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Toxic and essential elements changed in black-legged kittiwakes (*Rissa tridactyla*) during their stay in an Arctic breeding area

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HIGHLIGHTS

- Seasonality of Cd and Hg is closely related to seasonal dietary changes.
- Migration patterns influence the accumulation of Hg and Cd.
- The seasonality of Se and Hg was similar in kittiwakes.
- The seasonality of Zn was different in liver and muscle.
- Metallothionein followed the trend of Hg and Cd.

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ABSTRACT

Seasonal fluctuations in mercury (Hg), cadmium (Cd), zinc (Zn), copper (Cu) and selenium (Se) concentrations were studied in black-legged kittiwakes (*Rissa tridactyla*) from Kongsfjorden, Svalbard (79°57′N, 12°12′E). Element concentrations were determined in muscle and liver tissue in kittiwakes collected in May, July and October 2007. Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were analysed in muscle tissue to calculate trophic position (TP) and examine the possible influence of carbon source on element accumulation. Metallothionein (MT) concentrations in liver, as well as Hg and Cd concentration in size-fractionated liver supernatant were determined to evaluate the association between elements and MT.

Mercury concentrations declined from May through July to October in both tissues, while concentrations of Cd were similar in May and July and lower in October. A decline in TP between May and July, indicating a shift from fish-based diet towards an invertebrate-based diet explains the declining Hg concentration. The low Hg and Cd concentrations in October may be a result of an increased elimination, probably related to moulting.

Selenium decreased in the same manner as Hg in liver and muscle, possibly related to the formation of Se–Hg complexes. Zinc and Cu did not fluctuate in muscle tissue, whereas hepatic Zn concentrations where highest in May. Hepatic Zn concentrations were higher in females compared to males in May, possibly related to egg production.

Hepatic MT concentrations were lower in October compared to July, following the same trend as Hg and Cd. Cadmium was predominantly bound to the MT fraction of proteins in liver tissue, whereas Hg was associated with the larger proteins, indicating that MT was not sequestering Hg in the kittiwakes.

1. Introduction

Due to its high toxicity in a variety of different organisms and the fact that it is volatile and thereby able to enter atmospheric long range transport, mercury (Hg) is the element of highest concern regarding environmental effects (Dietz et al., 2013; Obrist et al., 2008; Scheuhammer,

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1987). Atmospheric Hg in its elemental form is transported from low latitudes towards the Arctic, where it is oxidized and deposited during spring (Steffen et al., 2008). When snow and ice melt, Hg is transferred to the marine environment, potentially resulting in a higher availability of Hg to marine organisms (Poissant et al., 2008). Methyl mercury (MeHg) is the Hg species most available for bioaccumulation and magnification in the food web. In birds, most of the ingested MeHg is absorbed across the intestinal wall, in contrast to the poorly absorbed inorganic Hg (Scheuhammer, 1987). MeHg has a biological half-life of 2–3 months in several species of birds, which is significantly longer than that of inorganic Hg (1–2 weeks) (Miller et al., 1960; Monteiro and Furness, 2001; Scheuhammer, 1987). Similar to Hg, cadmium (Cd) is a toxic non-essential metal that accumulates in biota including Arctic seabirds (Dietz et al., 1996; Savinov et al., 2003). Interactions between Cd and essential elements, particularly calcium (Ca) and zinc (Zn), are important mechanisms of Cd toxicity (Moulis, 2010).

The essential elements copper (Cu), zinc (Zn) and selenium (Se) are vital for the function of a variety of biomolecules like catalytic enzymes (Momcilovic, 2004; Peganova and Eder, 2004). The essential elements are closely regulated to avoid deficiencies and excess concentrations, but bioaccumulation of these elements may still occur depending on concentrations in the environment. The accumulation of essential elements may be influenced by interactions with non-essential elements, like the formation of Hg–Se complexes (Eisler, 2010). Increased Hg concentrations facilitate Se accumulation through complex formation, a process known to protect cells and organisms against Hg toxicity (Khan and Wang, 2009).

Metal-binding proteins protects against metal toxicity by sequestering toxic metal ions preventing them from reaching target molecules (Liu et al., 1991). Metallothionein (MT) is such a small metal binding protein found in most organisms (Beyer et al., 1996). Two isoforms of avian MT have been characterized, each constituted of approximately 63 amino acids (Nam et al., 2007). Metallothionein is known to play a protective role when an organism is subjected to chronic metal exposure with variable affinity for different metals (Merian et al., 2004; Nam et al., 2007).

Elements are absorbed and eliminated continuously through different routes with the combined rates of both processes determining the body burden at a given time. The primary route of absorption in seabirds is intestinally through ingested food, whereas elimination can take place through urine and faeces, moulting, skin exfoliation and egg laying (Monteiro and Furness, 2001; Nichols et al., 2010; Scheuhammer, 1987). In birds, the seasonal variation in element concentrations is species specific and dependent on many factors such as dietary changes, moulting sequence, migration patterns and reproduction strategies (Becker et al., 1994; Braune, 1987; Honda et al., 1986a). Knowledge about seasonal fluctuations of accumulated elements is important in spatial and temporal trend analysis to avoid erroneous conclusions based on asynchronous sampling. Element concentrations of internal tissues and feathers of black-legged kittiwake (*Rissa tridactyla*) have been reported, both from the Canadian Arctic (Braune, 1987; Campbell et al., 2005) and the Barents Sea area including Kongsfjorden, Svalbard (Jæger et al., 2009; Savinov et al., 2003). However, to our knowledge, the seasonality of internal element concentrations of kittiwakes has not previously been studied.

The aim of this study was to examine whether concentrations of toxic metals and essential elements changed in kittiwakes during their stay in the breeding area in an Arctic fjord between May and October. Fluctuations of element and the MT concentrations were explained in relation to diet, migration, sex and moulting.

2. Materials and methods

2.1. Study species

The black-legged kittiwake is a small pelagic gull with a global population of 6–8 million pairs of which 120000 pairs breed on the west coast of Spitsbergen, Svalbard (Frederiksen et al., 2012). The kittiwakes breeding on Svalbard migrate towards Greenland and Canada in the end of October, overwintering in a widespread area in the North Atlantic between November and April (Frederiksen et al., 2012; Schultner et al., 2014). Kittiwakes have a varied diet, preying on small pelagic fish like polar cod (*Boreogadus saida*) and capelin (*Mallotus villosus*), as well as pelagic amphipods (*Themisto* spp.) and euphausiids (*Thysanoessa* spp.) (Mehlum and Gabrielsen, 1993). Other small fish species (15–20 cm), polychaetes and pteropods are also frequent in the diet of kittiwakes (Hatch, 2013). Seasonal and spatial variations in kittiwake diet occur (Anker-Nilssen et al., 2000; Lønne and Gabrielsen, 1992). During the breeding season, adult kittiwakes in Kongsfjorden feed mainly on polar cod and krill (*Thysanoessa inermis*) (Mehlum and Gabrielsen, 1993).

2.2. Field sampling of birds

Field samples were collected in Kongsfjorden, Spitsbergen (79°57′N, 12°12′E) in May, July and October 2007. The field sampling was a part of a large project studying contaminant dynamics in polar areas (IPY-COPOL). Ten adult kittiwakes were sampled each month using a shot gun. Liver tissue samples were immediately dissected out and frozen in liquid nitrogen for MT analysis (in July and October). Subsequently, samples of liver and pectoralis muscle were collected for element and stable isotope analysis. The body mass (g) was recorded with an accuracy of 5 g (Table 1). Wing length (cm), gonys depth (mm), head–bill length (mm) and tarsus length (mm) were measured using a calliper. The sex was determined by gonad examination. Permission for sampling was given by the Governor of Svalbard.

2.3. Element analysis

Muscle and liver tissue were lyophilized for 24 h prior to digestion. Dry samples (~0.15 g) were transferred to PTFE-vials (18 mL) together with ultrapure water and nitric acid (4.2 g; HNO_3; Scanpure) prior to digestion using a high pressure microwave emitter (Milestone Ultra Clave, EMLS, Leutkirch, Germany). The samples were diluted in ultrapure water to a final volume of 60 mL (0.6 M HNO_3). Total Hg, Cd, Zn, Cu and Se were determined by high resolution inductively coupled plasma mass spectrometer (HR–ICP-MS; Thermo Finnigan model Element 2 instrument, Bremen, Germany). Samples of each tissue were grouped to limit carryover and randomized with respect to sampling month. The results are reported on dry-weight basis. No concentrations were below the detection limits. The average relative standard deviations (RSD) of multiple scans were below 3% for all elements. Blank samples and the standard reference materials bovine liver (NIST 1577b), oyster tissue (NIST 1566b) and chicken (GBW 10018) were included ($n > 6$). The recovery of Se was 114, 123 and 102% in bovine liver, chicken and oyster, respectively. Mercury was only certified in oyster tissue, with a recovery of 105%. Cadmium recovery in bovine liver and oyster tissue were 100 and 101%, respectively. Two samples were removed from the dataset due to possible contamination; one liver sample from July and one from October. These were identified by high Cd coinciding with high bismuth (Bi) values after analysis (results not reported).

2.4. Preparation of samples for metallothionein (MT) analysis

Prior to MT analysis, liver tissue samples (0.10–0.15 g) from July ($n = 10$) and October ($n = 9$) were homogenised in Tris-buffer (20 mM, pH 7.4; Sigma-Aldrich) in a 1:9 weight:volume ratio using a Potter–Elvehjem Teflon-glass homogeniser. The homogenate was centrifuged (10,000 × g, 10 min, 4 °C) and the supernatant analysed for MT. Aliquots of supernatant were stored at −80 °C prior to analysis. Care was taken to limit carryover and contamination, and the samples were kept on ice during the entire procedure to prevent protein degradation.

Table 1
Biometric data for kittiwakes (*Rissa tridactyla*) sampled in Kongsfjorden in May, July and October 2007. All values in mean ± sd.

Sampling date	Sex	n	Body mass (g)	Wing length (cm)	Gonys depth (mm)	Head-bill (mm)	Tarsus length (mm)
May 11th 2007	Male	4	474 ± 16	33.0 ± 0.9	11.1 ± 0.64	92.1 ± 3.8	42.0 ± 1.7
	Female	6	397 ± 57	31.0 ± 1.8	11.0 ± 0.28	87.1 ± 3.6[a]	39.9 ± 0.5[b]
July 21st 2007	Male	7	386 ± 41	32.3 ± 0.7	10.8 ± 0.35	90.8 ± 2.9	40.7 ± 1.1
	Female	3	366 ± 12	31.4 ± 0.4	10.4 ± 0.20	86.9 ± 3.0	37.7 ± 0.6
October 5th and 6th 2007	Male	8	461 ± 23	31.3 ± 1.4	10.5 ± 0.34	93.6 ± 2.1	38.9 ± 3.9
	Female	2	344 ± 27	30.8 ± 1.1	9.1 ± 0.64	86.1 ± 1.1	38.7 ± 0.2

[a] n = 4.
[b] n = 5.

2.5. Metallothionein quantification

The MT concentration in supernatant of liver samples was determined using the Cd saturation method described by Bartsch et al. (1990). The assay was performed in 1.5 mL Eppendorf tubes incubated on a Vibrax mixer between each step (1200 rpm; Heidolph, Swabach, Germany). The entire procedure was performed in a climate room (4 °C). Samples (100 μL) were mixed with acetonitrile (100 μL; C_2H_3N, Merck KGaA, Darmstadt, Germany) to denature large proteins. After 3 min incubation the samples were diluted in Tris buffer ("Buffer A"; 10 mM Tris–HCl, 85 mM NaCl, 7.4). A mixture of the radioactive isotope $^{109}Cd^{2+}$ and stable Cd^{2+} were added, allowing the isotopes to compete for binding sites on the MT proteins. After 3 min incubation Chelex 100-resin (Bio-Rad Laboratories, Hercules, CA, USA) was added to bind the excess ^{109}Cd. After 15 min incubation the samples were centrifuged (12,000 × g). Blanks containing "Buffer A" and Chelex and samples for total activity containing "Buffer A" and Cd-solution without Chelex were prepared for each run (n = 2). The activity of ^{109}Cd in the supernatant (900 μL) was measured using a gamma counter (Cobra II Auto-Gamma, Packard Instruments Company, Dowers Grove, IL, USA). The MT concentration was calculated by Eq. (1), where CPM_S is counts per minute activity in the sample, CPM_{Bg} is counts per minute activity in the blank and CPM_T is the counts per minute activity in the total sample without Chelex. A concentration of 263 nmol/mL Cd was added to the samples. Multiplication by 1/7 refers to 7 binding sites of Cd on MT; multiplication by 10 refers to dilution of the tissue homogenate, whereas multiplication by 1.49 refers to the dilution factor of the assay. The results are reported on wet-weight basis.

$$MT(\text{nmol/gw}\cdot\text{w}) = \frac{CPM_S - CPM_{Bg}}{CPM_T} * 263 \text{ nmol/mL} * {}^{1}\!/_{7} * 10 * 1.49. \quad (1)$$

2.6. Size-fractionation of liver homogenate

Gel filtration chromatography of supernatant of liver homogenate was performed using the method described by Andersen and Daae (1988). Radioactive Cd ($^{109}Cd^{2+}$; 1 μL) was equilibrated with liver supernatant (500 μL) before size fractionation on a sephadex G-75 gel column (Sigma-Aldrich, St. Louis, MO, USA). Absorbance at 254 nm was measured on the column eluate, indicating metal-thiolate bounds. The activity of ^{109}Cd in individual fractions was measured using a gamma counter (Cobra II Auto-Gamma). Six representative liver samples were examined. The flow rate was in the range 01:33 to 01:37 (mm:ss) for all six samples.

Pooled samples of supernatant were size-fractionated without the addition of ^{109}Cd. Each pooled sample consisted of 300 mL of supernatant from three kittiwakes sampled within the same month. The samples were thoroughly mixed before 700 μL were added to the sephadex G-75 gel column. The flow rate was in the range 01:55 to 01:58 (mm:ss) for all samples. Ultrapure HNO_3 (0.6 M, 0.9 ± 0.02 g) and ion exchanged water (4.76 ± 0.05) was added to each fraction prior to digestion using a block heater at 110 °C for 2 h. Diluted fractions (15 mL total volume) were analysed for Hg and Cd using HR–ICP-MS.

2.7. Stable isotopes of carbon and nitrogen

Stable isotope ratios were analysed in muscle tissue according to Hussey et al. (2010) as described by Hallanger et al. (2011). The ratios are expressed in δ notation as the deviation from standard in parts per thousand (‰) according Eq. (2):

$$\delta X(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \cdot 1000 \quad (2)$$

where δX is the delta value of ^{13}C and ^{15}N (‰) and R is the molar ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and in an international standard, respectively.

A trophic enrichment factor of 2.4‰ for ^{15}N between seabirds and their prey was assumed, resulting in the following equation: $\delta^{15}N_{bird} = \delta^{15}N_{prey} + 2.4$ (Mizutani et al., 1991). For the rest of the food web an enrichment factor of 3.8 was applied (Hobson and Welch, 1992). Trophic position (TP) for kittiwakes was calculated relative to *Calanus finmarchicus* at TP 2 by the following equation (data from Hallanger et al., 2011):

$$TP_{bird} = 3 + \frac{\delta^{15}N_{bird} - \left(\delta^{15}N_{C.finmarchicus} + 2.4\right)}{3.8}. \quad (3)$$

2.8. Statistical analysis

Statistical analyses were performed in R (v2.13.1; R Development Core Team). Equality of variance was tested using Levene's test. Uneven variance was indicated for Hg, Se and Se:Hg-ratio ($p < 0.05$). The distribution of variables was tested using QQ-plots and Shapiro–Wilk normality test. Deviation from normal distribution was found for Hg, Se, Se:Hg-ratio and TP. Hence, these variables were log-transformed to fulfil the criteria of normality and equal variance and parametric tests were applied. One-way ANOVA with Tukey HSD post hoc test and the *t*-test were used for group comparison. The level of significance was set to $p < 0.05$. However, $p < 0.1$ and $p > 0.05$ was included to indicate trends in the material. Linear models and Pearson correlations were used to examine the relationship between variables.

Literature values given on a wet-weight basis was recalculated to dry weight using the average dry content of liver samples (34.3 ± 7.7%, n = 40) and muscle samples (31.2 ± 2.8%, n = 59) included in the present paper and in Øverjordet et al. (submitted for publication).

3. Results and discussion

3.1. Seasonality of mercury and cadmium

Mercury concentrations declined in both liver and muscle of kittiwakes from May through July to October ($p < 0.001$; Fig. 1). This is in accordance with the decline in Hg concentrations from April through to

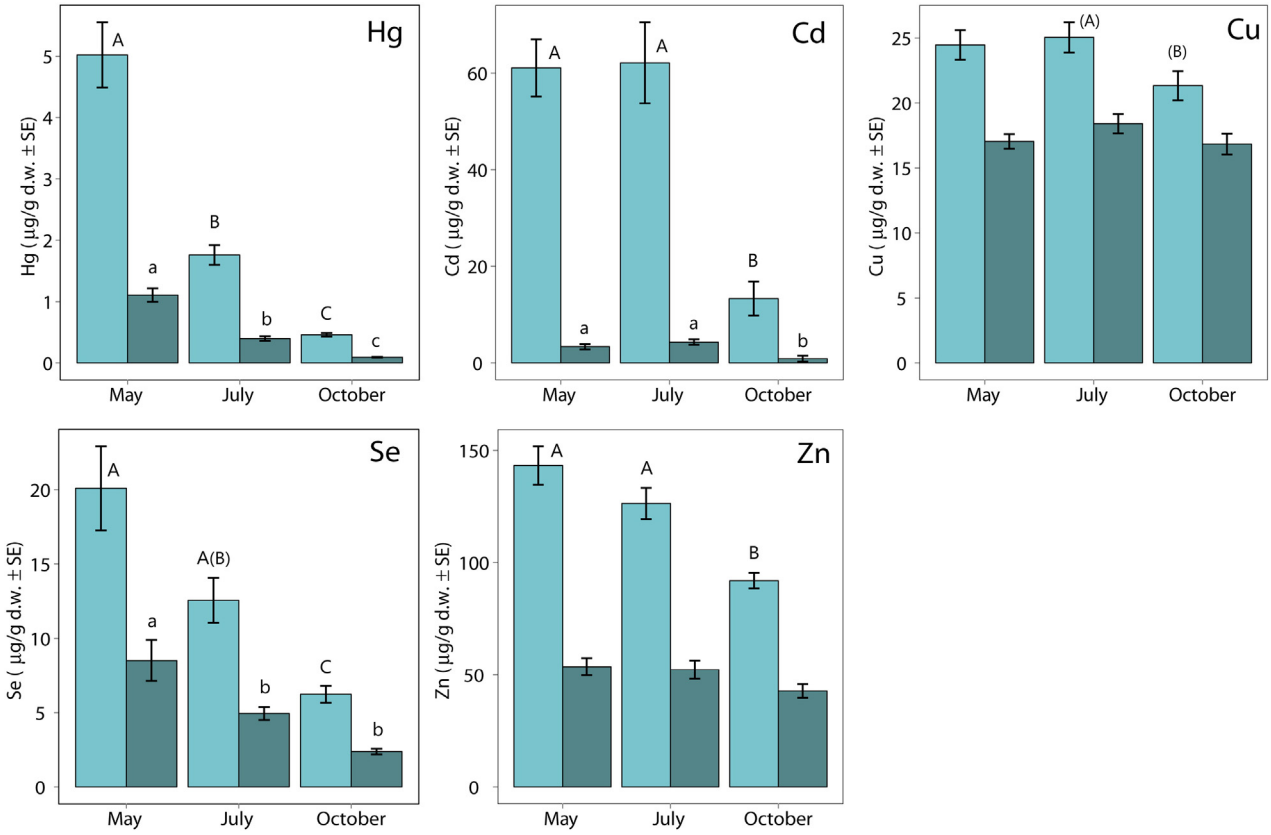

Fig. 1. Concentrations of Hg, Cd, Zn, Se and Cu (µg/g dry weight) in liver (light) and muscle (dark) of black-legged kittiwake (*Rissa tridactyla*) sampled in May, July and October in Kongsfjorden, Svalbard. Significantly different groups are indicated with upper- and lowercase letter for liver and muscle, respectively. Letter in parentheses indicate $0.1 > p > 0.05$. Both sexes are represented. $n = 10$, except liver in July and October where $n = 9$.

November in common guillemots (*Uria aalge*) and black-eared kites (*Milvus migrans lineatus*) (Honda et al., 1986a; Stewart et al., 1994). The concentrations of Hg in liver (5.02 ± 1.68 µg/g) and muscle (1.10 ± 0.35 µg/g) of the kittiwakes sampled in May were similar to concentrations reported in kittiwakes sampled in May and June 1998 in the Canadian Arctic (3.35 and 0.958 µg/g recalculated dry weight in liver and muscle, respectively; Borgå et al., 2006). Likewise, the Hg concentrations in liver (1.76 ± 0.49 µg/g) and muscle (0.40 ± 0.12 µg/g) in the kittiwakes sampled in July were similar to concentrations reported in kittiwakes sampled in Kongsfjorden in July 1991 (liver 1.95 µg/g; muscle: 0.43 µg/g; Savinov et al., 2003) and in July 2008 and 2009 (Liver: 2.27 ± 0.81 µg/g; Øverjordet et al., submitted for publication). The concentrations in the kittiwakes sampled in October (liver: 0.46 ± 0.09; muscle: 0.09 ± 0.01 µg/g) were to our knowledge lower than previously reported in this species (e.g., Borgå et al., 2006; Savinov et al., 2003). Based on the average elimination of hepatic Hg in kittiwakes between May and July (0.047 µg/day) and between July and October (0.017 µg/day), the half-time of Hg was 53 between May and July and 52 days between July to October. This was similar to the half-times found in great skuas (*Stercorarius skua*) and Cory's shearwaters (*Calonectris diomedea*) after dietary MeHg exposure (Bearhop et al., 2000; Monteiro and Furness, 2001).

The Cd concentrations in both liver and muscle were similar in May and July, but lower in October (Tukey HSD, $p < 0.001$; Fig. 1). Similar seasonal fluctuations have been found in common guillemots; Stewart et al. (1994) reported a significant rise in hepatic Cd concentration from April to June, followed by a decrease from June to November. The Cd concentrations in the kittiwakes sampled in July (4.30 ± 1.67 and 62.1 ± 25.2 µg/g in muscle and liver, respectively) were higher than reported in kittiwakes sampled in the Barents Sea and Kongsfjorden at the same time of year (Savinov et al., 2003).

3.1.1. The effect of diet composition

Mercury biomagnifies in Arctic marine food webs and the Hg concentrations in seabirds have been closely linked to diet composition and TP (Becker et al., 2002; Borgå et al., 2006; Campbell et al., 2005; Jæger et al., 2009; Lock et al., 1992; Øverjordet et al., submitted for publication). The kittiwakes collected in May were feeding at a higher TP (4.29 ± 0.08) than the kittiwakes collected in July (3.26 ± 0.10) and October (3.21 ± 0.10; $p < 0.001$). This suggests that the kittiwakes changed from diet dominated by fish to a diet predominantly constituted of invertebrates. Fish accumulate higher concentrations of Hg compared to crustaceans, predominantly as MeHg (Campbell et al., 2005; Jæger et al., 2009). Becker et al. (2002) found a negative correlation between the relative amount of crustaceans in the diet and Hg concentrations in 11 species of seabirds. Thus, the shift in TP between May and July could partly explain the difference in Hg concentrations between these two seasons (Fig. 1).

The Cd concentrations in kittiwakes were stable between May and July (Fig. 1). In muscle tissue, the mean Cd concentration seemed to be higher in July (4.30 µg/g) than in May (3.37 µg/g), but the difference was not significant. Crustaceans accumulate relatively high levels of Cd compared to fish (Macdonald and Sprague, 1988; Rainbow, 1989; Ritterhoff and Zauke, 1997). Thus, although a change in diet from fish to crustaceans lowers the dietary Hg exposure, it may maintain or increase the dietary Cd exposure. This could explain the divergent seasonality of Hg and Cd accumulation observed in the kittiwakes (Fig. 1). The decline in Cd between July and October seems contradictory, but this may be related to changes in dietary exposure or increased elimination, factors discussed in Sections 3.1.3 and 3.1.4. Similar fluctuations of Cd and Hg were found in common guillemots by Stewart et al. (1994): the concentration of Hg decreased, while the concentration of Cd increased in guillemots between April and June. This was attributed to a

higher consumption of zooplankton during the summer. In the present kittiwakes, the decline in TP combined with stable Cd and decreasing Hg concentrations between May and July suggests that dietary changes may explain parts of the seasonality of element concentrations in kittiwakes. The decline in Cd between July and October seems contradictory, but this may be related to changes in dietary exposure or increased elimination, factors discussed in Sections 3.1.3 and 3.1.4.

3.1.2. Dietary exposure in relation to migration

The higher concentration of Hg in kittiwakes collected in May than in July may also be related to different exposures in their wintering grounds compared to their breeding grounds at Svalbard (Frederiksen et al., 2012; Gonzalez-Solis et al., 2011). The relatively long turnover rate of stable isotopes in avian muscle tissue enables the assessment of metal concentration in relation to migration and diet. The winter diet of kittiwakes is not well known, but the higher TP of the kittiwakes sampled in May, shortly after their arrival in the breeding area, indicates that winter diet was dominated by fish. In addition, the range of hepatic Hg concentrations was wider in May (2.46–8.10 μg/g) than in July (1.12–2.78 μg/g) and October (0.37–0.61 μg/g) indicating larger individual variation in Hg accumulation in birds sampled in May. This suggests that distribution during winter influences Hg accumulation in individuals differently due to variations in feeding habits or similar feeding but different exposure due to local variation in contaminants. The use of the wintering habitat may vary significantly between individual kittiwakes (McKnight et al., 2011). Although kittiwakes breeding in Kongsfjorden mainly overwinter in the northwestern Atlantic off the coasts of Greenland and Newfoundland, their distribution during winter stretches across the Atlantic into the Norwegian Sea (Frederiksen et al., 2012). Mercury concentrations in capelin and polar cod are higher in the northwestern Atlantic compared to the Barents Sea and Svalbard (Braune et al., 2014a; Jæger et al., 2009). In addition, Hg concentrations are in general higher in polar cod (0.11–0.19 μg/g) than in capelin (<LOD–0.010 μg/g) in both areas (Braune et al., 2014a; Campbell et al., 2005; Jæger et al., 2009). Polar cod is a cold-water species associated with sea ice, while capelin is found in warmer Atlantic water. The diet of the kittiwakes foraging close to the ice edge of the Labrador Sea may contain a larger proportion of polar cod than capelin, resulting in elevated Hg accumulation in these kittiwakes. This would be consistent with the higher Hg concentrations in Brünnich's guillemots (*Uria lomvia*) of the more northern colonies at the Canadian Atlantic coast compared to those of the southern colonies, suggested to be related to spatial differences in polar cod and capelin consumption (Braune et al., 2014b). Although the overwintering location of the present kittiwakes is unknown, their migration pattern may have contributed to the higher and more variable Hg concentration within the kittiwakes sampled in May compared to the kittiwakes sampled in July and October.

3.1.3. Carbon source and element accumulation

Average TP of the kittiwakes sampled in October did not differ from that of the kittiwakes collected in July, indicating that the decline in Hg and Cd between these two months was less dependent on the TP of the prey. The $\delta^{13}C$ in kittiwakes were stable between May and July, but decreased significantly between July and October (Fig. 2). This indicates a diet less enriched in carbon of inshore origin in October compared to July (Post, 2002). Accumulation of Hg in some marine bird species has been related to inshore sources, indicated by positive correlations between $\delta^{15}N$–Hg residuals and $\delta^{13}C$ (Bond and Diamond, 2009). Terrestrial Hg and Cd may have had a higher influence on the bioaccumulation in prey items of kittiwakes in July compared to October, as they may have been feeding closer to the shore during the breeding period. The lower $\delta^{13}C$ in October indicates increased offshore feeding in the post-breeding period. Hence, parts of the decline in Hg and Cd in the kittiwakes may potentially be explained by a decrease in dietary exposure between the two months. Further investigations are recommended to determine whether the link between $\delta^{13}C$ and the influence of terrestrial elements is significant for seabirds in Svalbard.

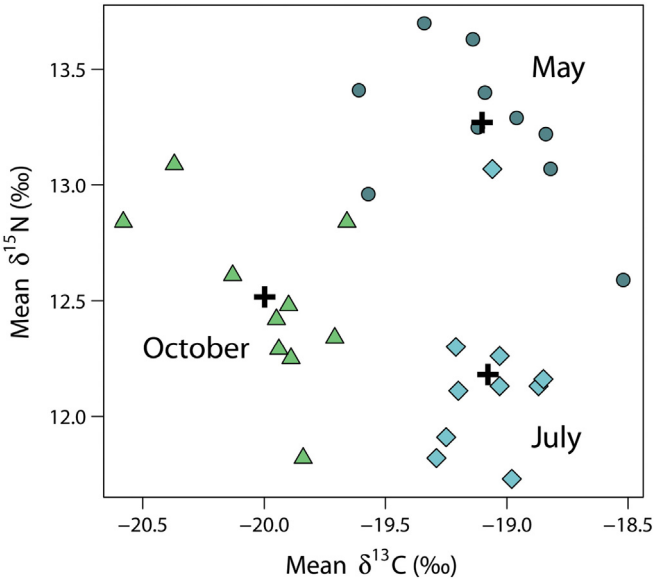

Fig. 2. Ratio of stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes (‰) in muscle tissue of kittiwakes (*Rissa tridactyla*) collected in May (○), July (◊) and October (Δ) in Kongsfjorden, Svalbard. The mean of each season is marked with a cross. n = 10.

3.1.4. The effect of moulting

Parts of the decrease in Hg and Cd between July and October may also be explained by increased elimination. Incorporation of Hg into growing feathers has been pointed out as an important excretory pathway in birds (Honda et al., 1986a; Monteiro and Furness, 2001). Kittiwakes moult their primary feathers twice a year: in March–April and from the end of August until the end of November (Hatch et al., 2009). In addition, the body feathers are moulted continuously from May to the end of November. High Hg concentrations have been reported in kittiwake feathers (Burger et al., 2008). In Cory's shearwaters exposed to MeHg, only 8% of the Hg was recovered in feathers of individuals exposed one month before the onset of moulting, compared to a recovery of 33% in feathers of individuals exposed during the moulting period (Monteiro and Furness, 2001). Elimination through faeces was assumed to be the most important elimination route of the shearwaters outside the moulting season. Hence, elimination by faeces and moulting of body feathers would facilitate a net decline in internal Hg concentrations throughout the breeding season in the kittiwakes, while the second moult of the primary feathers may have contributed to the decline between July and October.

3.1.5. Potential confounding factors

The uneven sex distribution of the kittiwakes caught in May, July and October could interfere with the seasonal comparison (Table 1). Several studies have shown that Hg and other elements are transferred from mother to eggs, which may lead to higher internal Hg concentrations in males than in females (Dietz et al., 2013; Miljeteig et al., 2010; Monteiro and Furness, 2001). Although sex differences in Hg concentrations have been linked to egg production (Becker et al., 2002; Robinson et al., 2012), some authors argue that the amount of Hg eliminated to eggs is negligible compared to the total body burden of the mother (Helander et al., 1982; Honda et al., 1986b). Mercury concentrations did not differ between males and females in the present study. It should be noted that the small sample size may limit the power to detect differences between sexes in the present study. However, similar results have been reported for several seabird species (Borgå et al., 2006; Stewart et al., 1994).

The age distribution within each group of kittiwakes may have influenced the seasonal variation in element concentration. Age determination is not possible in adult kittiwakes, making it difficult to assess the effect of age in this species. However, one bird caught in October was ring marked, with an age of at least four years. One other individual was a juvenile of the year, while the remaining birds were all more than one year old. The element concentrations in these individuals were similar to the element concentrations in the other kittiwakes. In most cases no correlation has been found between age and element concentrations in adult birds (Agusa et al., 2005; Burger et al., 1994, 2008; Hutton, 1981; Nielsen and Dietz, 1989). Hence, we assume that potential differences in age distribution did not confound the seasonality observed in Hg and Cd levels in the present study.

3.2. Seasonality of selenium

Hepatic Se concentrations of the kittiwakes followed the decreasing Hg concentration from May to October (Fig. 1); concentrations were lower in July compared to May (Tukey HSD, $p = 0.061$) and lower in October compared to July (Tukey HSD, $p = 0.003$). The correlation between Se and Hg was significant both in liver ($r = 0.741$, $p < 0.001$) and muscle ($r = 0.734$, $p < 0.001$). Significant correlations between Hg and Se have been reported in several Arctic seabird species (Borgå et al., 2006; Dietz et al., 2000; Nielsen and Dietz, 1989). In liver tissue of the present kittiwakes the molar ratio between Se and Hg (Se:Hg) was significantly lower in May (10.6 ± 4.8) compared to July (19.1 ± 7.4; Tukey HSD, $p = 0.008$) and in July compared to October (35.6 ± 12.0; Tukey HSD, $p = 0.006$). A strong negative correlation was found between the Se:Hg ratio and hepatic Hg ($r = -0.835$, $p < 0.001$) but not between the Se:Hg ratio and hepatic Se concentration, indicating that the Se:Hg molar ratio was mainly determined by the Hg concentration. Selenium is known to protect against Hg toxicity by the formation of Hg–Se complexes at Se:Hg molar ratios of >1 (Khan and Wang, 2009). At Se:Hg ratios of <1, the protective effect of Se is thought to be lost. Although some of the Se:Hg ratios were relatively low in May (5.25–18.76), none of the values were below the critical threshold of 1, indicating a low risk of Hg toxicity in the kittiwakes of the present study.

3.3. Seasonality of zinc and copper

Concentrations of Zn and Cu were expected to vary less than Cd and Hg, as essential metals are subjected to physiological regulation. As predicted, Zn and Cu concentrations in muscle tissue did not differ between the kittiwakes sampled in May, July and October (Fig. 1). The seasonality of hepatic Zn was similar to that of Cd, with comparable concentrations

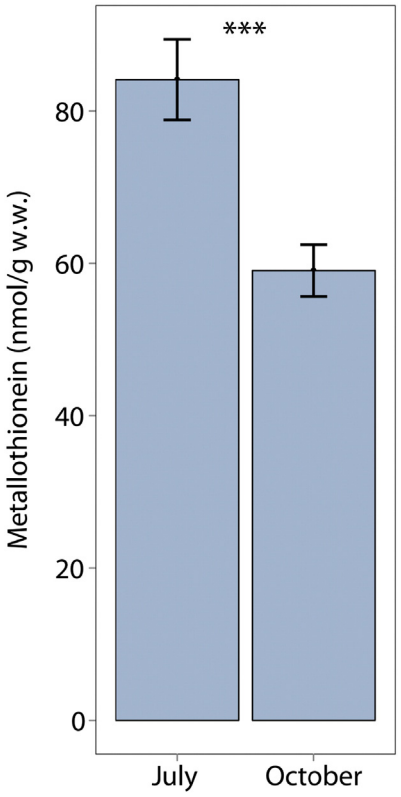

Fig. 4. Hepatic metallothionein concentrations (nmol/g wet weight) in kittiwakes (*Rissa tridactyla*) sampled in July and October in Kongsfjorden, Svalbard. Median concentrations were 917 and 643 μg MT/g wet weight in July and October, respectively. *** = $p < 0.001$.

in May (143 ± 27.1 μg/g) and July (126 ± 20.9 μg/g), and lower in October (92.0 ± 10.4 μg/g; Tukey HSD, $p < 0.01$). The decrease in hepatic Cu between July (25.0 ± 3.48 μg/g) and October (21.3 ± 3.35 μg/g) was however not significant (Tukey HSD, $p = 0.08$). Compared to Cd and Hg, the seasonality of Zn and Cu in liver tissue was not well reflected in muscle tissue of the kittiwakes (Fig. 1).

Birds undergo physiological changes in nutrient uptake, including that of elements like Zn and Cu, during reproduction (Farner and King, 1973). The kittiwake's breeding season spans from May to mid-August in Svalbard (Gabrielsen, 2009). Hence, an increased uptake of Zn linked to egg production may explain the higher hepatic concentrations in May and July compared to October (Fig. 1). This is supported by the higher

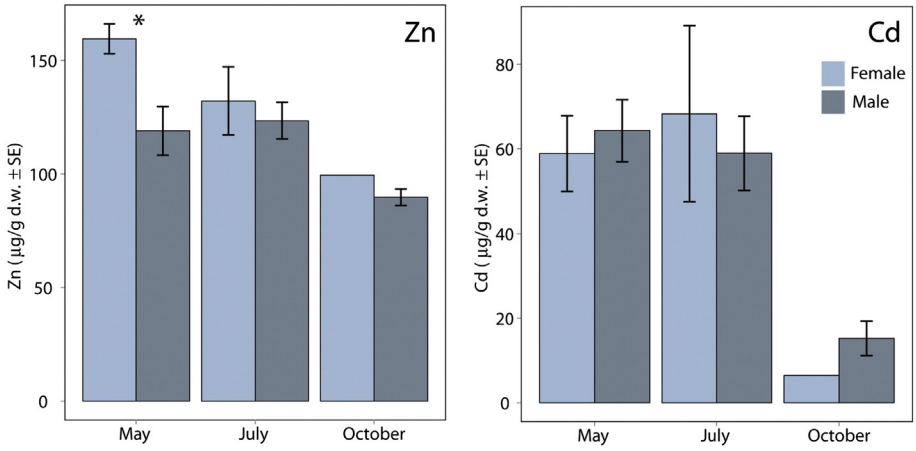

Fig. 3. Hepatic Zn and Cd concentrations (μg/g dry weight) in female (light) and male (dark) kittiwakes (*Rissa tridactyla*) sampled in May, July and October in Kongsfjorden, Svalbard. Significantly higher Zn concentrations in females compared to males in May (*; $p = 0.041$). Error bars are not shown for females in October ($n = 2$).

hepatic concentrations of Zn found in females (160 ± 16.0 μg/g) compared to males (119 ± 21.5 μg/g) in May (*t*-test, $p = 0.041$; Fig. 3). The higher concentrations of hepatic Zn in females may be a result of Zn associated with the egg yolk precursor vitellogenin (Vanderkist et al., 2000), supported by high levels of Zn transferred to eggs (Malinga et al., 2010; Miljeteig et al., 2010). Due to similarities in ionic properties, Cd and Zn tend to vary concordantly in nature and wildlife (Braune and Scheuhammer, 2008). Although a strong correlation was found between hepatic Zn and Cd ($r = 0.763$, $p < 0.001$), there was no evidence of higher Cd concentrations in females compared to males in May (Fig. 3).

3.4. Metallothionein

The concentration of MT was higher in July than in October (median 916.7 and 642.7 μg/g, respectively; $p < 0.001$, Fig. 4). Thus, the MT concentration followed the decrease of Hg, Cd and Zn concentrations between July and October (Fig. 1). These metals bind to MT and are known to induce MT synthesis after exposure in a variety of organisms (Valko et al., 2005). The MT concentrations were positively correlated to hepatic element concentrations in the following order (July and October samples combined): Cd ($r = 0.921$, $p < 0.001$) > Zn ($r = 0.858$, $p < 0.001$) > Hg ($r = 0.738$, $p < 0.001$) > Cu ($r = 0.593$, $p < 0.01$) > Se ($r = 0.479$, $p < 0.05$). The molar ratio between Se and Hg (Se:Hg) was negatively correlated to MT ($r = -0.553$, $p < 0.05$), suggesting that Se may have had an influence on the association between Hg and MT. Negative correlation between the Se:Hg ratio and MT have previously been reported in fish (Sørmo et al., 2011).

Size-fractionation of homogenised liver supernatant was performed to investigate the associations between Cd, Hg and MT (Fig. 5). Two absorbance peaks were detected, the largest indicating the high molecular weight fractions (HMW) and the smaller indicated the low molecular weight fractions (LMW). Tracing MT using radioactive ^{109}Cd detected the fractions containing metal binding proteins (MBP) between the two absorbance peaks (Fig. 5). Mercury was predominantly associated with the HMW fractions while most of the Cd was recovered in the MTP fractions both in July and in October (Fig. 5). The concentrations of both metals were lower in the fractions from October compared to July, corresponding to the decline in hepatic concentrations between the two seasons (Fig. 1). Similarly, most of the Hg was recovered in the HMW fraction of porcine liver after Hg exposure, with a minor recovery in the MBP fraction (Chen et al., 2006). The present results indicate that MT was not sequestering Hg in the kittiwakes, and the recovery of Hg in the HMW fraction could potentially disturb the function of larger proteins.

Metallothionein has mainly been studied in the kidneys of some Arctic seabird species: Braune and Scheuhammer (2008) reported highly variable MT concentrations in kidney tissue of five Arctic seabirds ranging between 177 and 5421 μg/g wet weight. Both the lowest and the highest concentrations were found within the same species, the Brünnich's guillemot, collected at different localities within the Canadian Arctic. In the same study they reported an average MT concentration of 1750 ± 1251 μg/g wet weight in kidney of black-legged kittiwakes. This is higher than the concentrations found in kittiwake liver in the present study. Hepatic concentrations of MT are expected to be lower than MT concentrations in kidney, due to a generally higher Cd

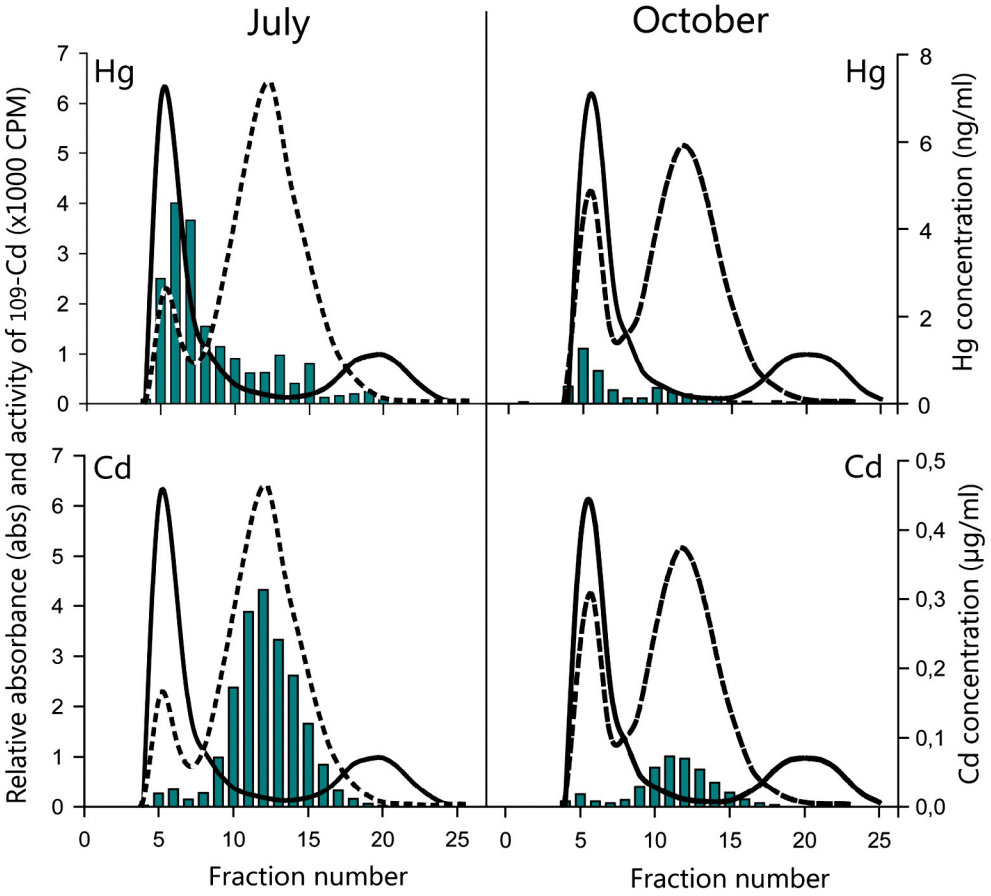

Fig. 5. Relative absorbance (abs 254 nm; solid line; left axis), activity of spiked ^{109}Cd (CPM; dashed line; left axis) and concentrations (bars; right axis) of Hg (ng/mL) and Cd (μg/mL) in protein fractions of homogenised liver tissue supernatant from kittiwake (*Rissa tridactyla*) sampled in July and October in Kongsfjorden, Svalbard. High molecular weight (HMW) protein fractions: 4–7; metal binding protein (MBP) fractions: 8–16; low molecular weight (LMW) protein fractions: 17–25.

concentration in kidney tissue. However, MT in liver and kidney of seabirds nesting on Reunion Island in the Western Indian Ocean did not differ significantly between the two organs in two out of three species (Kojadinovic et al., 2007). Thus, in some species the levels of MT in the kidney and the liver are similar. However, this relationship is not yet established in kittiwakes. Mean hepatic MT concentrations of 11,440 ± 6440, 6010 ± 4870 and 9220 ± 5860 μg/g dry weight were reported in Barau's petrels (*Pterodroma baraui*), Audubon's shearwaters (*Puffinus lherminieri*) and white-tailed tropicbird (*Phaethon lepturus*), respectively (Kojadinovic et al., 2007). These concentrations are all high compared to the mean hepatic MT concentrations of 1910 and 2722 μg/g dry weight (July and October, respectively) in the liver of the present kittiwakes. However, due to the influence of tissue type, species, season and geographical location on MT levels in seabirds, it is difficult to conclude whether the levels of MT in the present kittiwakes were different from the kittiwakes from the Canadian Arctic and other seabirds.

4. Conclusions

Seasonality was generally more pronounced for Hg and Cd than for the essential elements Cu, Zn and Se. Physiological regulation was probably the most important factor explaining the Zn and Cu concentrations, including the higher hepatic Zn concentrations in females compared to males in May. Mercury decreased consistently throughout the period from May to October. The decrease between May and July may be related to changes in diet composition and changes in exposure related to migration, while the decrease between July and October is possibly related to the second moulting of primary feathers between these months. The Cd concentrations were stable between May and July, suggested to be related to an increased proportion of zooplankton in the diet in July. The background for the decrease between July and October is less clear, but may be related to lower terrestrial influence of elements in October. Size-fractionations of liver homogenate supernatant revealed that most of the Cd was associated with MT, while most of the Hg was associated with larger proteins. This implies that MT has a minor role in sequestration of Hg in kittiwakes. Significant correlations between Hg and Se indicate that Se–Hg complexes formed that may have played a role in protection from Hg toxicity. The values of the Se:Hg molar ratio were relatively low in May compared to July and October; however, no values were below 1, suggesting low risk of Hg toxicity in the present kittiwakes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.09.058.

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