.543, p = 0.0236), with higher concentration in the West Greenland population and lower in the Baltic Sea. The range and mean concentrations of Cu in the Baltic Sea population were similar to previous findings in ringed seals from the Baltic Sea (Frank et al., 1992; Perttila et al., 1986). The West Greenland population had comparable levels to previous findings in adult ringed seals from the Northwater Polynya (Campbell et al., 2005) and Eastern Canadian Arctic (Wagemann et al., 1996). No reports on Cu levels in East Greenland have been found but concentrations are lower than those found in harp seal and hooded seals from the Bering Sea (Sonne et al., 2009).

The significant spatial difference observed for Cu may be related to the differences in diet among the populations. Ritterhoff and Zauke (1997) revealed accumulation of Cu in amphipods and copepods in water with extremely elevated concentrations, and such accumulation may also occur in water with lower Cu concentrations. Amphipods are possible prey species of Greenland ringed seal, and higher exposure to Cu through diet may partly explain the observed differences between populations. Ritterhoff and Zauke (1997) also reported similar patterns of Cd and Cu in amphipods, decapods, and copepods, and there was a positive correlation between these two elements (r = 0.643, p = 0.002) in this study as well. Copper correlates positively with $\delta^{13}C$ in this study (r=0.527, p=0.010) which may indicate that seals that feed more on benthic species are more exposed to Cu. Relatively high concentrations of Cu have been found in marine sediments from certain areas along the Greenland coast (Loring and Asmund, 1996), and benthic species can reflect local Cu levels. In addition, foraging on or near the sea floor may increase Cu exposure due to re-suspension of Cu rich sediments.

Copper is an essential element and important component in several enzymes, and as many essential elements it is potentially toxic at elevated concentrations. Stable Cu levels in tissues and organisms are maintained through control over the excretion of Cu, which occurs mainly through bile in mammals (Klaassen, 2008). Both depleted and elevated Cu concentrations have been associated with cellular susceptibility to oxidative stress (Gaetke and Chow, 2003). Binding of Cu to MT in liver has been proposed as a form of copper storage in addition to reduction of Cu toxicity at elevated levels (Gaetke and Chow, 2003; Klaassen, 2008). The observed Cu levels in ringed seal liver in this study are below the toxicity threshold levels

known for humans (Quakenbush and Citta, 2009), and similar to concentrations normally found in human livers (18-45 µg/g dw) (Gaetke and Chow, 2003). Though threshold levels are not known for ringed seals, the observed levels are likely non-toxic.

No spatial differences between populations were observed for other heavy metals such as Hg, Pb, and Zn.

4.2.2. Toxicity assessment

Zinc

The mean concentration of Zn were slightly higher, and the ranges wider, in this study compared to concentrations found in juvenile ringed seals by Frank et al. (1992) and Perttila et al. (1986) in the Baltic Sea. The West Greenland population had higher Zn concentration but similar range compared to those found in ringed seals from Central West Greenland (further south) by Sonne et al. (2009). Concentrations in ringed seals from the East Greenland were similar to previous findings in seals of comparable age by Dietz et al. (1998b). Zinc is an essential element important to growth and hormone regulation (Klaassen, 2008), and thus crucial in young and growing animals. In addition, Zn is also associated with a healthy immune system. Deficiency in Zn is more common than toxicity of Zn, and can result in impaired immune systems and consequently an increased sensitivity to infections and diseases (Klaassen, 2008).

The lack of spatial difference is consistent with the expectation of similar concentrations of closely regulated elements in individuals of the same species, and Zn is the only macromolecule living up to this expectation in this study.

Lead

The mean concentration of Pb in this study was extremely high compared to the other two populations. However, this concentration was the result of a single extreme value, and no spatial differences were apparent between the populations neither before nor after the removal of this outlier. The observed Pb concentrations within each population are similar to those observed by others in previous studies (Dietz et al., 1996; Fant et al., 2001; Frank et al., 1992; Wagemann et al., 1996).

Lead mimic Ca and Fe and is absorbed largely by the same routes as these essential macro-elements. Absorbed Pb is first distributed to the livers and kidney before redistribution to bone structures and hair, and Pb toxicity is primarily associated with other tissues than liver

(Klaassen, 2008). This distribution pattern complicates the assessment of the toxicity of the observed Pb levels, and trends that are statistically insignificant in liver may be stronger in other tissues.

The Baltic Sea has been polluted by Pb through both atmospheric and riverine transportation (Gusev, 2010; HELCOM PLC-Group, 2010), and concentrations have been found to exceed threshold limits (HELCOM, 2010a). Between 1990 and 2009, atmospheric deposition of Pb to the sea has decreased with 72 % (Gusev, 2010). Reduced levels of Pb have also been observed in livers of fish such as herring, cod (*Gadus morhua callaris*) and perch (*Perca fluviatilis*) over the past few decades (Bignert et al., 2011; Nørrevang Jensen, 2010). The same trend has not been obvious in other marine species in the Baltic Sea, such as blue mussel (*Mytilus edulis*) and eelpout (Rüdel et al., 2010). These trends may indicate that ringed seals in the Baltic Sea are less exposed to Pb than previously.

Lead pollution has also reach the Arctic, and cores of ice from the Greenland ice sheet have revealed increased Pb deposition in snow from the industrial revolution in the 1800's to the 1960s (Candelone et al., 1995). A total increase of 300 times from the Pb concentrations in prehistoric times have been estimated (Barrie et al., 1992). The increased atmospheric Pb concentrations have been attributed to the use of leaded gasoline, especially after World War II (Candelone et al., 1995). Other sources for atmospheric Pb are smelters and smokes from manufacturing industries (Barrie et al., 1992). Deposition of lead to marine waters and riverine transportation of Pb in melt water during spring and summer may have resulted in elevated Pb concentrations in marine environments. Mining activity in Greenland have also resulted in monitoring of Pb levels in marine organism in adjacent areas, and levels were found to be high in fish, prawns and blue mussel (Johansen et al., 1991). Comparisons of present day biota samples to prehistoric samples have indicated a considerable Pb increase in marine organisms (Barrie et al., 1992). This could also be true for ringed seals, and can contribute to the similar Pb levels in the three studied populations.

Mercury

Mercury concentrations in seals from the Baltic Sea in this study were lower than in other studies done on ringed seals from the Baltic Sea (Fant et al., 2001; Kari and Kauranen, 1978; Nyman et al., 2002; Perttila et al., 1986), and the range was similar to those observed by Frank et al. (1992). With the exception of the study done by Frank et al. (1992) seals in these studies were older, and age related accumulations can account for the previously observed

higher levels. The concentration of Hg in the East Greenland population is similar those observed previously in juvenile and subadult ringed seals in the same area, and generally lower than concentrations reported for adult animals from this area (Dietz et al., 1996; Riget et al., 2005). Seals from the West Greenland population had higher Hg concentrations compared to those found in seals of similar age in the same area. However, the concentrations found in seals in this study were in the same range as those measured for seals of all age groups (Campbell et al., 2005; Dietz et al., 1996; Riget et al., 2005). Higher mean concentrations have previously been found in seals from East Greenland than in seals from West Greenland (Dietz et al., 1998b), in contrast to the findings in this study.

Mercury is of environmental concern as it is a very toxic heavy metal, even at very low concentrations (HELCOM, 2010a). It can bind to both inorganic and organic compounds, and also occur in its metallic state. Thus, several routes of absorption are available for Hg, and the various speciations have different targets of toxicity (Klaassen, 2008). Mercury is also known to biomagnify in food webs, causing animals of higher trophic levels to accumulate higher concentrations of Hg compared to concentrations in their food (AMAP, 2005). Thus, a significant positive relationship between trophic level and Hg within an ecosystem has been observed in several previous studies (Campbell et al., 2005; Nfon et al., 2009; Rüdel et al., 2010). Logically, this same trend has also been observed in studies where $\delta^{15}N$ describe the trophic levels (Atwell et al., 1998; Jaeger et al., 2009). In this study, different ecosystems are compared and the same trend cannot be expected. Yet, the Baltic ringed seals accumulate comparable levels of Hg to the Greenland seals, despite having a lower trophic positioning. This may indicate higher Hg loads per trophic level in the Baltic Sea food web compared to the food web in Greenland. Considering the level of Hg pollution in the Baltic Sea this is not unlikely.

The transportation of Hg to the Arctic has increased during the 20^{th} century as anthropogenic Hg emissions of this element increased. This has resulted in higher Hg exposure for Arctic organisms. Tissues from several animals species in the Arctic, including ringed seals, have been observed to reflect this increase of Hg, and the trend has been more pronounced in the Greenland and Canadian Arctic compared to other Arctic areas (AMAP, 2011; Riget et al., 2007). Comparable exposure levels would explain the similar Hg concentrations observed between the three populations. In addition, as Hg accumulates with age in ringed seals (Smith and Armstrong, 1978) and as this study was based on juvenile animals, it is possible that these

animals have not yet accumulated Hg levels representative for each populations environmental load.

Selenium

Among the variables analysed the only variable that correlated significantly with age was Se ($r=0.521$, $p=0.011$). This relationship of Se concentrations increasing with age have been observed in several marine mammals, including ringed seal (Hansen et al., 1990; Mackey et al., 2003; Smith and Armstrong, 1978), and appear to be very sensitive considering the relatively short age interval among the sampled seals in this study. The Se levels were lower than previous finding in seals from the Bothnian Bay (Fant et al., 2001; Kari and Kauranen, 1978) but lack of age data in these studies makes the comparisons uncertain as the higher Se levels may be a result of accumulation with age. Perttila et al. (1986) also found higher Se levels in ringed seals of varying age from the Gulf of Finland. The range and mean concentrations of Se slightly exceeded those observed in juveniles in Swedish waters (Frank et al., 1992), and the Se levels from the Greenland populations are similar to those found in adult ringed seals from the same areas (Dietz et al., 1998b) (assuming misspelling of µg as mg).

Selenium is an essential element important for antioxidant systems as well as hormone homeostasis. In addition, it can form complexes with several metals such as Hg, As, Cu, and Cd, and thus affect the toxic effect of these metals as well eliminating its own potential toxicity. Chronic exposure of high Se concentrations can have both neurological and dermal effects, while Se deficiency can cause increased susceptibility to infections and cancer (Klaassen, 2008). Recently, correlations between Ag and Se have been found in liver tissues from other marine mammals (Ikemoto et al., 2004), and in this study Ag was the only other element besides Hg that positively correlated with Se ($r = 0.434$, $p = 0.039$). This indicate that Se also interact with Ag in ringed seals.

No spatial differences were found for Se in this study. As for other essential elements Se concentrations are regulated, and the lack of spatial variation would be explained by similar individual biological requirements.

4.2.3 Detoxification of mercury

Hg:Se

Ringed seals are exposed to Hg primarily through diet. In fish methyl mercury are accumulated on muscles where it is the dominating Hg compound (Cappon and Smith, 1981)

and ringed seals are thus exposed to fairly high levels of this highly toxic compound. Contrary to its prey, ringed seals accumulate Hg mainly in the liver. In this tissue there is a preponderance of inorganic Hg, indicating the occurrence of demethylating processes (Koeman et al., 1975; Smith and Armstrong, 1978). Several studies have found strong relationships between Hg and Se in marine mammal tissues (Kari and Kauranen, 1978; Koeman et al., 1975; Perttila et al., 1986), and a molar ratio of 1:1 between these elements are often found in marine mammals (Ikemoto et al., 2004; Nigro and Leonzio, 1996). A strong correlation between Hg and Se was also found in this study ($r=0.805$, $p<0.001$), but the molar ratio of Hg and Se varied from 0.189-0.91 and indicated and excess of Se in liver. The young age of the sampled animals may be a factor for the lack of a stronger molar ratio. As Hg accumulates with age, the molar ratio may be more equimolar in adults. Hg correlated with Hg:Se ($r=0.681$, $p<0.001$), while Se did not. This may indicate increased formation of Hg-Se complexes with higher Hg concentrations in the presence of stable (or closely regulated) Se concentrations.

The presence of Se in 1:1 molar ratio with Hg has been credited for the lack of Hg toxicity in marine mammals, even at high Hg concentrations. Selenium and Hg have been known to have detoxifying effects on each other since the 1960's, and several potential mechanisms for this effect have been proposed (Cuvin-Aralar and Furness, 1991). The observed 1:1 molar ratios of Hg and Se have spurred the hypothesis of formation of Hg-Se complexes, and both organic and inorganic complexes have been proposed (Cuvin-Aralar and Furness, 1991; Nigro and Leonzio, 1996). Evidence of the formation of mercury selenide (tiemannite) have been found in many cetaceans and also in pinnipeds. However, other mechanism may also contribute to the detoxification of Hg by Se (Nigro and Leonzio, 1996).

Other mechanisms for the excretion and detoxification of high Hg levels have been found for seals. For instance, the accumulation of Hg in fur offers an annual opportunity for the excretion of Hg and other heavy metals during moult (Wenzel et al., 1993). Detoxification of Hg can also occur through binding to MT, and these additional mechanisms may contribute to the lower Hg:Se ratios observed in this study (Nigro and Leonzio, 1996).

5 Conclusion

The present study reports concentrations for a wide range of chemical elements from three distinct ringed seal populations. Samples of liver tissue were collected from juvenile seals from West Greenland, East Greenland and the Baltic Sea. Differences in chemical element concentrations between these populations were investigated, and revealed spatial trends for several elements. Generally, differences were found between the Baltic Sea population and one or both of the Greenland populations. Chemical elements known to be of environmental concern in the Baltic Sea (e.g. Pb and Hg) did not show any spatial difference between the three populations. This is unexpected and contradicts the set hypothesis, but is comprehensible when combined with observed trophic trends, though the relatively young age of the animals studied may also conceal spatial trends of metals that accumulate with age and thus would be apparent in adult animals. Stable isotope ratios of C and N were used to examine dietary variations between the populations. The hepatic ratios of $\delta^{13}C$ and $\delta^{15}N$ expressed spatial differences between all three populations indicating substantial variation of dietary composition between Baltic seals and seals from Greenland. In addition, differences were seen between East and West Greenland seals as well. Large ecosystem variations can account for the difference seen between the Baltic Sea population and the Greenland populations. The two Greenland populations inhabit comparable ecosystems consisting of many of the same species. Thus, the observed variances are suggested to be caused by different dietary compositions of the same prey species. The Baltic ringed seal population was found to occupy a lower trophic level, and to have a stronger preference for pelagic prey, than the ringed seal populations from Greenland. These factors can contribute to the Baltic ringed seal retaining hepatic concentrations of possibly harmful chemical elements within ranges similar to those observed in less polluted areas.

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Appendix A – Biometric data

Table A: Biometric data of the sample collection, including sex, age, found date, and individual identifictation.

Population	ID	Found	Sex	Age (years)
Baltic Sea	42	01.10.2009	F	2
	126	16.05.2008	F	1
	141	05.06.2008	F	1
	147	30.05.2008	M	1
	171	16.06.2008	M	3
	176	28.05.2008	M	3
	186	28.05.2008	M	2
East Greenland	203	05.04.2008	M	1
	204	03.04.2008	F	1
	205	05.04.2008	F	1
	206	05.04.2008		2
	207	05.04.2008	M	3
	208	10.04.2008	F	2
	209	11.04.2008	M	2
	210	10.04.2008	M	3
	211	09.04.2008	F	1
	212	11.04.2008	M	3
West Greenland	195	22.05.2008	M	3
	197	22.05.2008	M	1
	198	22.05.2008	F	3
	200	23.05.2008	M	2
	201	22.05.2008	M	1
	202	23.05.2008	F	2

Appendix B - Reference material

Table B: Reference material of Scallop GBW10024 was used to determine the accuratcy of the analysis of liver samples by the HR-ICP-MS. Certified values for the elements are listed as well as the per cent accuracy of measured mean concentrations. All values are given in $\mu g \cdot g^{-1}$.

Chemical element	Isotope	Mean±standard deviation	Certified value	Accuracy (%)
Ag	109	0.00564±0.00211	nc	
Al	27	72.4±18.8	156	46.4
As	75	3.95±0.334	3.6	110
B	11	14.3±2.24	12	119
Ba	137	0.531±0.0542	0.62	85.7
Ca	43	700±74.5	750	93.3
Cd	114	1.30±0.0830	1.06	123
Ce	140	0.0458±0.00770	0.053	86.4
Co	59	0.0435±0.00182	0.047	92.5
Cr	52	0.132±0.0209	0.28	47.3
Cs	113	0.0128±0.000319	0.014	91.3
Cu	63	1.25±0.0546	1.34	93.0
Fe	57	31.3±1.62	41	76.5
Ga	69	0.0166±0.00381	nc	
Hg	202	0.0510±0.00793	0.04	128
K	39	20200±7140	11500	176
La	139	0.0270±0.00450	0.037	73.1
Mg	25	1790±126	1740	103
Mn	55	19.5±0.747	19.2	101.
Mo	98	0.0536±0.00326	0.066	81.1
Na	23	4800±431	4600	105
Nb	93	0.00272±0.000557	nc	
Nd	146	0.0190±0.00262	0.025	76.1
Ni	60	0.124±0.0141	0.29	42.8
P	31	8570±1020	8800	97.4
Pb	208	0.0736±0.00220	0.12	61.3
Pr	141	0.00512±0.000797	0.006	85.4
Rb	85	5.47±0.249	5.1	107

…table B continues…

Chemical element	Isotope	Mean±standard deviation	Certified value	Accuracy (%)
S	34	17400±1560	15000	116
Sb	121	0.00275±0.000414	0.014	19.6
Se	82	1.56±0.123	1.5	104
Se	78	1.55±0.132	1.5	103
Sm	147	0.00385±0.000570	0.0048	80.2
Sn	118	0.119±0.00669	0.13	91.2
Sr	88	5.99±0.323	6.5	92.1
Th	232	0.00752±0.00109	0.012	62.7
Ti	47	0.738±0.107	6	12.3
Tl	205	0.00233±0.000159	0.0025	93.2
U	238	0.00507±0.000337	0.0073	69.5
V	51	0.239±0.0155	0.36	66.4
W	182	0.00131±0.000676	nc	
Y	89	0.0829±0.00593	0.107	77.5
Zn	67	81.6±6.91	75	109

Appendix C – Method detection limits

Table C: Method detection limits in $\mu g \cdot g^{-1}$, found by comparing instrument detection limit and 3 x standard deviation of blank samples, and choosing the highest value.

Element	Isotope	Resolution	MDL	Element	Isotope	Resolution	MDL
Ag	109	MR	$3 \cdot 10^{-3}$	**Nb**	93	HR	$1.4006 \cdot 10^{-5}$
Al	27	MR	$3 \cdot 10^{-2}$	**Nd**	146	LR	$1.2 \cdot 10^{-3}$
As	75	HR	$3.75 \cdot 10^{-3}$	**Ni**	60	MR	$2.2703 \cdot 10^{-3}$
B	11	Lr	$1.2 \cdot 10^{-2}$	**P**	31	MR	0.48841008
Ba	137	MR	$2.6092 \cdot 10^{-3}$	**Pb**	208	LR	$5.5538 \cdot 10^{-4}$
Ca	43	MR	1.5	**Pr**	141	LR	$4.5 \cdot 10^{-5}$
Cd	114	LR	$3 \cdot 10^{-4}$	**Rb**	85	MR	$4.2501 \cdot 10^{-3}$
Ce	140	LR	$3 \cdot 10^{-5}$	**S**	34	MR	3.0739296
Co	59	MR	$1.0296 \cdot 10^{-3}$	**Sb**	121	MR	$4.3381 \cdot 10^{-4}$
Cr	52	MR	$1.5953 \cdot 10^{-3}$	**Se**	82	LR	$1.0563 \cdot 10^{-2}$
Cs	133	LR	$2.7806 \cdot 10^{-4}$	**Sm**	147	LR	$7.5 \cdot 10^{-5}$
Cu	63	MR	$9.1061 \cdot 10^{-3}$	**Sn**	118	LR	$5.2362 \cdot 10^{-4}$
Fe	57	MR	1.92199	**Sr**	88	MR	$3.75 \cdot 10^{-3}$
Ga	69	MR	$1.05 \cdot 10^{-3}$	**Th**	232	LR	$7.5 \cdot 10^{-5}$
Hg	202	LR	$5.5506 \cdot 10^{-2}$	**Ti**	47	MR	$8.1040 \cdot 10^{-3}$
K	39	HR	0.98780712	**Tl**	205	LR	$5.9674 \cdot 10^{-5}$
La	139	MR	$3. \cdot 10^{-4}$	**U**	238	LR	$6.2753 \cdot 10^{-5}$
Mg	25	MR	$6.5971 \cdot 10^{-2}$	**V**	51	MR	$6.9546 \cdot 10^{-4}$
Mn	55	MR	$2.3708 \cdot 10^{-2}$	**W**	182	LR	$1.5 \cdot 10^{-4}$
Mo	98	MR	$3 \cdot 10^{-3}$	**Y**	89	LR	$6 \cdot 10^{-5}$
Na	23	MR	1.5	**Zn**	66	MR	$5.8828 \cdot 10^{-3}$

Appendix D – Mean concentrations, median and ranges for macronutrients and rare earth metals

Table D3: Mean concentrations and standard deviations, median and ranges of the macronutrients within each of the three populations. All data are given in $\mu g \cdot g^{-1}$ dry weight

		Ca	Fe	K	Mg	Na	P	S	Zn
BS	**Mean±SD**	162±46.3	1370±608	11400±748	654±42.6	3520±491	9400±554	8590±493	141±71.0
	Median	156	1050	11700	660	3500	9600	8700	121
	Range	108-242	791-2380	10200-12200	589-703	2970-4270	8180-9790	7530-9040	82.8-296
	n	7	7	7	7	7	7	7	7
EG	**Mean±SD**	116±11.9	1020±516	7820±1350	598±64.7	2910±564	8270±929	8600±529	134±22.3
	Median	111	979	8070	598	2890	8430	8600	136
	Range	100-134	346-1720	6000-10000	464-677	1860-3620	6630-9530	7810-9260	93.3-160
	n	10	10	10	10	10	10	10	10
WG	**Mean±SD**	160±24.3	558±239	13100±1100	734±36.0	2860±125	10400±990	11000±779	174±15.6
	Median	150	676	13100	727	2830	9980	10800	173
	Range	139-205	252-752	11300-14600	703-804	2740-3100	9500-12200	10200-12300	157-197
	n	6	6	6	6	6	6	6	6

Table D4: Mean concentrations ± standard deviation (SD), median and ranges of rare earth elements within the three ringed seal populations. All values are given in $\mu g \cdot g^{-1}$. Due to analytical errors the n varies within the East Greenland population.

		Y	Ce	Pr	Nd	Sm	La
BS	**Mean±SD**	0.00478±0.00316	0.0536±0.0447	0.00529±0.00453	0.0208±0.0176	0.00275±0.00212	0.0371±0.0273
	Median	0.00468	0.0498	0.00475	0.0197	0.00274	0.0293
	Range	0.000657-0.00858	0.00950-0.138	0.000953-0.0142	0.00338-0.0547	0.000521-0.00661	0.0171-0.0969
	n	7	7	7	7	7	7
EG	**Mean±SD**	0.00258±0.00173	0.0336±0.0197	0.00352±0.00231	0.0147±0.0101	0.00228±0.00148	0.0169±0.00865
	Median	0.00213	0.0308	0.00286	0.0123	0.00174	0.0158
	Range	0.00116-0.00673	0.0118-0.0757	0.00123-0.00871	0.00518-0.0381	0.00108-0.00577	0.00635-0.0370
	n	8	8	8	8	8	10
WG	**Mean±SD**	0.00245±0.000807	0.0642±0.0311	0.00618±0.00290	0.0215±0.0103	0.00285±0.00124	0.0356±0.0174
	Median	0.00253	0.0609	0.00533	0.0179	0.00243	0.0316
	Range	0.00133-0.00341	0.0366-0.122	0.00373-0.0116	0.0124-0.0407	0.00176-0.00507	0.0213-0.0682
	n	6	6	6	6	6	6

Appendix E – Mean concentrations, median and rages for chemical elements

Table E1: The mean ± standard deviation (SD), and ranges of trace metals in the ringed seal populations. All values are given in $\mu g \cdot g^{-1}$ dry weight.

		Ag	Al	As	Cd	Cr	Cu	Hg
BS	**Mean±SD**	0.309±0.273	3.72±5.76	0.249±0.0517	0.638±0.501	0.0215±0.0455	16.0±10.7	13.5±8.89
	Median	0.201	0.901	0.232	0.624	0.00444	10.5	12.1
	Range	0.0817-0.761	0.268-15.7	0.190-0.320	0.110-1.57	0.000798-0.124	8.08-37.2	2.93-27.6
	n	7	7	7	7	7	7	7
EG	**Mean±SD**	0.183±0.0944	0.309±0.127	5.72±0.978	34.9±14.7	0.733±0.846	28.2±12.2	12.14±7.95
	Median	0.172	0.290	5.55	32.3	0.407	26.9	9.77
	Range	0.0512-0.329	0.151-0.507	4.09-7.40	17.2-60.1	0.0554-2.68	12.8-52.1	5.30-29.1
	n	10	10	10	8	10	10	8
WG	**Mean±SD**	0.672±0.470	1.03±0.691	1.71±1.15	25.7±13.5	0.191±0.195	38.3±28.5	18.7±8.31
	Median	0.612	0.803	1.42	24.7	0.125	33.5	16.8
	Range	0.252-1.54	0.445-2.30	0.773-3.95	8.04-43.7	0.0484-0.574	13.2-93.8	10.6-31.0
	n	6	6	6	6	6	6	6

		Mn	Mo	Rb	Se	Sn	Sr	V
BS	**Mean±SD**	9.74±2.30	1.27±0.228	7.30±1.17	9.69±6.95	0.195±0.120	0.173±0.125	0.209±0.110
	Median	11.0	1.22	7.73	7.03	0.249	0.137	0.179
	Range	7.16-12.7	1.03-1.75	5.48-8.65	2.93-24.2	0.0483-0.346	0.0657-0.444	0.0801-0.369
	n	7	7	7	7	7	7	7
EG	**Mean±SD**	9.55±2.03	2.05±0.322	5.10±1.13	10.9±4.88	0.129±0.0484	0.227±0.0472	0.614±0.215
	Median	10.2	2.05	5.07	10.1	.0118	0.226	0.587
	Range	6.38-11.8	1.49-2.57	3.68-6.62	5.72-22.2	0.0728-0.215	0.165-0.331	0.295-1.11
	n	10	10	10	10	8	10	10
WG	**Mean±SD**	9.65±1.65	2.36±0.800	7.24±0.844	12.3±4.02	0.0230±0.00724	0.316±0.186	0.289±0.0642
	Median	9.37	2.11	7.09	10.9	0.0254	0.257	0.286
	Range	7.94-12.6	1.68-3.84	6.26-8.34	8.72-19.0	0.0135-0.0305	0.182-0.684	0.0199-0.378
	n	6	6	6	6	6	6	6

Table E2: The mean ± standard deviation (SD), and ranges of trace metals in the ringed seal populations for low concentration elements. All values are given in $ng \cdot g^{-1}$ dry weight

		B*	Ba*	Co*	Cs*	Ga*	Nb*	Ni*
BS	**Mean±SD**	59.0±118	182±443	98.6±127	37.5±17.9	1.84±1.12	0.325±0.422	63.3±135
	Median	16.3	9.15	54.9	46.0	1.34	0.169	11.9
	Range	6.00-325	130-1190	27.7-385	9.79-52.5	1.06-4.26	0.0980-1.27	7.93-370
	n	7	7	7	7	7	7	7
EG	**Mean±SD**	92.1±60.8	13.4±16.1	50.0±9.19	70.1±14.4	1.68±0.485	0.0815±0.0788	20.2±7.56
	Median	99.1	6.42	50.1	67.6	1.62	0.0609	17.8
	Range	6.00-185	3.50-56.4	36.2-66.3	56.2-100	1.08-2.69	0.00700-0.267	12.9-31.9
	n	10	10	10	8	10	10	10
WG	**Mean±SD**	98.1±38.5	16.9±12.1	52.7±7.62	61.7±18.2	1.56±0.606	0.132±0.177	46.7±61.5
	Median	84.5	13.8	51.5	60.8	1.35	0.0313	26.2
	Range	60.7-154	5.63-38.1	44.5-64.5	41.6-92.0	1.20-2.79	0.00700-0.378	9.73-171
	n	6	6	6	6	6	6	6

		Pb*	Sb*	Th*	Ti*	Tl*	U*	W*
BS	**Mean±SD**	282±637	6.41±7.82	0.353±0.464	58.8±89.1	18.8±12.2	0.210±0.155	15.7±39.8
	Median	33.7	3.37	0.141	26.1	18.2	0.194	0.645
	Range	6.51-1720	1.16-23.7	0.0375-1.22	15.0-260	5.13-38.4	0.0314-0.423	0.0750-106
	n	7	7	7	7	7	7	7
EG	**Mean±SD**	40.6±12.2	33.9±9.81	0.0540±0.0272	48.7±25.6	1.80±0.636	1.21±0.517	0.523±0.132
	Median	44.1	35.4	0.0375	39.5	1.74	1.05	0.531
	Range	20.5-51.7	17.4-46.5	0.0375-0.103	30.4-117	1.08-2.81	0.729-2.33	0.217-0.604
	n	8	10	10	10	8	8	8
WG	**Mean±SD**	20.7±3.63	39.7±12.3	0.320±0.249	137±87.6	1.91±0.850	0.682±0.226	0.397±0.172
	Median	21.3	41.0	0.241	108	1.75	0.719	0.366
	Range	16.5-25.0	22.4-53.0	0.0841-0.798	55.9-294	1.05-3.29	0.406-0.974	0.217-0.604
	n	6	6	6	6	6	6	6

Appendix F – Mean concentrations, median and ranges for stable isotopes, age and molar Hg:Se

Table F1: Mean concentrations ± standard deviation (SD) and rages for additional variables.

		Age	d 13C	d 15N	HgSe
BS	**Mean±SD**	2.11±0.957	–22.2±0.341	13.1±0.223	0.584±0.292
	Median	2.16	-22.2	13.1	0.652
	Range	1.13-3.21	–22.6- –21.6	12.9-13.5	0.189-0.910
	n	7	7	7	7
EG	**Mean±SD**	1.9±0.880	–20.6±0.283	14.5±0.649	0.361±0.222
	Median	2.02	-20.6	14.4	0.400
	Range	1.01-3.03	–21.1- –20.3	13.7-15.7	0.000879-0.749
	n	10	10	10	10
WG	**Mean±SD**	2.15±0.894	–18.4±0.303	16.3±1.07	0.579±0.0899
	Median	2.15	-18.5	16.6	0.576
	Range	1.15-3.15	–18.7- –17.9	14.1-17.1	0.479-0.683
	n	6	6	6	6

Appendix G – Pearsons' correlations

Pearsons' correlations for all variables in liver (log transformed data). Note that for the chemical elements Y, Cd, Sn, Cs, Ce, Pr, Nd, Sm, W, Hg, Tl, Pb and U n=21. For all other variables n=23.

	Age	**$\delta^{13}C$**	**$\delta^{15}N$**	**B**	**Se**	**Zn**	**Ca**	**Y**	**Cd**	**Mo**	**Sn**	**Cs**	**Ce**	**Pr**	**Nd**	**Sm**	**W**	**Hg**	**Tl**	**Pb**	**Th**	**U**	**Na**
Age					0.521																		
$\delta^{13}C$			**0.845**	0.518					**0.736**	**0.700**	**-0.759**	0.499							**-0.697**			0.544	
$\delta^{15}N$		**0.845**							**0.613**	0.456	**-0.749**	0.439							**-0.724**				-0.470
B		**0.518**				0.422			**0.811**	0.523		**0.703**					0.454		-0.453			**0.808**	
Se	0.521						-0.472			0.449								**0.805**					-0.506
Zn				0.422													**0.587**				0.507		
Ca					-0.472														0.467		**0.546**		**0.575**
Y													**0.806**	**0.814**	**0.853**	**0.860**	**0.601**				**0.560**		
Cd		**0.736**	**0.613**	**0.811**						**0.823**		**0.741**							**-0.764**			**0.925**	
Mo		**0.700**	0.456	0.523	0.449				**0.823**			0.435							**-0.637**			**0.721**	
Sn		**-0.759**	**-0.749**																0.516	0.437			0.466
Cs		0.499	0.439	**0.703**					**0.741**	0.435												**0.750**	
Ce								**0.806**						**0.994**	**0.982**	**0.956**					**0.579**		
Pr								**0.814**					**0.994**		**0.992**	**0.971**					0.533		
Nd								**0.853**					**0.982**	**0.992**		**0.989**					0.493		
Sm								**0.860**					**0.956**	**0.971**	**0.989**						0.457		
W						**0.587**		**0.601**												**0.823**	0.493		
Hg					**0.805**																		
Tl		**-0.697**	**-0.724**	-0.453			0.467		**-0.764**	**-0.637**	0.516											**-0.587**	**0.625**
Pb											0.437						**0.823**						
Th						0.507	**0.546**	**0.560**					**0.579**	0.533	0.493	0.457	0.493						
U		0.544		**0.808**					**0.925**	**0.721**		**0.750**							**-0.587**				
Na			-0.470		-0.506		**0.575**				0.466								**0.625**				

	Mg	Al	P	S	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Ga	Rb	Sr	Ag	Sb	Ba	La	K	As	Nb	Hg:Se
Age																							
δ13C				**0.756**	**0.637**		0.525		**-0.564**			0.527			**0.544**		**0.691**				0.435		
δ15N				**0.659**	0.429		**0.543**		**-0.653**						**0.562**	0.450	**0.677**				0.445	-0.509	
B					**0.623**		**0.619**				0.454		**0.559**				0.505	0.439			0.434		0.499
Se																0.434					0.187		
Zn	0.525	0.451	0.462	**0.617**	**0.724**			0.486		**0.730**	**0.686**	0.466	0.441			**0.539**		0.520					
Ca	**0.588**	0.510	**0.536**			-0.494								0.477						**0.645**	**-0.564**		
Y		**0.633**								**0.573**			**0.595**					0.545	**0.602**				**0.565**
Cd					0.501	**0.709**	**0.889**					**0.643**		-0.445	**0.554**		**0.879**				**0.873**	-0.466	
Mo				0.492	0.419	**0.534**	**0.641**					**0.700**					**0.643**				**0.639**		
Sn				**-0.686**					**0.623**							-0.479							
Cs						**0.559**	**0.753**								**0.594**		**0.716**				**0.664**		
Ce		0.465			0.460														**0.824**				**0.574**
Pr					0.449														**0.810**				**0.587**
Nd																			**0.771**				**0.578**
Sm																			**0.696**				0.547
W		**0.635**			0.470					**0.895**	**0.655**		**0.695**					**0.826**					0.463
Hg					0.462											0.523							**0.681**
Tl		**0.605**				-0.551	**-0.627**							0.491			**-0.708**				**-0.773**	0.544	
Pb									0.473	**0.722**	0.512		**0.586**					**0.783**				0.455	
Th	0.507	**0.861**	0.486	0.415	**0.557**					**0.565**			0.510	0.475				**0.607**	0.483	0.488	-0.428		
U					0.455	**0.794**	**0.896**					**0.637**		-0.485	0.535		**0.804**			-0.455	**0.809**		
Na									0.484												-0.439		**0.606**

	Age	d13 C	d15 N	B	Se	Zn	Ca	Y	Cd	Mo	Sn	Cs	Ce	Pr	Nd	Sm	W	Hg	Tl	Pb	Th	U	Na
Mg						0.525	**0.588**														0.507		
Al						0.451	0.510	**0.633**					0.465				**0.635**		**0.605**		**0.861**		
P						0.462	**0.536**														0.486		
S		**0.756**	**0.659**			**0.617**				0.492	**-0.686**										0.415		
Ti		**0.637**	0.429	**0.623**		**0.724**			0.501	0.419			0.460	0.449			0.470	0.462			**0.557**	0.455	
V							-0.494		**0.709**	**0.534**		**0.559**							-0.551			**0.794**	
Cr		0.525	**0.543**	**0.619**					**0.889**	**0.641**		**0.753**							**-0.627**			**0.896**	
Mn						0.486																	
Fe		**-0.564**	**-0.653**								**0.623**									0.473			0.484
Co						**0.730**		**0.573**									**0.895**			**0.722**	**0.565**		
Ni				0.454		**0.686**											**0.655**			0.512			
Cu		0.527				0.466			**0.643**	**0.700**												**0.637**	
Ga				**0.559**		0.441		**0.595**									**0.695**			**0.586**	0.510		
Rb							0.477		-0.445										0.491		0.475	-0.485	
Sr		**0.544**	**0.562**						**0.554**			**0.594**										0.535	
Ag			0.450		0.434	**0.539**					-0.479							0.523					
Sb		**0.691**	**0.677**	0.505					**0.879**	**0.643**		**0.716**							**-0.708**			**0.804**	
Ba				0.439		0.520		0.545									**0.826**			**0.783**	**0.607**		
La								**0.602**					**0.824**	**0.810**	**0.771**	**0.696**					0.483		
K							**0.645**														0.488	-0.455	
As		0.435	0.445	0.434			**-0.564**		**0.873**	**0.639**		**0.664**							**-0.773**		-0.428	**0.809**	-0.439
Nb			-0.509						-0.466										0.544	0.455			
Hg:Se				0.499				**0.565**					**0.574**	**0.587**	**0.578**	0.547	0.463	**0.681**					**0.606**

	Mg	Al	P	S	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Ga	Rb	Sr	Ag	Sb	Ba	La	K	As	Nb	Hg:Se
Mg		0.452	**0.893**	**0.715**	0.454									0.475						**0.691**			0.478
Al	0.452		0.434		0.453	-0.487				**0.657**	0.421		**0.591**	0.496				**0.695**	0.473	0.461	**-0.596**	0.470	
P	**0.893**	0.434		**0.717**										**0.614**						**0.642**			**0.568**
S	**0.715**		**0.717**		**0.562**				-0.471			0.455		0.494		0.519				**0.603**			
Ti	0.454	0.453		**0.562**						0.480	**0.687**	0.470	**0.536**			0.480	0.436	**0.650**					
V		-0.487					**0.653**							**-0.581**			**0.626**			**-0.535**	**0.773**		
Cr						**0.653**					0.416	**0.607**			0.523		**0.851**			-0.454	**0.838**		
Mn																							
Fe				-0.471												-0.514							
Co		**0.657**			0.480						**0.641**		**0.624**					**0.781**					
Ni		0.421			**0.687**		0.416			**0.641**			0.482			**0.563**		**0.638**					
Cu				0.455	0.470		**0.607**									0.422	0.449				0.482		
Ga		**0.591**			**0.536**					**0.624**	0.482							**0.700**					
Rb	0.475	0.496	**0.614**	0.494		**-0.581**													0.418	**0.713**	**-0.620**		
Sr							0.523										**0.743**						
Ag				0.519	0.480				-0.514		**0.563**	0.422											
Sb					0.436	**0.626**	**0.851**					0.449			**0.743**						**0.760**		
Ba		**0.695**			**0.650**					**0.781**	**0.638**		**0.700**										
La		0.473												0.418						**0.634**	**-0.538**		
K	**0.691**	0.461	**0.642**	**0.603**		**-0.535**	-0.454							**0.713**					**0.634**		**-0.633**		
As		**-0.596**				**0.773**	**0.838**					0.482		**-0.620**			**0.760**		**-0.538**	**-0.633**		-0.430	
Nb		0.470																			-0.430		
Hg:Se	0.478		**0.568**																				