Migration Patterns Affect Element Concentrations in an Arctic Seabird

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Cover photo: Arctic skua (*Stercorarius parasiticus*), by Christoffer Høyvik Hilde.
ABSTRACT

Arctic seabirds have often been used to assess metal levels in remote regions. Most Arctic-breeding seabirds are migratory, and differences in wintering locations and migration patterns have been suggested to affect their element concentrations. In a separate part of this project, the migration patterns and wintering areas in a Svalbard-breeding population of Arctic skuas (*Stercorarius parasiticus*) were determined (America, West Africa, the Gulf of Guinea and the Mediterranean). In the present study, the effects of winter areas on feather and blood concentrations of mercury (Hg), selenium (Se), cadmium (Cd), lead (Pb) and zinc (Zn) were investigated in this population of Arctic skuas. Relationships between feather and blood concentrations were examined, and the temporal consistency of each element in the Arctic skuas was quantified by calculating their repeatabilities (REPs). Blood samples (collected during the breeding seasons from 2009-2013) and feather samples (from 2010 to 2013) from 23 breeding adult Arctic skuas were analysed for elements. There were significant differences in feather concentrations of all the elements among Arctic skuas migrating to the different wintering areas. Feather concentrations of Hg, Se and Cd had high REPs, which implies similar individual dietary exposure to these elements within wintering areas among years. Blood concentrations of Hg and Se differed significantly among the wintering Arctic skuas, and the individual levels of these elements remained consistent between the years. There were significant relationships between feather and blood concentrations of Hg, Se, Cd and Zn. These relationships suggest that concentrations of these elements obtained during the winter affected circulating blood levels during the breeding season. Hg and Se seemed to be released from storage tissues into the bloodstream in concentrations reflecting winter levels, while Cd and Zn blood levels remained relatively similar among Arctic skuas with different migration strategies. Results concerning effects of migration on Pb blood concentrations were inconclusive because feather and blood concentrations did not correlate. High REP of Pb in blood indicates similar individual exposure to Pb in Kongsfjorden among years. Mean concentrations of Hg and Se were lower in blood of female Arctic skuas than in males, and the mean concentration of Hg was higher in blood of Arctic skuas breeding on islands of Kongsfjorden than in birds breeding on the tundra. Year was a significant predictor for Zn in feathers and blood. The results of this study emphasize the importance of considering migration patterns and wintering locations, in addition to factors such as sex, year and breeding location, when evaluating year-round and local exposure to potentially toxic elements.
Arktiske sjøfugler har ofte blitt anvendt i vurderinger av metallnivåer i avside sliggende områder. De fleste sjøfugler som hekker i Arktis er migrerende, og forskjeller i overvintringslokaliteter og migrasjonsmønstre har tidligere blitt antydet å påvirke variasjoner i elementkonsentrasjoner hos slike sjøfugler. I en separat del av dette prosjektet ble migrasjonsstrategiene og overvintringsområdene til en Svalbard-hekkende populasjon av tyvjo (Stercorarius parasiticus) beregnet (Amerika, Vest-Afrika, Guineabukta og Middelhavet). I dette studiet ble effekten av vinterområder på fjær- og blodkonsentrasjoner av kvikksølv (Hg), selen (Se), kadmium (Cd), bly (Pb) og sink (Zn) undersøkt i denne tyvjo-populasjonen. Forhold mellom fjær- og blodkonsentrasjoner ble undersøkt, og den årlige konsistensen til hvert element kvantifisert ved å beregne deres repeterbarhet (REP). Blodprøver (innsamlet hver hekkesesong fra 2009-2013) og fjærprøver (fra 2010-2013) fra 23 hekkende, adulte tyvjøer, ble analysert for elementer. Det var signifikante forskjeller i fjærkonsentrasjoner av alle elementer blant tyvjoer som migrerte til de ulike vinterområdene. Fjærkonsentrasjoner av Hg, Se og Cd hadde høye REPs, som indikerer lignende individuell eksponering via diett innad i hvert vinterområde, hvert år. Blodkonsentrasjoner av Hg og Se var signifikant forskjellige mellom tyvjoer som migrerte til de ulike vinterområdene, og disse elementene hadde høye REPs. Fjærkonsentrasjoner av Hg, Se, Cd og Zn var positivt korrelert med blodkonsentrasjoner av disse elementene, som tyder på at nivåer av disse elementene som blir absorbert om vinteren kan påvirke sirkulerende blodnivåer under hekkesesongen. Hg og Se så ut til å bli frigitt fra lagringsorganer til blodstrømmen i konsentrasjoner som reflekterer vinterkonsentrasjoner, mens blodnivåene av Cd og Zn var relativt like mellom tyvjoer med ulik migrasjonsstrategi. Pga. sannsynligheten for ekstern forurensing av fjærprøvene, kan ikke effekter av migrasjon på Pb-konsentrasjoner vurderes ut fra resultatene fra dette studiet. Blodkonsentrasjonene av Pb var relativt stabile mellom år innad i individene, som kan tyde på lignende eksponering i Kongsfjorden hvert år. Det var lavere blodkonsentrasjoner av Hg og Se i hunner enn i hanner, og høyere Hg i blod hos tyvjoer som hekket på øyer enn hos de som hekket på tundraen innad i Kongsfjorden. År var også en signifikant forklaringsvariabel for blod- og fjærkonsentrasjoner av Zn. Resultatene av dette studiet viser viktigheten av å ta hensyn til migrasjonsmønstre og overvintringslokaliteter, i tillegg til faktorer som kjønn, år og hekkelokalitet, når en evaluerer total og lokal eksponering til potensielt giftige elementer.
ACKNOWLEDGEMENTS

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Finally, I am very thankful to my family and my friends for all the support you have given me during my years as a student. Special thanks go to my ecotox classmates for fun and friendship during the master’s programme, and to Christoffer Høyvik Hilde for all feedback and help with modelling and R.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>CEBC</td>
<td>Centre d'Etudes Biologiques de Chizé</td>
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<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>GLS</td>
<td>Global location sensing</td>
</tr>
<tr>
<td>HP-ICP-MS</td>
<td>High resolution inductively coupled plasma mass spectrometry</td>
</tr>
<tr>
<td>Hg</td>
<td>Mercury</td>
</tr>
<tr>
<td>HNO₃</td>
<td>Nitric acid</td>
</tr>
<tr>
<td>MR²</td>
<td>Marginal R squared: proportion of variance explained by fixed factors in a linear mixed model</td>
</tr>
<tr>
<td>N</td>
<td>number of individuals</td>
</tr>
<tr>
<td>n</td>
<td>number of observations</td>
</tr>
<tr>
<td>NINA</td>
<td>Norwegian Institute for Nature Research</td>
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<tr>
<td>NTNU</td>
<td>Norwegian University of Science and Technology</td>
</tr>
<tr>
<td>p</td>
<td>Probability of rejecting the null hypothesis</td>
</tr>
<tr>
<td>Pb</td>
<td>Lead</td>
</tr>
<tr>
<td>POP</td>
<td>Persistent organic pollutant</td>
</tr>
<tr>
<td>REP</td>
<td>Repeatability</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Se</td>
<td>Selenium</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>VAR</td>
<td>Variance</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
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1. Introduction

Metal pollution, due to local and global redistribution by anthropogenic activities such as mining and smelting, is considered a serious environmental problem in many regions of the world (Walker, 2001). Seabirds have often been used to study metal levels in remote ecosystems, such as in the Arctic (Wenzel and Gabrielsen, 1995, Norheim, 1987, Sagerup, 2009). Most seabirds that breed in Arctic regions are migratory, and therefore spend parts of the year away from their breeding areas (Gaston, 2004). Migratory seabirds may be exposed to varying levels of contaminants during their annual cycle resulting from exploitation of different food webs year-round (Fort, 2014). As it is challenging to track individual birds throughout their annual migration cycles (Lavoie, 2014), few studies have assessed migration in relation to potentially toxic substances, such as metals and metalloids.

Ecotoxicological important metals, such as mercury (Hg), cadmium (Cd) and lead (Pb) (Casarett and Doull, 2013), have been detected at elevated levels in Arctic biota (AMAP, 2005, Dietz, 1996, Sagerup, 2009). Through different mechanisms, these toxic elements can e.g. affect the reproductive, the neurological and the hepatic systems in birds and other organisms (Eisler, 1988, Burger and Gochfeld, 1997, Scheuhammer, 1987). However, also essential elements may be of toxicological concern. The metalloid selenium (Se) is essential in maintaining biological functions (Ohlendorf and Heinz, 2009), though it has also been reported to cause adverse effects in birds at elevated levels (Hoffman, 2002). Se has a high affinity for Hg, and these two elements are known to decrease the toxic effects of the other through complex interactions involving binding through a direct linkage (Falnoga, 2006, Khan and Wang, 2009). The essential element zinc (Zn) is necessary in e.g. feather formation (Burger, 1993), though excess levels of this metal have caused poisoning events in birds (Beyer, 2004).

The total body burden of an element in a bird is dependent on the combined rates of absorption, which is mostly determined by diet (Lavoie, 2014), and elimination, which occurs e.g. through egg laying, skin exfoliation and moulting (Casarett and Doull, 2013, Scheuhammer, 1987). Though element levels in blood are presumed to reflect immediate dietary exposure (Geens, 2010), variations in element concentrations in seabirds may be influenced by differences in migration strategies and winter areas, and by factors such as sex, year and breeding location (Borgå, 2012, Lavoie, 2014, Øverjordet, 2015a). If a migratory bird does not eliminate the
elements it is exposed to during the winter by the time it reaches its breeding site, the total body burden, and thus the circulating blood levels in the breeding bird may be influenced by concentrations obtained during the winter (Lavoie, 2014). Through elimination processes, elements may be subsequently released from storage tissues, such as liver and kidneys, into the bloodstream in concentrations that reflect the winter concentrations. Such effects of migration on element concentrations are likely influenced by the toxicokinetic properties (absorption, distribution, metabolism and elimination) of the elements (Casarett and Doull, 2013).

Feathers may be used to assess past exposure in birds, as elements are excreted into feathers during their formation (Burger, 1993). When feather growth is completed, the feathers become metabolically isolated from the rest of the body, and they therefore represent the circulating blood element concentrations at the time of their development (Burger, 1993, Bearhop, 2000). To use feathers as a proxy for assessing element exposure during the winter, the selected study species must moult during winter, and there should be a consistent relationship between the element levels in feathers and other tissues (Burger, 1993). There are generally high correlations between element levels in feathers and in the liver, kidneys, blood and other tissues in seabirds (Burger, 1993).

Leat et al. (2013) expressed a need for thorough analysis of the effect of wintering area on contaminant concentrations within a seabird species, which includes individuals with different migration strategies. The Arctic skua (Stercorarius parasiticus) is a migrating seabird with kleptoparasitic behaviour (Wiley and Lee, 1999, Furness, 1987). It breeds in arctic regions, and spends the nonbreeding part of the year (September through May) migrating and overwintering (Furness, 1987). Relationships between feather and blood element concentrations have not previously been determined in Arctic skuas. However, body feather moultting usually occur during the winter in this species (Wiley and Lee, 1999, Furness, 1987), which implies that body feather element concentrations likely reflect the blood concentrations of the wintering birds. In a Svalbard-breeding population of Arctic skuas, global location sensing (GLS) loggers have been used to track the migration strategies of individual birds during several consecutive years (2009-2013) (Moe et al., unpublished data). The individuals from this population spend the winter in one of four distinct coastal or offshore areas: Central and South America (hereafter called “America”), West Africa, the Gulf of Guinea, and the Mediterranean. The birds were observed to migrate to the same wintering area year after year, demonstrating relatively low within-individual variation and high among-individual variation in the migration strategies of this Arctic skua population (Moe et al., unpublished data). The tendency of the Arctic skuas to
consistently return to the same wintering area every year may be reflected in the element concentrations of the birds. By calculating the *repeatability* (REP), the statistical degree of consistency of the element concentrations in all the birds, among several years, can be quantified. The REP of an element is expressed as a ratio between 0 and 1, and is the proportion of variation in element concentration that is due to differences among individuals, compared to the total variation, which is the sum of the among and the within variation (Wolak, 2012, Lessells and Boag, 1987).

The population of Arctic skuas breeding in Kongsfjorden is an appropriate system to investigate the importance of long-distance migration on variations in element concentrations during the winter and summer seasons. The population consists of repeatedly tracked individuals with different migration strategies, and the determined wintering areas represents a wide range of feeding habitats and dietary exposure to elements. Investigating effects of migration on element concentrations in Arctic skuas during several years can contribute to a wider understanding of year-round and local exposure to potentially toxic elements in this Arctic seabird.

2. Objectives

In this study, element concentrations in body feather and whole blood samples from the GLS-tracked, Svalbard-breeding population of Arctic skuas were analysed. The samples were obtained during the breeding season over several years from the same individuals. The aims of the present study were to investigate if the feather and blood concentrations of Hg, Se, Pb, Cd and Zn differed in Arctic skuas migrating to America, West Africa, the Gulf of Guinea and the Mediterranean, and to assess how consistent each element remained in the birds during all years of sampling. It was hypothesized that the elements would generally have high REPs due to the individual consistency in migration strategies of this species, though the REPs were anticipated to be influenced by the toxicokinetics properties of the elements (Casarett and Doull, 2013).

It was presumed that body feathers is an appropriate proxy for assessing winter exposure to elements in Arctic skuas, as correlations between feather and blood element concentrations in seabirds are generally high. Nevertheless, relationships between feather and blood concentrations were investigated to confirm the presence of such correlations. It was hypothesized that feather concentrations would differ in birds migrating to the different winter locations, because the wintering areas used by the Arctic skuas represent a wide range of
different feeding habitats and dietary element exposure. Blood concentrations were expected to differ among Arctic skuas migrating to the different wintering if the birds did not entirely eliminate the element concentrations obtained during the winter before being sampled during the breeding season. It was also investigated if sex, year and breeding location within Kongsfjorden, along with wintering area, affected feather and blood concentrations of elements in Arctic skuas.

3. Methods

3.1. Sampling
Breeding adult Arctic skuas (N=23, 12 males, 11 females) were captured at their breeding sites on the islands and on the southern and northern side of Kongsfjorden (c. 79°N, 12°E, Figure 1), Svalbard, between June and July from 2009 to 2013. The Arctic skuas were captured on the nest, using a trap with a remote-triggered noose, or by using a handheld net cannon.

Biometric measurements of head and bill (mm), tarsus (mm), wing (mm), body mass (g) and inner and outer tail feathers (mm) were recorded. All birds were ringed, and a 2.5 g GLS logger was attached to the bird’s tarsus, connected to a plastic ring with a cable tie. GLS loggers record ambient light, from which the timing of sunset and sunrise, and the longitude and latitude can be calculated (Delong, 1992, Phillips, 2004). Approximately 2 mL of blood was sampled from the brachial vein of each bird. The samples were frozen at -20 °C each day after returning from the field, and kept frozen until analysis. The total number of blood samples collected were 64,
from 23 different individuals, during the period 2009-2013. The birds were sexed using molecular methods described by Weimerskirch et al. (2005) at the Centre d’Etudes Biologiques de Chizé (CEBC). Four to five dorsal feathers were collected from each bird during the period 2011-2013, and kept in plastic zip-lock bags. No feathers were collected in 2009, and feather samples from 2010 consisted of a single feather per sample. The total number of feather samples were 53, obtained from the same 23 individuals from which the blood samples were collected. Sampling and handling of the birds were in accordance with the regulations of the Norwegian Animal Welfare Act.

The tracking data from the GLS loggers were analysed in a separate part of this project (Moe et al., unpublished data), and the wintering area for each individual included in this study was determined. The wintering areas are presented as a part of the results in this thesis. The tracking data made it possible to test for any association between individual migration strategies and element concentrations in the obtained feather and blood samples.

3.2. Element analysis

Element analysis consisted of preparation, digesting and quantification of metals and metalloids from whole blood and body feather samples. The analysis was performed at the Department of Chemistry, NTNU, in February 2014.

3.2.1. Preparation and digestion of samples

Preparation and digestion of feather samples were performed following the method described by Dauwe et al. (2003), with some minor modifications. Feathers collected in 2010 were cut in two lengthwise with a Titanium knife, which was rinsed in acetone between each cutting. The feathers were placed in Teflon microwave vessels before the exact weight (22.50 ± 8.82 mg) was determined. The feathers were then soaked in acetone for 1-1.5 hours. The acetone was poured out and the feathers were rinsed three times with deionized MilliQ water to remove loosely adherent external contamination. To minimize contamination of the samples, the feathers were not dried before digestion. Feathers were digested in 3 mL 50 % nitric acid, HNO₃ (v/v, UltraPure grade, distilled in SubPur from Milestone, Bergamo, Italy, at the Department of Chemistry, NTNU, Trondheim, Norway). Whole blood samples were placed in Teflon microwave vessels, and the exact weight (344.38 ± 148.62 mg) was determined. The samples were then added 1 ml concentrated HNO₃, similarly to the procedure described by Thompson and Dowding (1999). Blood and feather samples were digested in a high performance Microwave reactor, UltraClave from Milestone, Bergamo, Italy, for two hours (Appendix A).
3.2.2. Analysis of elements using high-resolution inductive coupled plasma mass spectrometry (HP-ICP-MS)

Following the digestion procedure, the samples were diluted with deionized MilliQ water. Feather samples were diluted to a final volume of 30.00 mL ± 0.44 mL, blood samples to 15.43 mL ± 0.42 mL (exact volume noted for each sample).

The quantification of total element content in blood and feathers was determined with HR-ICP-MS, using ELEMENT 2 from Thermo Electronics, Bremen, Germany, at NTNU in Trondheim, Norway. All samples were run with blanks and appropriate standards. Certified reference material was analyzed to verify the accuracy of the method, using chicken material (GBW 10018, China), and whole blood (Seronorm™ Trace Elements Whole Blood 3, Norway). A more appropriate whole blood reference material would be Seronorm™ Trace Whole Blood 1, as this level is more relevant for Arctic skua blood.

All samples were run in triplicates, and the relative standard deviation (RSD) was instrumentally calculated for each sample (data point). The equation for calculating RSD is as follows (IUPAC, 1976).

\[
\text{RSD} (\%) = \frac{\text{standard deviation}}{\text{mean of triplicates}} \times 100
\] (1)

The elements that were selected for further analyses were Hg, Se, Pb, Cd and Zn. All elements had RSD below 25 % (Appendices B and C).

3.3. Statistical analyses

Statistical analysis was carried out in R (v. 3.0.1 : the R Development Core Team).

All analyses in this study were performed with linear mixed models, using the R package “nlme” (Pinheiro, 2013). In such models, explanatory variables (hereafter called “predictors”) are either non-random (fixed effects) or random (random effects). The random effects allows for the use of repeated measurements of the same individuals, and account for different numbers of measurements caused by some inconsistency in annual recapture. Individual identity was therefore entered as a random effect in all linear mixed models. The assumptions of linear mixed models were satisfied without transforming the data in this study.

Outliers were assessed using plots of fitted values vs. standardized residuals of linear mixed models with individual identities as random factors (Appendix D). A residual value of 2.7
standard deviations (SD) was decided based on these plots. The data points which had a residual value >2.7 SD were considered to be outliers, and not used in the analyses. Outliers are marked in red in Appendices B and C.

Mean concentrations (µg/g ± SE) of each element in blood and feathers were calculated using linear mixed models to adjust for the different number of measurements of individual Arctic skuas. The means are the intercepts from models with element concentrations as response variables, and no predictors, and represents all years of sampling. Concentrations are given in dry weight (dw) for feathers and wet weight (ww) for whole blood.

The following wintering areas were defined from the GLS logger data: America, West Africa, the Gulf of Guinea and the Mediterranean (Appendix E). Winter area, year and sex were included as variables in linear mixed models to explain variations in element concentrations in feathers. Breeding location was also included in the blood models, as we had observed in the field that Arctic skuas breeding on islands and the tundra had slightly different diets. It was not expected that this factor would significantly affect feather concentrations due to the small geographical scale within Kongsfjorden. To confirm this, breeding location was initially included in the feather models, and was removed when no effect was observed. Body mass did not correlate with any other biometric measurements, and therefore best represented the body condition of the birds. Body mass was also initially included as a predictor in the models, but was excluded from all models when no effects were found. Analysis of variance (ANOVA) were performed on the outputs of models analysing variations in feather and blood concentrations. Estimates, degrees of freedom, total number of individuals (N), number of observations (n) and p-values from linear mixed models are presented in Appendix F. Tukey’s post hoc tests were run to assess the significant differences between each winter area, and between each year (only for Zn). Tukey’s tests were performed using the packages “lsmeans” and “multcomp” in R (Lenth, 2015).

The REP's of each element in the birds were calculated using the following equation:

$$\text{REP} = \frac{\text{VAR among}}{(\text{VAR among} + \text{VAR within})}$$ (2)

(Lessells and Boag, 1987). “VAR among” signifies the variance of element concentrations between the random effect terms (individuals) in a linear mixed model. “VAR within” is the variance in element concentrations which is due to differences within the “group” (all
measurements of one individual, all years). These variance components were obtained from linear mixed models with elements in feathers or blood as response variables, and no predictors, using the function “VarCorr” in R.

To investigate whether there were relationships between the feather and blood concentrations of the elements, feather concentrations were used as predictors in linear mixed models with blood concentrations as response variables. Slopes and intercepts for producing feather vs. blood plots were obtained from these models. Marginal ($\text{MR}^2$) R squared values were obtained using MuMIn package (Barton, 2015), and is interpreted as the variance explained by the fixed factors in a linear mixed model (Nakagawa and Schielzeth, 2013).
4. Results

The wintering areas used by the Svalbard-breeding population of Arctic skuas (Table 1, Appendices E and G) were determined from GLS logger data in a separate part of this project (Moe et al., unpublished data). All birds were highly consistent in their individual migration strategies (Appendix H).

Table 1. Winter areas used by the population of Arctic skuas (Stercorarius parasiticus) breeding in Kongsfjorden, Svalbard, during the summers of 2009 to 2013. Number of individuals (N) is included. Winter areas were determined from tracking data from GLS loggers (Moe et al., unpublished data).

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<th>Winter area</th>
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<tbody>
<tr>
<td>America</td>
<td>13</td>
</tr>
<tr>
<td>West Africa</td>
<td>6</td>
</tr>
<tr>
<td>Gulf of Guinea</td>
<td>3</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>23</strong></td>
</tr>
</tbody>
</table>

The mean concentrations of the investigated elements (Table 2) were detected in feathers (dw) at concentrations following the order: Zn > Se > Hg > Pb > Cd. In blood (ww), the order was Se > Zn > Hg > Pb > Cd.

There were significant differences in feather element concentrations of all elements among Arctic skuas migrating to different wintering areas (Table 3, Appendix F). The single individual migrating to the Mediterranean had higher mean concentrations of feather Hg and Pb than birds wintering in the other areas (Figure 2). Birds wintering in West Africa had higher mean feather Hg concentration than the birds wintering in America and the Gulf of Guinea. The Arctic skuas migrating to West Africa had lower mean Se and Zn feather concentrations than birds wintering in America. The birds migrating to the Gulf of Guinea had higher feather Cd concentrations than birds in America and West Africa.

Zn concentrations in feathers were significantly affected by year (Table 3). The mean feather Zn concentrations was lower in 2010 than in the other years (Appendix I). Year did not influence feather concentrations of other elements than Zn.
Table 2. Mean ± SE and ranges of concentrations (µg/g) of Hg, Se, Pb, Cd and Zn in feathers (dw) and blood samples (ww) from male and female Arctic skuas (*Stercorarius parasiticus*) breeding in Kongsfjorden. Feather samples were collected each breeding season from 2010 to 2013, blood were sampled each season from 2009 to 2013. The means are intercepts from linear mixed models with element concentrations in feathers and blood, respectively, as response variables, and no predictors. Individual identities were set as random factors. Number of individuals of each sex (F, M), total number of individuals (N) and number of measurements of all individuals (n) are given.

<table>
<thead>
<tr>
<th>Element</th>
<th>F, M</th>
<th>N</th>
<th>n</th>
<th>Mean ± SE (µg/g)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feathers (dw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>11, 12</td>
<td>23</td>
<td>53</td>
<td>2.289 ± 0.467</td>
<td>0.340 - 12.500</td>
</tr>
<tr>
<td>Se</td>
<td>11, 12</td>
<td>23</td>
<td>53</td>
<td>6.166 ± 0.718</td>
<td>0.850 - 15.970</td>
</tr>
<tr>
<td>Pb</td>
<td>11, 11</td>
<td>22</td>
<td>50</td>
<td>0.193 ± 0.034</td>
<td>0.017 - 1.063</td>
</tr>
<tr>
<td>Cd</td>
<td>11, 12</td>
<td>23</td>
<td>52</td>
<td>0.009 ± 0.001</td>
<td>0.001 - 0.030</td>
</tr>
<tr>
<td>Zn</td>
<td>11, 12</td>
<td>23</td>
<td>52</td>
<td>135.038 ± 2.445</td>
<td>75.110 - 158.090</td>
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<tr>
<td>Blood (ww)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Hg</td>
<td>11, 12</td>
<td>23</td>
<td>64</td>
<td>0.335 ± 0.020</td>
<td>0.160 - 0.650</td>
</tr>
<tr>
<td>Se</td>
<td>11, 11</td>
<td>22</td>
<td>62</td>
<td>17.110 ± 1.587</td>
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<tr>
<td>Pb</td>
<td>11, 12</td>
<td>23</td>
<td>62</td>
<td>0.006 ± 0.000</td>
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</tr>
<tr>
<td>Cd</td>
<td>11, 12</td>
<td>23</td>
<td>63</td>
<td>0.001 ± 0.000</td>
<td>0.0002 - 0.004</td>
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<tr>
<td>Zn</td>
<td>11, 12</td>
<td>23</td>
<td>64</td>
<td>4.790 ± 0.052</td>
<td>3.970 - 5.850</td>
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Table 3. Results from ANOVAs of linear mixed models analysing concentrations of elements in feathers (dw) of Arctic skuas (*Stercorarius parasiticus*) breeding in Kongsfjorden. Feather samples were collected during each breeding season from 2010 to 2013. Total number of individuals (N) and number of measurements of all individuals (n) are included.

<table>
<thead>
<tr>
<th>Element</th>
<th>Predictor</th>
<th>N</th>
<th>n</th>
<th>F</th>
<th>Df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg</td>
<td>Winter area</td>
<td>23</td>
<td>53</td>
<td>85.013</td>
<td>3, 18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td></td>
<td></td>
<td>2.646</td>
<td>3, 27</td>
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<td></td>
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<td>Se</td>
<td>Winter area</td>
<td>23</td>
<td>53</td>
<td>4.099</td>
<td>3, 18</td>
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<td>1.707</td>
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<td>0.277</td>
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<td>Pb</td>
<td>Winter area</td>
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<td>50</td>
<td>8.054</td>
<td>3, 17</td>
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<td></td>
<td>2.446</td>
<td>3, 25</td>
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<td>0.236</td>
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<td>Cd</td>
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<td>52</td>
<td>4.344</td>
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<td>Winter area</td>
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<td>52</td>
<td>5.402</td>
<td>3, 18</td>
<td>0.008</td>
</tr>
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<td>Year</td>
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<td></td>
<td>7.457</td>
<td>3, 26</td>
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<td>0.080</td>
<td>1, 18</td>
<td>0.781</td>
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Figure 2. Mean feather concentrations (µg/g ± SE, dw) of Hg, Se and Pb in Arctic skuas (Stercorarius parasiticus) migrating to four wintering areas (AM = America, WA = West Africa, GG = the Gulf of Guinea, ME = the Mediterranean). Means are intercepts from linear mixed models with feather concentrations as response variables and no predictors. Hg and Se: AM: N=13, n=30, WA: N=6, n=12, GG: N=3, n=8, ME: N=1, n=3. Pb: AM: N=13, n=29, WA: N=5, n=11, GG: N=3, n=7, ME: N=1, n=3. Groups without a common letter (a, b, c) are significantly different (p≤0.05).
Figure 2. cont. Mean feather concentrations (µg/g ± SE, dw) of Cd and Zn in Arctic skuas (*Stercorarius parasiticus*) migrating to four wintering areas (AM = America, WA = West Africa, GG = the Gulf of Guinea, ME = the Mediterranean). Means are intercepts from linear mixed models with feather concentrations as response variables and no predictors. Cd and Zn: AM: N=13, n=29, WA: N=6, n=12, GG: N=3, n=8, ME: N=1, n=3. Groups without a common letter (a, b) are significantly different (p≤0.05).
Table 4. Results from ANOVA of linear mixed models analysing concentrations of elements in blood (ww) from Arctic skuas (Stercorarius parasiticus) from Kongsfjorden. Blood samples were collected during each breeding season from 2009 to 2013. Total number of individuals (N) and number of measurements of all individuals (n) are included.

<table>
<thead>
<tr>
<th>Element</th>
<th>Predictor</th>
<th>N</th>
<th>n</th>
<th>F</th>
<th>df</th>
<th>p</th>
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<tbody>
<tr>
<td>Hg</td>
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<tr>
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<td></td>
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<td></td>
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<tr>
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<td>5.247</td>
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<tr>
<td>Se</td>
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<td>62</td>
<td>7.402</td>
<td>3.16</td>
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<tr>
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<td>0.153</td>
<td>1.16</td>
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<td>Pb</td>
<td>Winter area</td>
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<td>62</td>
<td>1.689</td>
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<tr>
<td></td>
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<td>Sex</td>
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<td>1.038</td>
<td>1.17</td>
<td>0.322</td>
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<td></td>
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<td>3.123</td>
<td>1.17</td>
<td>0.095</td>
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<tr>
<td>Cd</td>
<td>Winter area</td>
<td>23</td>
<td>63</td>
<td>0.666</td>
<td>3.17</td>
<td>0.585</td>
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<td>1.557</td>
<td>4.36</td>
<td>0.207</td>
</tr>
<tr>
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<td>0.132</td>
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<td>0.721</td>
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<td>1.237</td>
<td>1.17</td>
<td>0.282</td>
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<tr>
<td>Zn</td>
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<td>0.630</td>
<td>3.17</td>
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<td>4.204</td>
<td>4.37</td>
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<td>0.244</td>
<td>1.17</td>
<td>0.628</td>
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<td>0.187</td>
<td>1.17</td>
<td>0.671</td>
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</tbody>
</table>
Figure 3. Mean blood concentrations (µg/g, ± SE, ww) of Hg and Se in Arctic skuas *Stercorarius parasiticus* migrating to four wintering areas (AM = America, WA = West Africa, GG = the Gulf of Guinea, ME = the Mediterranean). Means are intercepts from linear mixed models with feather concentrations as response variables and no predictors. Hg: America: N=13, n=34, West Africa: N=6, n=16, the Gulf of Guinea: N=3, n=10, the Mediterranean: N=1, n=4. Se: America: N=12, n=32, West Africa: N=6, n=16, Gulf of Guinea: N=3, n=10, the Mediterranean: N=1, n=4. Groups without a common letter (a, b, c) are significantly different (p≤0.05).
Wintering areas and sex were significant predictors of blood concentrations of Hg and Se (Table 4, Appendix F). When sex was removed as a predictor, winter area remained significant (Hg: p<0.001, Se: p = 0.006). As in feathers (Figure 2), the individual wintering in the Mediterranean had the highest mean Hg blood concentration (Figure 3), though Hg levels in the birds migrating to America and West Africa did not differ in blood. The Arctic skuas wintering in the Gulf of Guinea had higher Hg blood concentrations compared to the birds from America and West Africa. The mean Se blood concentration in birds wintering in West Africa was significantly lower than birds from America and the Gulf of Guinea, similarly to the mean feather concentration of these birds, which differed significantly from birds wintering in America.

Male Arctic skuas had higher mean blood concentrations of Hg and Se than females (Appendix F and J). There were no effects of sex on blood concentrations of Pb, Cd or Zn (Table 4).

The birds breeding on the islands in Kongsfjorden had higher mean Hg blood levels than the birds breeding on the tundra (Appendix F and K). Breeding location did not influence blood concentrations of other elements than Hg (Table 4).

Year significantly affected Zn blood concentrations (Table 4). The mean Zn blood concentration was significantly lower in 2010 than in 2012 (Appendix F and I). Year did not influence concentrations of Hg, Se, Pb or Cd.

There were significant relationships between feather concentrations (dw) and blood concentrations (ww) of Hg (estimate: 0.027 ± 0.006, df: 26, p<0.001, MR²: 0.337), Se (estimate: 1.361 ± 0.272, df: 26, p<0.001, MR²: 0.416), Cd (estimate: 0.029 ± 0.014, df: 24, p=0.046, MR²: 0.088) and Zn (estimate: 0.007 ± 0.003, df: 25, p=0.05, MR²: 0.078) (Figure 4). There was no relationship between feather and blood concentrations of Pb (p=0.678, MR²: 0.003). As no feather samples were obtained in 2009, no comparisons could be performed with the blood samples collected this year. When Hg concentrations of the individual migrating to the Mediterranean (in ring) was removed from the Hg plot, the relationship between Hg in feathers and blood was still significantly positive (N=21, n=46, estimate: 0.030 ±0.011, df: 24, p = 0.010, MR²: 0.108).
Figure 4. Scatter plots showing the significant relationships between feather (dw) and blood concentrations (ww) of Hg, Se, Cd and Zn (µg/g) in Arctic skuas (Stercorarius parasiticus) breeding in Kongsfjorden from 2010-2013. Regressions lines obtained from linear mixed model outputs are included. Hg: N=22, n=49. Se: N=22, n=48. Cd: N=22, n=47. Zn: N=22, n=48.
Table 5. REP of Hg, Se, Pb, Cd and Zn in feathers and blood of Arctic skuas (Stercorarius parasiticus) breeding in Kongsfjorden from 2009-2013. REPs were calculated using equation (2). The variance components were obtained from linear mixed models with element concentration as response variables and no predictors.

<table>
<thead>
<tr>
<th>Element</th>
<th>Feathers</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VAR among</td>
<td>VAR within</td>
</tr>
<tr>
<td>Hg</td>
<td>4.741</td>
<td>0.542</td>
</tr>
<tr>
<td>Se</td>
<td>10.865</td>
<td>1.942</td>
</tr>
<tr>
<td>Pb</td>
<td>0.006</td>
<td>0.044</td>
</tr>
<tr>
<td>Cd</td>
<td>1.436*10^{-5}</td>
<td>1.598*10^{-5}</td>
</tr>
<tr>
<td>Zn</td>
<td>0.019*10^{-6}</td>
<td>311.000</td>
</tr>
</tbody>
</table>

The REPs of the examined elements were generally high, though they varied between elements, and between blood and feathers (Table 5). The highest REPs were observed for Hg and Se in feathers (0.897 and 0.848) and in blood (0.610 and 0.728). Further, Pb had relatively high REP in blood (0.503), and relatively low in feathers (0.125). Cd had relatively high REP in feathers (0.473), and low REP in blood (0.016). Zn had low REPs in both blood and feathers (<0.001).
5. Discussion

The aims of the present study were to investigate if the feather and blood concentrations of Hg, Se, Pb, Cd and Zn differed in Arctic skuas migrating to America, West Africa, the Gulf of Guinea and the Mediterranean, and to determine the consistency of these element concentrations in the birds during the years of sampling. Relationships between feather and blood concentrations were also investigated. There were significant differences in feather concentrations for all elements among Arctic skuas migrating to the different winter areas. Additionally, blood concentrations of Hg and Se differed among wintering Arctic skuas. The feather concentrations of Hg, Se, Cd and Zn were significantly related to the blood concentrations. Hg, Se and Cd had high REP\textsuperscript{s} in feathers, and Hg, Se and Pb had high REP\textsuperscript{s} in blood. Sex also affected Hg and Se in blood, and breeding location significantly explained variations in blood Hg concentrations. Year was a significant predictor for Zn in feathers and blood. The results of this study gives emphasis to the importance of considering migration patterns and wintering locations, in addition to factors such as year, sex and breeding location, when evaluating year-round and local exposure to potentially toxic elements.

5.1. Effects of migration on feather element concentrations in Arctic skuas

As element concentrations in predatory birds are mostly determined by diet (Lavoie, 2014), the differences in feather element concentrations in Arctic skuas migrating to the different wintering areas likely result from varying dietary exposure among the winter locations. However, this assumption depends on the certainty of which the feathers used in this study correctly reflect element concentrations obtained in the actual wintering areas of the Arctic skuas, and on whether the feathers accurately reflect blood concentrations at the time of feather growth.

Like most other long-distance migratory seabirds, Arctic skuas undergo partial moulting of their body feathers during migration, though the main moulting phase is commenced after they arrive in their wintering areas (Wiley and Lee, 1999, Svensson, 2010). The feathers used in this study may therefore have been developed during different periods of the birds’ annual cycles, such as during migration. The accurate growth period of the feathers could have been assessed by analysing the feather samples for stable isotopes, which could have provided information about the prey and approximate geographic location of the Arctic skuas during feather growth (Lavoie, 2015, Dahl, 2003). The high feather REP\textsuperscript{s} of Hg, Se and Cd indicates that the feathers
used in the present study are grown in a specific geographic area each year, in which the exposure to these elements were highly similar within each individual from 2010 to 2013. As moulting in Arctic skuas mainly occur after they reach their overwintering locations, rather than during migration (Wiley and Lee, 1999), it is likely that the feathers used in this study are grown in the wintering areas. As each feather sample (except feathers from 2010) consisted of four or five feathers, variations in growth time within each sample should be accounted for.

Feather concentrations of Hg are considered to accurately and consistently reflect blood concentrations at the time of feather growth (Dauwe, 2003, Jaspers, 2004, Bearhop, 2000), an assumption which must be fulfilled to use feathers to assess past exposure (Burger, 1993). However, there are discrepancies concerning the accuracy of which feather concentrations of other elements, such as Se, Cd, Pb and Zn, reflect blood concentrations at the time of feather growth. External contamination, despite similar washing procedures as was performed in the present study, has been reported to affect relationships between blood and feathers in several raptor species and a passerine species (Dauwe, 2003, Jaspers, 2004, Geens, 2010). However, the significant relationships between feather and blood concentrations of Hg, Se, Cd and Zn in the present study indicate that Arctic skua feathers may be used to assess winter exposure to these elements in this species.

The lack of a relationship between feather and blood concentrations of Pb indicates that body feathers is an inappropriate proxy for assessing dietary Pb exposure in Arctic skuas. Previous studies have reported varying results concerning such relationships in other bird species (Geens, 2010, Scheifler, 2006), and Scheuhammer (1987) discouraged the use of feathers to assess dietary Pb exposure due to the high risk of external contamination. Though there were differences in feather Pb concentrations in Arctic skuas migrating to the different wintering areas, it is not possible to discern whether these differences are caused by varying external contamination among the birds, or by varying dietary exposure. The low feather REP for Pb shows that feather levels were dissimilar within the individual birds among years, and is further indicative of varying degrees of external contamination. Therefore, possible effects of migration on Pb concentrations will not be further assessed here.

The differences in dietary exposure to elements among the wintering areas may result from variable levels of pollution between these locations, though it is important to note that the differences may have been caused by variable non-anthropogenic sources. Redistribution of elements caused by e.g. large-scale ocean currents, erosion, run-off from large riverine systems
(Mareano, 2013) or variations in atmospheric depositions (AMAP, 2005) is likely occurring, and may explain the differences in element exposure between the wintering locations. It is therefore difficult to evaluate to which extent the patterns of the elements in the present study represent spatial variations in anthropogenic pollution. Nevertheless, the most obvious differences in feather concentrations among Arctic skuas migrating to the different wintering areas will be assessed here.

The Mediterranean is a semi-enclosed basin with high levels of Hg from both natural and anthropogenic sources (Bernhard, 1988, Kayhan, 2007). The high mean feather concentration of Hg in the single female migrating to the Mediterranean may therefore result from generally high levels of Hg in this region. However, it is problematic that only one individual represents such a large area, even though it has been repeatedly sampled (three feather samples, four blood samples). This female may be repeatedly overwintering close to a point source of pollution, or it might consistently feed on specific types of prey with high Hg levels during the winter. This would mean it would be erroneous to include the entire Mediterranean region as the female’s wintering area, and that conclusions concerning general Hg levels in the Mediterranean should not be drawn from these results. When removing the individual wintering in the Mediterranean, wintering area was still a significant predictor for Hg feather concentrations (p<0.001).

The feather concentrations of the essential elements Se and Zn in Arctic skuas migrating to West Africa were lower than in birds from America, which may result from generally low element levels in West African aquatic systems (Biney, 1994). However, it is also possible that these birds are experiencing difficulties in regulating and maintaining optimal levels of these essential elements, caused by e.g. more unfavourable environmental conditions in West Africa than in America. This was not investigated in the present study.

The higher Cd feather levels in the Arctic skuas migrating to the Gulf of Guinea compared to birds wintering in America and West Africa may be explained by higher local levels of Cd in this region caused by run-off from the Congo River (Mwanamoki, 2014) into the Angola basin, an area which borders to the Gulf of Guinea. However, the higher levels in this region compared to the other winter areas may also result from differences in global Cd distribution caused by non-anthropogenic redistribution processes.

The wintering areas of the Arctic skuas in this study are regions separated by thousands of kilometres, and they thus represent a wide range of feeding habitats and dietary exposure to elements. Differences in element concentrations in feathers were therefore anticipated.
Overwintering location and differences in feeding ecology during the winter has previously been reported to affect feather concentrations of Hg in Little auks (Allé allé) (Fort, 2014), Double-crested cormorants (Phalacrocorax auritus) (hereafter called “Cormorants”) and Caspian terns (Hydroprogne caspia) (hereafter called “Terns”) (Ofukany, 2012, Lavoie, 2015). Wintering locations may affect feather element concentrations, and thus blood levels at the time of feather growth, in several long-distance migratory seabird species, as these usually undergo moulting when in their wintering areas (Svensson, 2010). Therefore, future studies should assess possible effects of migration when evaluating year-round contamination risk in migratory seabirds.

5.2. Consistency in element exposure in the wintering areas

The high REPs of Hg, Se and Cd in Arctic skua feathers show that individual variation in these element concentrations were low between 2010-2013. This suggests that the individual dietary exposure for Hg, Se and Cd within each winter area remained relatively consistent from year to year, and that levels of exposure to these elements during future winter seasons may be similar to the levels reported in the present study. Similarly, Hg concentrations in winter feathers of Cormorants and Terns were consistent between years, possibly due to similarities in feeding habitats and thus dietary exposure to Hg among years (Lavoie, 2015). High REPs of elements were anticipated in the Arctic skuas because of the high individual consistency in migration strategies, and accordingly high consistency in individual dietary inputs of elements among years. The low REP for Zn in feathers show that individual variation in feather concentrations of this metal is high between years, though this is not necessarily due to varying individual exposure within the wintering areas each year. Zn is needed in feather formation during moulting (Burger, 1993), and the low REP suggests that physiological regulation of Zn varies individually between years in Arctic skuas.

5.3. Effects of migration of blood element concentrations in Arctic skuas

There were significant differences between blood concentrations only for Hg and Se among Arctic skuas migrating to the different wintering areas in this study. This winter area effect indicate that levels of Hg and Se obtained during the winters influenced the total body burden, and thus the circulating blood levels, of these elements during the breeding seasons. The feather concentrations of Hg and Se, which represents the winter concentrations, explained approximately 38% and 42%, respectively, of the variation in blood concentrations during the breeding season. Hg and Se seem to be released from storage tissues into the bloodstream during the breeding season in concentrations that reflect the winter concentrations. This is further
indicated by the similarities between the mean feather and blood concentrations in the Arctic skuas migrating to the different wintering areas. Hg concentrations obtained during winter has previously been reported to affect Hg concentrations during breeding in the Cormorants and Terns breeding in Lake Ontario, Canada (Lavoie, 2014). Additionally, accumulation of Hg in Little auks (Fort, 2014) and POPs in Great skuas (Stercorarius skua) (Leat, 2013) during the winter has been reported to affect blood or plasma concentrations of these compounds during the breeding season. For the essential element Se, of which an optimal blood concentration is continuously maintained (Geens, 2010), the observed effect of migration in Arctic skuas may be occurring due to interactions between Hg and Se. The two elements bind through a direct linkage in a process that reduces toxicity of both (Khan and Wang, 2009, Mulder, 2012). The high REPs of Hg and Se in both feathers and blood suggest that the effect of migration on blood concentrations in breeding Arctic skuas is relatively consistent among years. Future studies should assess possible effects of migration when evaluating local exposure to elements in migratory seabirds.

The blood concentrations of Cd and Zn did not significantly differ among Arctic skuas migrating to the wintering areas, though winter feather concentrations significantly affected blood concentrations of these metals. However, feather concentrations explained a relatively low amount of variation (9% and 8%, respectively) in blood concentrations of Cd and Zn, which may be a reason for the lack of significant differences among the Arctic skuas with different migration strategies. Cd is mainly stored in the liver, has a long biological half-life, and is excreted to a very small degree (Scheuhammer, 1987). Elimination rates of Cd from the liver seem to be similar in the Arctic skuas, which results in relatively low variation among the Arctic skuas migrating to the different wintering areas. Interestingly, the low REP of Cd in blood indicates that these amounts varied individually between years, which may be due to variations in individual excretion capacity or in physiological condition. The low variation in Zn blood concentrations among Arctic skuas migrating to the different wintering areas may be explained by physiological regulation of this essential element (Casarett and Doull, 2013), resulting in a similar concentration of Zn in all birds. The low REP of Zn in blood suggest that Arctic skuas showed high individual variation between years in regulation of Zn, which may depend on physiological condition or other factors not assessed in the present study.

As effects of migration on Pb concentrations could not be assessed here, the results of this study may only provide information about the Pb exposure during breeding. The high REP of Pb in blood shows that blood concentrations of Pb remained relatively stable within each individual
between 2009 and 2013, which suggests that individual dietary exposure within Kongsfjorden was relatively similar among these years.

5.4. Effects of sex, breeding location and year on element concentrations

The lower concentrations of Hg and Se identified in females may indicate that egg excretion of these elements occur in Arctic skuas. Lower Hg blood concentrations due to egg excretion has previously been suggested to occur in Arctic skuas (Stewart, 1997), and female birds generally have lower Hg concentrations than males (Robinson, 2012). Se may be excreted along with Hg in Arctic skuas, due to interactions between the two elements. The observed sex difference could also be due to dietary aspects. Males might spend more time foraging than females, or they may obtain larger prey items (Burger, 1993), which could also result in higher Hg and Se concentrations in males than in females.

The Arctic skuas breeding on islands had higher blood levels of Hg than the birds breeding on the tundra. In the field, we consistently observed that the Arctic skuas breeding on the tundra obtained food items, mainly Polar cod (*Boreogadus saida*), from Kittiwakes and Arctic terns (*Sterna paradisaea*), while the island-breeding Arctic skuas consumed Common eider (*Somateria molissima*) eggs, in addition to Polar cod. The mean Hg concentration in Common eider eggs from Kongsfjorden was approximately nine times higher in 2012 (Fenstad, A., 2012, unpublished data) than concentrations in Polar cod from Kongsfjorden during 2005-2006 (Jæger, 2007). This difference in dietary strategies may thus explain the higher Hg blood concentrations in the island-breeding Arctic skuas.

Both blood and feather concentrations of Zn were significantly affected by year in the Arctic skuas of this study. The results suggest that the Arctic skuas excreted less Zn into their feathers in 2010 than in the other years. The mean concentrations of Zn in blood was similarly low in 2010, though only compared to 2012. The differences in concentrations of Zn between years may be due to inter-annual variability in prey availability within the wintering areas and in Kongsfjorden each year. Within Kongsfjorden, it has been reported that variations in trophic position from year to year resulted in varying concentrations of elements in Kittiwakes breeding in Kongsfjorden (Øverjordet, 2015a). As Arctic skuas consistently parasite Kittiwakes, varying element concentrations in Kittiwake prey may result in varying dietary exposure to e.g. Zn in Arctic skuas between years. However, the mean in yearly concentrations were small in blood and feathers, which shows that the Arctic skuas were regulating the levels of this essential element.
5.5. Implications of findings and future perspectives

Due to difficulties in tracking migratory seabirds throughout their annual cycles (Lavoie, 2014), there has generally been few studies assessing effects of bird movements on concentrations of elements or other contaminants. Recent developments in the use of GLS loggers, often in combination with stable isotope analyses, have contributed to an increasing number of studies assessing migratory movements of birds (Frederiksen, 2012, Leat, 2013, Gilg, 2013). Results from such studies should be used in combination with investigations of contaminant levels to assess whether differences in migration strategies affect concentration of potentially toxic substances in other seabird species.

A common assumption concerning element concentrations in blood is that they reflect immediate, dietary concentrations (Geens, 2010). The influence of winter concentrations of elements on blood levels during breeding in Arctic skuas implies that this assumption may not always be accurate. If one wishes to investigate local exposure, effects of migration on element levels in blood should be considered. However, as the influence of winter concentrations on blood levels has been reported to change during the breeding season in several seabird species, accounting for such effects may be challenging. The effect of winter concentrations on circulating blood levels of Hg became reduced in both Cormorants and Terns breeding in Canada, though this reduction occurred earlier in Terns than in Cormorants (Lavoie, 2014). Additionally, effects of migration on Hg and Se blood levels in Kittiwakes was suggested to become reduced during the breeding season (Øverjordet, 2015b). Such temporal changes likely vary with absorption and elimination rates of elements. To investigate if effects of migration changes during time, the study species should be repeatedly sampled during the breeding season. Such repeated sampling is not always possible, and almost impossible to perform on the Arctic skuas, due to their evasive behaviour during capture. Therefore, it may be challenging to account for local exposure to elements in birds, and one should obtain as much information as possible about effects of other seasons before making conclusions about such exposure.

In addition to increasing the time of which the Arctic skuas may be exposed to potentially harmful element levels, the observed effect of migration on element levels may also indirectly affect other species at Svalbard. Seabirds have been reported to carry species-specific mixtures of metals, varying in relation to foraging habitats and locations, to their breeding sites (Carravieri, 2014, Michelutti, 2010). Concentrations of e.g. Hg in aquatic sediments may reach toxic levels resulting from deposition from eggs, carcasses, faeces and feathers (Blais, 2005). As Hg and Cd are of environmental concern, biological transportation of these metals to the
Arctic by migratory birds, in addition to atmospheric transport and other types of anthropogenic redistribution (AMAP, 2005), must be considered when evaluating total contamination Arctic organisms may face.
6. Conclusions

In the present study, feather concentrations of Hg, Se, Cd and Zn differed among Arctic skuas migrating to America, West Africa, the Gulf of Guinea and the Mediterranean, which was hypothesized from the wide range of different feeding habitats and dietary element exposure these wintering areas represent. Feather levels of Hg, Se and Cd remained relatively consistent within each individual among years, as was expected from the consistent individual migration strategies of the Arctic skuas. The low feather REP of Zn was suggested to be due to variation in individual regulation of this essential element.

The concentrations of Hg, Se, Cd and Zn that the Arctic skuas obtained during the winters influenced the blood concentrations of these elements during the breeding seasons, suggesting that the Arctic skuas did not entirely eliminate the concentrations of these elements obtained during the winters before being sampled during the breeding seasons. The high REPs of Hg and Se in both feathers and blood suggest that the effect of migration on blood concentrations in breeding Arctic skuas is relatively consistent among years. Results concerning effects of migration on Pb levels were inconclusive because of the lack of a significant relationship between feather and blood concentrations of Pb. The results of the present study shows that effects of migration, in addition to factors such as sex, year and breeding location, should be accounted for when evaluating both year-round and local exposure to potentially toxic elements.
7. References

AMAP 2005. AMAP assessment 2002: Heavy metals in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, xvi + 265.

FENSTAD, A. A. 2012. Unpublished data on mercury concentrations in Common eider eggs from Kongsfjorden, Svalbard. NTNU.


MOE, B. Unpublished data from GLS loggers: Arctic skuas from Kongsfjorden, Svalbard, 2009-2013. NINA.


8. Appendices

Appendix A. Procedure and temperature program for UltraCLAVE, Milestone, Bergamo, Italy.
Appendix B: Concentrations of elements in feathers (µg/g, dw) of Arctic skuas (*Stercorarius parasiticus*) (N=23) from Kongsfjorden, sampled during the breeding seasons from 2010-2013. RSD (%) is included. Outliers (red) were excluded from all analyses. Means are not corrected for repeated measurements of each individual.

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Appendix C: Concentrations of elements in blood (µ g/g, ww) of Arctic skuas (*Stercorarius parasiticus*) (N=23) from Kongsfjorden, sampled during the breeding seasons from 2009-2013. RSD (%) is included. Outliers (red) were excluded from all analyses. Means are not corrected for repeated measurements of each individual.

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Appendix D. Example of plot for determining outliers for Hg (left) and Zn (right) feather concentrations: fitted values vs. standardized residuals. All residuals for Hg concentrations had values < 2.7 SD, while one measurement of Zn of individual with ring number 6223846 (7.462 µg/g, dw) had residual value < 2.7 SD. This data point was excluded from the analyses.

Appendix E. Illustration of general winter areas used by the Svalbard-breeding (red star) population of Arctic skuas (*Stercorarius parasiticus*) during the winters of 2009-2013. AM: America. WA: West Africa. GG: Gulf of Guinea. ME: the Mediterranean.
Appendix F. Estimates ± SE, degrees of freedom and p-values from linear mixed models with element concentration in feathers or blood as response variables, winter category, year, sex and breeding location as predictors. Total number of individuals (N) and number of measurements of all individuals (n) are included. For feathers, intercept is females wintering in America in 2010. For blood, intercept is females breeding on islands, wintering in America in 2009. Wintercat2: West Africa. Wintercat3: Gulf of Guinea. Wintercat4: Mediterranean.

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<th>Blood</th>
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Appendix G. Winter areas of all Arctic skuas (*Stercorarius parasiticus*) included in this study. Winter areas were determined from GLS data in a separate part of this project.

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Appendix H. Example of individual Arctic skua (*Stercorarius parasiticus*) with consistent migration strategy (ring number: 6217924). The maps show two positions per day each day during the period 1st of December (start) to 31st of January (end) in A: 2009/2010, B: 2010/2011, C: 2011/2012 (Moe et al., unpublished data).

Appendix J. Mean concentrations (µg/g ± SE) of Hg and Se in blood of Arctic skua (*Stercorarius parasiticus*) males and females from Kongsfjorden sampled during the breeding seasons from 2009-2013. Hg, males: N = 12, n = 31, females: N = 11, n = 33. Se, males: N = 11, n = 29, females: N = 11, n = 33. Groups without a common letter (a, b) are significantly different (p≤0.05).
Appendix K. Mean concentrations (µg/g ± SE) of Hg in Arctic skuas (*Stercorarius parasiticus*) breeding on islands (N = 7, n = 22) and on the tundra (N = 16, n = 42) in Kongsfjorden sampled during the breeding seasons from 2009-2013. Groups without a common letter (a, b) are significantly different (p≤0.05).