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Exposure of a small Arctic seabird, the little auk (*Alle alle*) breeding in Svalbard, to selected elements throughout the course of a year

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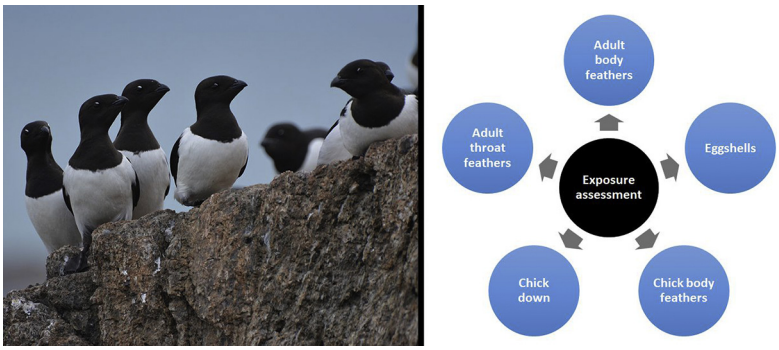
HIGHLIGHTS

- Elemental concentration was examined in feathers and eggshells of little auks.
- Differences in pre- and post-breeding exposure found for part of the elements
- High mercury level found in adults throat feathers
- Chick down can be a valuable indicator of chicks maternal input.

GRAPHICAL ABSTRACT



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ABSTRACT

The Arctic marine ecosystem can be altered by processes of natural and anthropogenic origin. Spatio-temporal variation in species exposure to contamination is still poorly understood. Here, we studied elemental concentrations in the non-lethally collected samples from the most numerous seabird in European Arctic, the little auk (*Alle alle*) nesting in one breeding colony in Svalbard. This seabird spent the breeding season in the high-Arctic zone and the non-breeding period in sub-Arctic areas what may implicate spatio-temporal variation in elements bioaccumulation. We determined concentrations of 19 elements in adults feathers to determine levels of exposure during part of the pre-breeding ($n = 74$) and post-breeding ($n = 74$) seasons, feathers from nestlings ($n = 18$) to determine local contamination, and chick down ($n = 16$) and post-hatching eggshells ($n = 18$) to determine maternal input to offspring. During the pre-breeding period adults accumulated in their feathers significantly more Hg (one third of feathers exceeded the established toxicity threshold), Se and Mn compared to the post-breeding period. It reflects a higher exposition of birds to contaminants in pre-breeding moult areas outside the High Arctic compared to the post-breeding moult in the High Arctic. Sex differences in adult feathers representing the post-breeding period were found only for Ca and Zn with higher values in females. Chick down was characterized by high levels of several essential elements, an intermediate level of Hg and Se, and the highest Se:Hg molar ratios of all groups. Chick body feathers had

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the highest level of Cu and K among all the studied groups. Post-hatching eggshells were characterized by high Sr level (exceeding 2000 μg/g). Concentrations of several non-essential elements (Bi, Cd, Cr, Hg, Ni and Pb) in them were below method detection limits.

1. Introduction

The Arctic is currently undergoing dynamic climate changes (Box et al., 2019), which have an impact on both biological systems and contaminant pathways (Macdonald et al., 2005). Although remote Arctic areas are mostly non-industrialised and sparsely occupied, they can still be exposed to multiple pollutants (MacDonald et al. 2005; AMAP, 2005). The long-range transport of contaminants is considered to be a significant factor affecting toxic metal levels in the High Arctic zone (AMAP, 2005, 2011; Halbach et al., 2017; Zaborska et al., 2017). Additionally, some elements entering the ecosystem can originate from natural sources (volcanic eruptions on Iceland, rock weathering) and can also be produced by local human activities, both past and present, including runoffs from active and shut-down coal mines, ship transportation and increased tourism (Rose et al., 2004; Granberg et al., 2017).

As top predators, seabirds are considered as a good model group for studying the bioaccumulation and biomagnification of elements (Burger et al., 2008). Moreover, they play an important role in Arctic ecosystems by being two-environmental organisms; foraging at sea and breeding on land, they may easily transport various types of matter, including contaminants, from marine areas to terrestrial ones (Stempniewicz et al., 2007; Burger et al., 2007).

The aim of this study was to examine the concentrations of certain elements in samples collected from the High Arctic seabird, the little auk (*Alle alle*). We took advantage of the fact that birds excrete the elements into external tissues such as feathers (changed regularly during the moulting, e.g. Jaspers et al., 2004; Burger et al., 2008; Fort et al., 2016; Pacyna et al., 2017), and eggshells (females; Lam et al., 2005). Thus, sampling those allowed us to examine birds exposure to contaminants relatively non-invasively. Since little auks moult twice a year in different time and area, various feather types can be used to trace changes in exposure to elements over an annual cycle and to examine changes in food-chain contamination (Fort et al., 2016). We sampled throat and body feathers: throat feathers are changed during the partial pre-breeding moult (Fort et al., 2014; SEATRACK), thus represent contamination exposure during the wintering; body feathers are moulted after the breeding (Fort et al., 2014, 2016; SEATRACK). Elemental concentrations in chick feathers, being affected by fewer factors (including the fact that food comes from a more restricted foraging area of the parents during the breeding season) can be used to examine local contamination effect (Evers et al., 2005). Chick down and post-hatching eggshells can be used to examined females contamination prior to egg-laying, thus also a maternal effect in the contamination level of the chicks (Ackerman et al., 2016). Sampling the body and throat feathers of adults, post-hatching eggshells, and the body feathers and down of chicks we were able to build a full-year picture of the spatio-temporal exposure of the local population to various elements, including contaminants.

Due to differences in the adults moulting location (outside the breeding colony, i.e. various quarters of the Northern Atlantic, including sub-arctic area) and chicks (breeding colony, High Arctic) we expected higher concentration of various elements in adults. Also, since the isotopic niches (reflecting foraging niches) occupied by little auks from the colonies in East Greenland and Spitsbergen change throughout their annual cycle (Fort et al., 2010), we also expected differences between adults feathers being moulted in different time (pre- and post-breeding). Due to sex specific deposition of the elements into egg, we also expected sex differences in elemental concentration in post-breeding feathers of adults. Additionally, owing to the fact of sampling the body feathers of the same adult individuals in two consecutive breeding seasons we could examine temporal patterns of individual contamination levels.

2. Methods

2.1. Studied species

The little auk (or dovekie) is a small seabird (body mass 150–180 g), breeding colonially, and exclusively in the High Arctic. The female lays one egg annually, in early to mid-June, which is incubated ca 30 days by both parents (Stempniewicz, 2001). The little auk is a specialised zooplanktivore, and its diet is composed mostly of *Calanus* sp. copepods, amphipods and krill, and supplemented by small fish (Stempniewicz, 2001; Rosing-Asvid et al., 2013; Øverjordet et al., 2015). The chick diet is dominated by copepods of the *Calanus* genus (Steen et al., 2007; Wojczulanis et al., 2006; Jakubas et al., 2011). In winter, when the availability of *Calanus* sp. decreases dramatically due to its migration to greater depths (Fort et al., 2010), little auks switch to higher trophic level prey, such as larger amphipods, krill or small fish (Fort et al., 2010; Stempniewicz, 2001; Rosing-Asvid et al., 2013).

The little auk has been proven to be a valuable bio-indicator of the temporal trends of environmental contamination within the Arctic pelagic zone (Borgå et al., 2006; Jæger et al., 2009; Fife et al., 2015; Fort et al., 2016). It has been recognized as the most numerous seabird in the European part of the Arctic (Stempniewicz, 2001) and a keystone species for the functioning of the Arctic terrestrial ecosystem through its transportation of nutrients from the sea to the land (Stempniewicz et al. 2007). Thus, the knowledge about little auks contamination is important for understanding the circulation of elements in both marine and terrestrial Arctic environments.

2.2. Studied area and types of sampled tissues

The study was performed in the little auk breeding colony on the Ariekammen slope in Hornsund (SW Spitsbergen, 77°00′ N, 15°33′ E). This breeding site is considered to be one of the largest breeding aggregations of this species in Svalbard (Keslinka et al., 2019; Fig. 1).

Two general types of samples were collected: feathers and post-hatching eggshells. To collect feathers from adults, individuals were captured by hand when present in the nest during incubation or early chick-rearing periods. Post-breeding body feathers, from the back, (hereafter POSTBR) and pre-breeding feathers, from the throat (PREBR) were collected from each individual. Usually, both members of a pair were sampled resulting in an equal sex ratio in the collected data set. Pre-breeding moult, which involves the replacement of head and throat feathers occurs during the spring (March–April) when the birds are still outside the breeding grounds (Fort et al., 2014; SEATRACK). Individuals have been located at that time in the north-east coast of Iceland or further south in the North Atlantic (Fort et al., 2013; SEATRACK). A complete moult that involves the replacement of the entire body plumage is exhibited shortly after the breeding season (September), probably in the vicinity of the breeding areas (Fort et al., 2014, 2016; SEATRACK). Individuals have been located at that time close to the marginal ice zone, in the Greenland Sea or in the Barents Sea south of the Franz Josef Land (Fort et al., 2010, 2013, 2014, 2016; SEATRACK). From nestlings we collected down and newly grown feathers: down (hereafter CHDOW) was collected from chicks at 7–14 days old and body feathers from the same chicks at 14–23 days

Fig. 1. Study site; map source: toposvalbard.npolar.no; photo by KWJ.

old (hereafter CHBFR). Residual post-hatching eggshells (hereafter EGGSCH) were also collected from focal nests just after hatching (little auk parents remove eggshells from the nest burrow a few days after hatching, thus our collection could not affect the birds' welfare). For 20 adults sampled in 2017, body feathers collected in the previous season (2016) were available. They were used to investigate an inter-annual variation in elemental concentration.

After collection, feathers were kept at an ambient temperature in sealed string bags. Eggshells were kept in plastic containers in a refrigerator (−20 °C).

2.3. Sex determination

Due to the lack of considerable morphological dimorphism in the little auk, sex of the studied individuals was determined molecularly (Jakubas and Wojczulanis, 2007) using other feather samples. The DNA was extracted using a Sherlock kit (A&A Biotechnology, Gdynia, Poland). We performed PCR using a primer pair F2550 and R2718, with 50 °C annealing temperature (Griffiths et al., 1998). These primers amplify a 430-bp fragment on the W chromosome (in females only), and a 600-bp fragment on the Z chromosome (in both sexes) (Griffiths et al., 1998). This size difference was clearly visible under UV-light when the fragments were dyed with Midori green and separated on a 2% agarose gel.

In total, feathers from 74 adult birds were analysed, but a reliable sex determination was only possible for 54 individuals (26 males and 28 females).

2.4. Sample preparation

All feather samples were cleaned once with acetone and twice with deionised water (Mili-Q Water, France) to remove external contaminations, and air-dried for about 24 h. Next, the dry feathers were homogenised by being cut into small parts and weighed to the nearest 0.01 mg (mean sample mass of adult body feathers: 24.33 ± 5.81 mg, throat: 7.65 ± 2.07 mg, chick feathers: 16.09 ± 4.32 mg, chick down feathers: 27.21 ± 12.16 mg). In the case of eggshells, the internal membrane was discarded, as it was too contaminated, and samples were washed with acetone and deionised water. Samples were left to dry for 24 h, then crushed into small pieces and weighed (mean 203.30 ±

4.10 mg). All samples were placed individually into a clean Teflon vessel with 65% HNO_3 (Merck, Suprapure). Digestion was carried out using a high-pressure microwave emitter (Microwave Digestion System, Anton Paar) (see detailed description in Pacyna et al., 2019a). For quality control, blank samples were run with every batch. To ensure the accuracy of the obtained results, a Certified Reference Material (CRM - Human hair ERM-DB001, Sigma Aldrich) was run in triplicate. The elemental concentration was determined by means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS 2030 Shimadzu, Japan), with the exception of calcium and magnesium in eggshells, which were analysed on ICP-OES 9820 (Shimadzu, Japan). Details regarding measurement conditions and parameters are described in previous studies (for ICP-MS, see Pacyna et al., 2019a; for ICP-OES, see Pacyna et al., 2019b).

2.5. Quality control (QA/QC)

Results for the CRM ERM-DB001 analysis were in agreement with certified values, with recoveries reaching 92–106% (values were certified for As, Cd, Cu, Hg, Pb, Se and Zn). To check the accuracy and recoveries of other elements absent from this CRM, a treatment used before by Pacyna et al. (2019b) was applied. The accuracy of the analyses was verified by means of two CRMs: Trace metals ICP - sample 1 and Trace metals ICP - sample 2 with recoveries 96–109% (certified values include all elements except Hg). Calibration of the ICP-MS was performed with the ICP IV multi element standard (Sigma Aldrich Merck group, Poznan, Poland) and with single-element standards for As, Hg, Sb, Se, Mo and V (Sigma Aldrich Merck group, Poznan, Poland). Additionally, for ICP-MS Sc, Rh, Tb and Ge in 1% HNO_3 ≥ 99.999% trace metals basis (Sigma Aldrich Merck group, Poznan, Poland) were used as internal standards. Deionised water was obtained from the Milli-Q Direct 8 Water Purification System (Merck Millipore) and applied for sample (pre)treatment and dilutions. The ICP-OES 9820 (Shimadzu, Japan) calibration was completed with single-element standards (Sigma Aldrich Merck group, Poznan, Poland) containing 1000 mg L^{-1} of Ca and Mg of which calibration standard solutions were made. The limit of detection (LOD) and quantification (LOQ) values were calculated as the concentrations corresponding to signals equal to, respectively, three and ten times the standard deviation of the blank solution signal. LOD and LOQ were in the ranges 0.004–0.92 and 0.01–3.07 $ng \cdot g^{-1}$, respectively. Values were blank corrected by the mean value of all the blank samples. Values below LOD were replaced with half the LOD value. All results are reported as $\mu g \cdot g^{-1}$ dry weight (dw). For statistical analysis, the arithmetic mean was calculated if at least 65% of the samples had concentrations of the compound >LOD.

2.6. Statistical analyses

Although feather samples were collected from family members, potentially imposing dependency in the data set, they were treated as independent data points in all the analyses. This is because feathers of both types (pre- and post-breeding) collected from adult males and females have grown independently (both in a geographical and temporal sense). In addition, all the individuals were represented in each sample group in similar numbers, thus the issue of possible pseudoreplication could be considered negligible. To examine the level of contamination of chemical elements in little auks in respect to sample type and birds sex, a data mining approach was applied, using various methods:

1) A multivariate (for all trace elements together) principal component analysis (PCA) was used to assess similarity of elemental concentrations in the studied sample groups. This technique reduces the number of variables to a few new ones called factors, representing groups of elements with correlated concentrations. Since the concentrations of all elements were measured in the same units ($\mu g \cdot g^{-1}$ dw), the PCA on a variance–covariance matrix was performed.
2) A multivariate PERMANOVA (non-parametric MANOVA based on the Bray–Curtis measure; Anderson, 2001) was used to examine various relationships between the sample types and element concentrations. In this analysis concentrations of all elements were treated as response variables and tissue types, representing various stages of little auk life-history (i.e. PREBR, POSTBR, CHDOW, CHBFR, EGGSCH) were treated as explanatory variables.
3) Kruskal–Wallis tests, with *post-hoc* Mann-Whitney *U* tests was used to compare concentrations of particular elements in feather types and eggshells.

The molar ratio of Se and Hg in the examined samples was also analysed. This is because both elements can be bioaccumulated in significant amounts (Borghesi et al., 2016). Se is an essential element which level is physiologically regulated within the body and plays an important role in the organism's proper functioning, including protection against the adverse effects of Hg, by the creation of an Hg—Se complex (Hg binds to Se with an extraordinarily high affinity; Berry and Ralston, 2008; Khan and Wang, 2009; Øverjordet et al., 2015). Hg toxicity is observed when Hg has a substantial molar excess of Se (Berry and Ralston, 2008). We used the Kruskal–Wallis tests, with *post-hoc* Mann-Whitney *U* tests, to compare the molar ratio, Se:Hg between the sample types. Eggshells and chick feathers were excluded from this analysis because of the high representation of samples with Hg or Se concentrations <LOD (see Table 1).

To analyse sex differences in elemental concentrations in post-breeding feathers of adults, *t*-tests were used. To investigate inter-annual consistency in elemental concentrations in feathers of the same individuals captured in 2016 and 2017 Pearson correlation was used.

PCA, and PERMANOVA analyses were performed on log(x + 1) transformed data in PAST software (Hammer et al., 2001) and all other analyses in R software (R Core Team, 2018).

3. Results

Elemental concentrations in all samples are reported in Table 1. In the case of Bi in chick body feathers, the results suggested possible external contamination, as extraordinary variations were found (65% of samples were < LOQ, range was from <LOD–92.40 $\mu g \cdot g^{-1}$ dw).

3.1. Differences in all elemental concentrations between the studied types of samples

Principal component analysis (PCA) revealed that 75.7% of the total variance in the elemental concentrations in the studied types of samples was explained by the two axes. The first axis (explaining 55.4% of total variance) was the most correlated with K ($r = 0.625$), Ca ($r = 0.495$) and Sr ($r = 0.406$), and the second axis (explaining 20.3% of total variability) with K ($r = -0.712$) and Ca ($r = 0.463$) (Fig. 2). PREBR and POSTBR clustered in similar positions and partly overlapped with CHDOW. EGGSCH and CHBFR clustered in position different from any other groups not overlapping with any of them (Fig. 2).

PERMANOVA revealed that concentrations of all the studied elements differed significantly between the studied types of samples, i.e. feathers PREBR, POSTBR, CHDOW, CHBFR and EGGSCH ($F = 133.6$, $p = 0.001$; Bonferroni–corrected post hoc pairwise comparisons: all $p = 0.001$).

3.2. Differences in particular elements between the studied types of samples

Kruskal–Wallis inter-group tests comparing the concentrations of particular elements in the studied types of samples, i.e. feathers PREBR, POSTBR, CHDOW, CHBFR and EGGSCH revealed significant differences for all 19 elements ($p < 0.05$) (Supplementary Materials 1, Figs. ES1–ES8). *Post-hoc* U Mann-Whitney tests revealed that most of

Table 1
Elemental concentration in little auk samples mean ± sd (median; min-max) μg·g^{-1} dw; all sampled individuals; Se:Hg molar ratio calculated only when >65% of both Se and Hg were above LOD.

Element	Adult body (n = 74)	Adult throat (n = 74)	Chick body feather (n = 18)	Chick down (n = 16)	Eggshells (n = 18)
As	**0.20 ± 0.20****(0.15; <LOD-14.43)	**68% < LOD; 80% < LOQ**	**1.38 ± 2.83*** (0.31; <LOD-11.50)	**1.43 ± 1.08** (1.33; 0.10–3.38)	**0.06 ± 0.04** (0.05; 0.02–12.00)
Ba	**2.88 ± 2.99** (2.63; <LOD- 13.86)	**3.59 ± 5.41*** (0.65; <LOD- 21.80)	**65% < LOD**	**65% < LOD**	**9.53 ± 3.48** (9.77; 5.01–16.88)
Bi	**90% < LOD**	**90% < LOD**	**65% < LOQ (possibly contaminated)**	**2.43 ± 3.21** (0.91; 0.05–9.50)	**88% < LOD**
Ca	**155 ± 59** (151; 70–308)	**101 ± 83*** (83; 6.18–455)	**30.20 ± 12.10** (26.90; 12.98–65.10)	**238 ± 66** (235; 158–440)	**41,669 ± 8583** (43,571; 27,763–57,862)
Cd	**0.31 ± 0.21*** (0.24; <LOD- 2.15)	**92% < LOQ**	**72% < LOQ**	**0.62 ± 0.46** (0.53; 0.18–1.82)	**83% < LOQ**
Co	**75% < LOD**	**75% < LOD**	**82% < LOD**	**82% < LOD**	**0.23 ± 0.09** (0.21; 0.13–0.48)
Cr	**0.74 ± 1.40*** (0.12; <LOD- 12.3)	**1.38 ± 2.20*** (0.30; <LOD- 11.80)	**72% < LOQ**	**68% < LOD**	**66% < LOD**
Cu	**17.30 ± 5.98** (16.70; 8.81–58.35)	**16.70 ± 10.90**** (14.30; 2.56–67.97)	**28.50 ± 6.44** (28.50; 19.07–42.23)	**12.50 ± 2.99** (13.03; 5.99–17.35)	**0.51 ± 0.31** (0.52; 0.09–1.07)
Fe	**58 ± 111*** (20.40; <LOD- 597)	**102 ± 201**** (25.40; <LOD- 1289)	**44.60 ± 55.90** (15.80; <LOD- 192)	**23.50 ± 12.60*** (22.10; <LOD-152)	**11.10 ± 6.94** (9.42; 2.60–27.11)
Hg	**1.39 ± 0.40** (1.38; 0.58–2.36)	**5.02 ± 3.32** (4.27; 1.45–17.20)	**0.57 ± 0.17** (0.52; 0.25–0.96)	**1.11 ± 0.41** (1.08; 0.64–2.03)	**100% < LOD**
Mg	**536 ± 112** (531; 319–861)	**542 ± 156** (521; 280–1324)	**152 ± 29** (156; 114–226)	**544 ± 110** (546; 340–749)	**1563 ± 237** (1557; 1118–2000)
Mn	**0.52 ± 0.66**** (0.27; <LOD- 27.59)	**3.12 ± 4.29** (0.57; <LOD-17.60)	**84% < LOD**	**5.49 ± 4.13** (4.29; 0.48–16.71)	**0.82 ± 0.43** (0.65; 0.27–1.93)
Ni	**85% < LOD**	**85% < LOD**	**100% < LOD**	**100% < LOD**	**88% < LOD**
Pb	**1.23 ± 1.66*** (0.68; <LOD- 10.98)	**1.53 ± 2.43**** (0.41;<LOD-20.22)	**66% < LOD**	**0.32 ± 0.42** (0.08; <LOD- 1.24)	**80% < LOD**
Se	**1.03 ± 0.44*** (1.03; <LOD-2.14)	**2.63 ± 1.61** (2.55; <LOD- 7.56)	**66% < LOD**	**2.53 ± 0.59** (2.51; 1.45–3.76)	**0.28 ± 0.11** (0.27; 0.12–0.46)
Sr	**14.00 ± 3.60** (13.40; 7.20–25.70)	**11.20 ± 4.44** (10.40; 5.76–37.87)	**4.80 ± 1.11** (4.54; 3.22–7.23)	**23.40 ± 4.76** (24.10; 15.40–31.77)	**2093 ± 267** (2052; 1810–2720)
V	**0.10 ± 0.09**** (0.08; <LOD- 1.57)	**0.18 ± 0.16**** (0.12; <LOD- 1.16)	**0.31 ± 0.66*** (0.02; <LOD- 2.18)	**0.08 ± 0.09*** (0.06; <LOD-1.11)	**0.01 ± 0.01** (0.004; <LOD- 0.03)
Zn	**155 ± 50** (154; 78–254)	**158 ± 99** (136; 62–696)	**105 ± 18** (106; 70–132)	**63.90 ± 15.10** (61.10; 38.25–97.66)	**1.65 ± 0.92** (1.35; 0.60–3.83)
K	**98% < LOQ**	**98% < LOQ**	**819 ± 458** (829; <LOD- 1522)	**83% < LOQ**	**428 ± 72** (412; 304–517)
Se:Hg	**2.27**	**1.91**	–	**6.49**	–

*One outlier excluded; ** two outliers excluded.
Significant differences are bolded ($p < 0.05$).

differences were significant ($p < 0.05$) (Electronic Supplementary Materials 1, Figs. ES1–ES7).

The Se:Hg molar ratio was highest in chick down feathers (Table 1) with significant statistical differences between groups (PREBR vs POSTBR, $p < 0.05$; PREBR vs CHDOW $p < 0.0001$; POSTBR vs CHDOW $p < 0.0001$; Kruskal-Wallis, $p < 0.001$).

3.3. Differences in elemental concentration (by sex and year of sampling)

Ca and Zn concentrations in the POSTBR body feathers of females were significantly higher than in males. Concentrations of Hg also tended to be higher in females, but results were not statistically significant ($p = 0.057$). No significant sex differences were found in concentrations of other studied elements in POSTBR body feathers (Table 2).

An analysis of inter-annual consistency in elemental concentrations in the same individuals sampled both in 2016 (side body feathers) and 2017 (back feathers) revealed no statistically significant relationships, except for a positive correlation for Pb (Table 3).

4. Discussion

The elemental exposure of the most numerous High Arctic seabird throughout the year was characterized based on non-lethally collected samples. Several significant differences between the concentrations of the studied elements were found (only elements of known relevance and toxicity are discussed).

4.1. Concentration of selected elements in adults

4.1.1. Hg and Se

Hg is an element of primary concern in marine environment. In the present study, 35% of all the analysed adults feathers representing the pre-breeding period exceed the Hg toxicity threshold value for feathers (5 μg·g^{-1} dw; Burger and Gochfeld, 1997), with 11% of individuals having concentrations >10 μg·g^{-1} dw. Lack of samples with Hg levels exceeding 5 μg·g^{-1} dw during the post-breeding period suggests lower Hg contamination in breeding colonies area, located in the High-Arctic zone. One should remember that the established toxicity threshold associated with Hg adverse effect (Burger and Gochfeld, 1997) in birds is a guideline and real effect on avian organism may differ between species. However, one third of individuals with elevated level of Hg during the pre-breeding period is alarming.

Previous study on adult little auks breeding in Greenland has also shown higher Hg concentrations in feathers grown during the pre-breeding period (2.27–3.73 μg·g^{-1} dw) compared to the post-breeding period (1.00–2.11 μg·g^{-1} dw) (Fort et al., 2016). In the present study conducted in Svalbard, even higher values were found, i.e. 1.45–17.2 (mean ± SD: 5.02 ± 3.32 μg·g^{-1} dw) during the pre-breeding and 0.58–2.36 (1.39 ± 0.40 μg·g^{-1} dw) the post-breeding period. These results may be explained by the higher exposure of little auks during the pre-breeding time (reflecting contamination outside breeding grounds), when they are more exposed to human activities (oil drilling, oil spills, etc.) compared to the High-Arctic post-breeding moulting areas. A study of multi-year (between 2006 and 2014) changes in Hg concentration in the body feathers of little auks breeding in East Greenland revealed an increase in accumulation of this element at a rate of 3.4% per year between 2006 and 2014 (Fort et al., 2016).

Se is an essential trace element for proper organism functioning, but in excess may as well have toxic effects on vertebrates (Burger et al., 2013). Se concentration in the feathers of adults followed the trend found for Hg, with significantly higher concentrations in throat feathers, revealing a higher Se exposure during the pre-breeding period. A high hepatic Se:Hg molar ratio has previously been reported for the little auk (17.23–29.63 depending on sampling year and region), suggesting a relatively low risk of Hg toxicity (Øverjordet et al., 2015). The present study revealed that 35% of pre-breeding feather samples and only 13% of

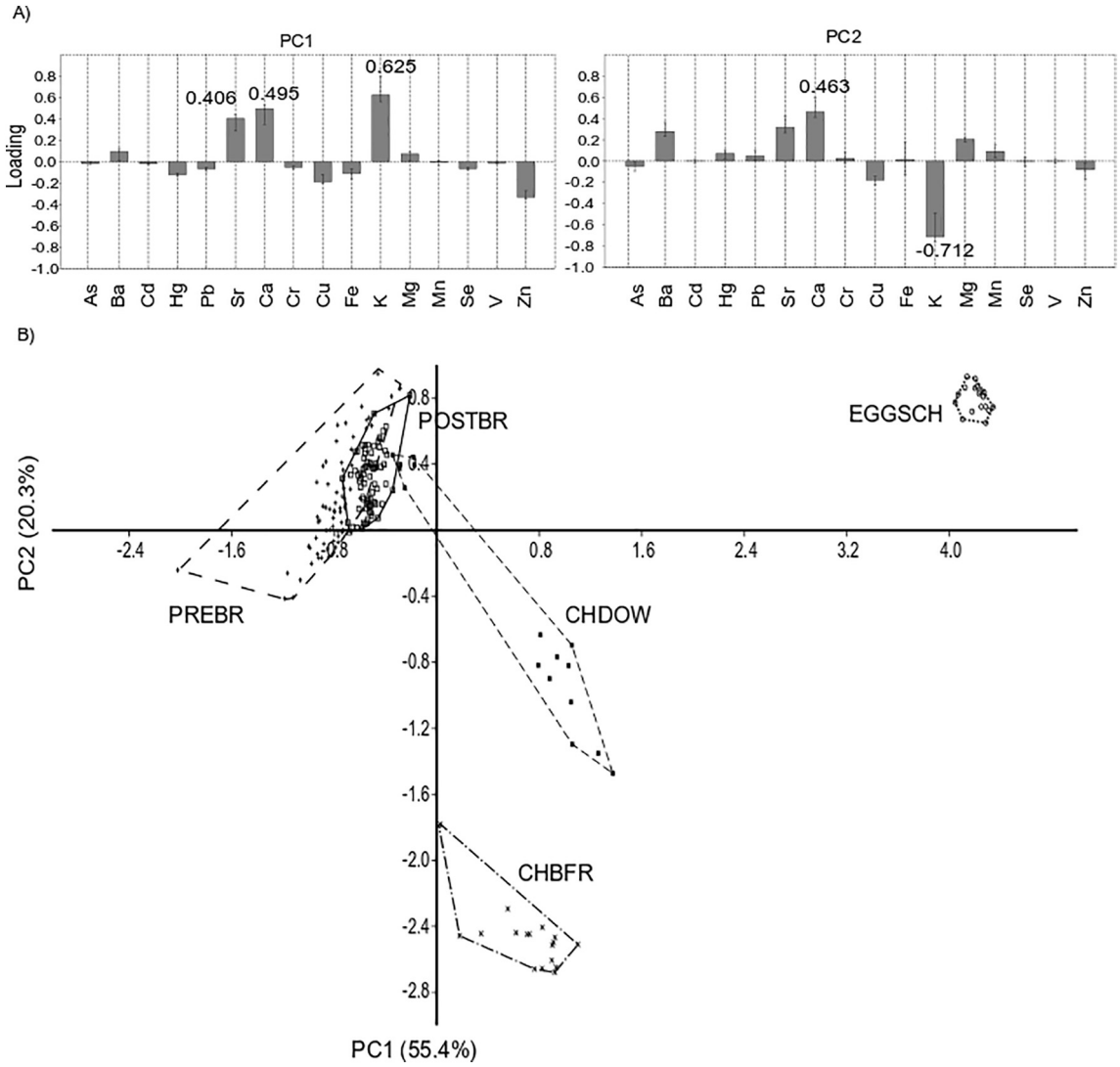

Fig. 2. Principal component analysis (PCA) biplot of elemental concentrations in the studied types of samples, i.e. pre-breeding feathers (PREBR), post-breeding feathers (POSTBR), chick down (CHDOW), chick body feathers CHBFR and eggshells (EGGSCH), based on log(x + 1) transformed data. A) The loadings of each studied elements for two first axes (PC1 on left and PC2 on right). B) Points representing the scores of each sample and convex hulls for particular types of samples. Points grouping close together correspond to observations that have similar scores on the PCA components.

post-breeding feather samples were characterized by a Se:Hg molar ratio lower than 1, suggesting possible toxic effects of Hg in those groups of birds (Lucia et al., 2016).

Table 2
Summary of elements concentration [$\mu g \cdot g^{-1}$ dw] in adults body feathers. Males ($N = 26$) and females($N = 27$) difference analysed with *t*-test.

Element	Male_mean	Male_SD	Female_mean	Female_SD	*t*	*p*
As	0.77	2.80	0.28	0.49	−0.88	0.386
Ba	2.44	3.30	3.12	2.89	0.81	0.424
Ca	135	49.60	169	57.90	2.31	**0.025**
Cd	0.40	0.42	0.28	0.24	−1.35	0.185
Cr	0.94	1.66	0.73	1.45	−0.48	0.631
Cu	17.20	3.60	18.00	8.81	0.44	0.662
Fe	61.60	130	62.20	122	0.02	0.986
Hg	1.27	0.44	1.49	0.36	1.95	0.057
Mg	532	116	552	108	0.66	0.514
Mn	0.53	0.64	0.52	0.79	−0.03	0.979
Pb	1.56	2.58	1.45	1.88	−0.18	0.859
Se	1.26	1.73	1.06	0.45	1.87	0.120
Sr	13.60	3.90	14.60	3.89	1.03	0.309
V	0.61	2.65	0.12	0.12	−0.96	0.346
Zn	137	46.90	168	51.00	2.39	**0.021**

4.1.2. Cd

The Cd concentration in the adult feathers was generally very low (mean $0.31 \pm 0.21\ \mu g \cdot g^{-1}$ dw in body feathers; <LOQ in head feathers). Cd is naturally abundant in the North Pacific, which may result in a net

Table 3
Inter-annual consistency in element concentrations (Pearson correlation) in the body feathers of adult little auks captured both in 2016 and 2017.

Element	*r*	*t*	*p*
Ba	0.20	0.84	0.414
Ca	0.39	1.75	0.098
Cd	−0.04	−0.18	0.861
Cr	0.14	0.58	0.573
Cu	0.31	1.35	0.196
Fe	0.42	1.91	0.074
Hg	0.04	0.18	0.862
Mg	0.14	0.58	0.571
Mn	0.26	1.10	0.289
Pb	0.55	2.68	**0.016**
Se	0.35	2.65	0.160
Sr	0.36	1.60	0.129
V	0.22	0.91	0.374
Zn	0.20	0.85	0.408

flux of Cd-rich water to the Atlantic side of the Arctic Ocean, potentially leading to high Cd levels in Arctic water (Øverjordet et al., 2015). Cd is easily absorbed by the chitinous structure of zooplankton (Dietz et al., 1996; Øverjordet et al., 2015). However, feathers are not the main Cd accumulation target tissue, as only about 30% of the body load is deposited in them, while the highest concentration is usually found in the kidneys and liver (Friberg et al., 1979; Burgos-Núñez et al., 2017).

4.1.3. Pb

Generally, sampled adult individuals were characterized by relatively low levels of Pb in the keratinized tissues. Here, no difference in Pb concentration was found, neither between the body and head feathers, nor between females and males during the post-breeding period.

However, during the post-breeding period, 10% of feathers from adults exceeded the Pb levels in feathers identified as the toxic threshold (4 $\mu g \cdot g^{-1}$ dw, Burger and Gochfeld, 1997), with the maximum value of 11 $\mu g \cdot g^{-1}$ dw. During the pre-breeding period, 16% of individuals had Pb concentrations >4 $\mu g \cdot g^{-1}$ dw, with the highest value >20 $\mu g \cdot g^{-1}$ dw. Although the percentage of individuals exceeding Pb toxicity levels was relatively low, a special attention should be paid in future studies, as climate conditions may alter in the Arctic, resulting in changes in birds' exposure to this (and others) element.

4.1.4. Other elements

Generally, concentrations of non-essential elements in feathers of the studied adult little auks were low. Arsenic is a ubiquitous metalloid classified as toxic and, although it does not biomagnify (Hargreaves et al., 2011), but it can accumulate in bird tissue (such as liver, bone and feathers) in a dose-dependent way (Sánchez-Virosta et al., 2018). Here, As levels <0.2 $\mu g \cdot g^{-1}$ dw in body feathers, and <LOD in throat feathers were found. When excluding outliers, levels of As, Pb, and Cd did not suggest any potential ecotoxicological risk.

Sr, being a Ca analogue, accumulates mainly in Ca-rich structures (Chowdhury and Blust, 2001), with some evidence of trophic biomagnification (Campbell et al., 2005). Here, small but significant differences were found for Sr concentration, between the throat (11.20 ± 4.44 $\mu g \cdot g^{-1}$ dw) and body feathers (14.00 ± 3.60 $\mu g \cdot g^{-1}$ dw).

The highest absolute elemental concentration in both back and throat feathers were found for Mg, Ca and Zn. These elements are necessary for a proper feather formation and are regulated mostly by homeostatic processes (McGraw et al., 2003; Bocher et al. 2003).

4.2. Temporal variation in levels of elements in respect to sex

Exposure to contaminants may differ between females and males due to sex-specific diets, and spatial and/or temporal differences in stop-over or wintering strategies (Hargreaves et al., 2010). In addition, females may eliminate some toxic elements by sequestration into their eggs (Lam et al., 2005). We found significant sex differences in the levels of Ca and Zn in adults feathers representing the post-breeding period with females having higher concentrations of both elements compared to males. Since, isotopic niches of little auk males and females revealed a lack of significant sex differences through the year (Fort et al., 2010) this result could be explained on the ground of sex-specific elements processing.

4.3. Pathways of elemental input in chicks

Elemental allocation from the female organism to the chick down serves as a major elimination pathway for Hg accumulated by the mother (Wenzel et al., 1996). Se transferred from females plays a significant role in prenatal Hg intoxication, reducing its toxicity (Berry and Ralston, 2008; Hargreaves et al., 2011). Both Hg and Se levels as well as the molar ratio of the two elements were higher in chick down than chick body feathers (Table 1). However, as elevated Se level can also be toxic for organism it is difficult to predict its full toxicological effect (Khan and Wang, 2009; Hargreaves et al., 2011).

Ca levels tend to increase in a little auk chick's body after the 10th day of its life, suggesting intense ossification of the skeleton at that time (Taylor and Konarzewski, 1992). The nutritional deficiencies in chicks' diets, especially in Ca, could result in delayed growth. Thus Ca concentration being very high in the down (238 ± 66 $\mu g \cdot g^{-1}$ dw) compared to the chick body feathers (30.20 ± 12.10 $\mu g \cdot g^{-1}$ dw) could be related to a protective maternal effect and Ca absorption from the shell (Orłowski et al., 2017). Similarly, Mg and K levels could be under a strong maternal influence. The level of these two elements have been reported to be relatively stable throughout the little auk's early development (Taylor and Konarzewski, 1992). We found significant differences between down (maternal input) and chick feathers (current ingestion).

Both Cu and Zn levels in the organism can be regulated metabolically (Wenzel et al., 1996), and both were found in relatively high concentrations in the chick body feathers (28.50 ± 6.44 and 105 ± 18.1 $\mu g \cdot g^{-1}$ dw, respectively). Nestling plumage still has a blood connection, therefore it may contain more active metals, like Cu and Zn, when compared to pure keratin (Costa et al., 2013). High Cu concentrations may be connected with physiological development processes (Nygård et al., 2001). This could also be the result of dietary exposure, as Cu and Zn levels can be high in herbivorous calanoids (Battuello et al., 2017), auks preferred prey items.

Elevated levels of As in nestlings may negatively affect skeletal and wing growth rate, through an interaction between As and the mineral fraction of the bone. Thus, this may potentially affect negatively fledging success and chick survival (Sánchez-Virosta et al., 2018). Approximately 20% of the studied chicks had As levels in their body feathers >3 $\mu g \cdot g^{-1}$ dw, but high variability was found (min-max <LOD−11.5), which could be partially a result of external contamination by As. More studies would be useful to conclude whether As constitutes a toxicological risk for young little auks.

4.4. Eggshells

The eggshell protects the embryo from the external environment and serves as an important source of Ca for its skeletal growth and calcification (Reynolds et al., 2004; Reynolds and Perrins, 2010). The developing embryo absorbs Ca and other elements firstly from the yolk, and then from the eggshell. The eggshell is the main source of Ca and Mg during embryogenesis (Orłowski et al., 2017).

The metabolic pathway of Ca in the eggshell may be interrupted by toxic elements (Dauwe et al., 1999; Mora et al., 2007). Especially considering the fact that females may excrete some non-essential inorganic elements into the egg (Mora et al., 2007; Dauwe et al., 1999). Egg content has a significantly higher Hg concentration than the hardened part of the eggshell (Peterson et al., 2017). However, strong positive correlations have been found between the total Hg concentration in eggshells and in the egg content, with the relationship being species-dependent, and also dependent on the age of the embryo and cleanliness of the eggshell (Peterson et al., 2017). In our study both Hg and Pb concentrations were below analytical limits, while As was present at very low level (mean 0.06 ± 0.04 $\mu g \cdot g^{-1}$ dw).

We found exceptionally high Sr concentration (min-max: 1737–2720 $\mu g \cdot g^{-1}$ dw). Values for the little auk were much higher than reported for some passerines (70–1360 $\mu g \cdot g^{-1}$ dw; Mora et al., 2007, Ruuskanen et al., 2014). Sr mobilization in body is closely associated with mobilization of Ca (Kottferova et al., 2001; Mora et al., 2007). Also a high Sr concentrations have been reported for some polar invertebrates (Campbell et al., 2005). Thus, high absorption of Ca in the little auk female intestines during the egg production, and corresponding increase in the absorption of Sr may be a reason of the elevated Sr values patterns.

4.5. Limitations of our study

Besides Hg, most elemental concentrations in the feathers of adults may be biased because of external contamination, which cannot be fully removed even after extensive cleaning (Dauwe et al., 2003; Borghesi et al., 2016). Thus, it should be noted that adult feathers in particular are less reliable as indicators for internal uptake in the case of Cd, Co, Fe, Li, Pb, Se, Mn, Cr, or V (Hargreaves et al., 2010; Borghesi et al., 2016), and may give instead information on integrated exposure. This may be the reason for detecting some outliers observed in feathers collected from adults, especially in case of As, Ba, Bi, Cd, Cr, Cu, Fe, Mn, Pb and Se. However, it is not possible to judge whether the particular outlier is caused by external contamination or high internal uptake. Here, all feathers were treated with the same cleaning procedure proven to be effective (Dauwe et al., 2003; Jaspers et al., 2004; Pacyna et al., 2019a). Thus, although we cannot fully reject the possibility of externally contaminated samples, this risk was reduced as much as possible.

Internal elemental deposition depends on several factors including feather physiology. It is known that some elements are incorporated into the feather as part of the building blocks of the keratin, while others can enter the developing feather cells in proportion to their abundance in the blood stream (Bortolotti, 2010). Deposition of some elements is time-dependent and depends on feather growth rate. Time-dependent scale, which is based on feather measurements not mass, can be very useful for flight feathers that grow in a highly linear fashion, with relatively uniform elongation during growth (Bortolotti, 2010). Here as we used very small body and throat feathers, as well as chick down and body feathers we applied mass dependent scale measuring elemental concentration, to enable a uniform comparison between them. In the case of some elements it could cause mass dilution bias, but this phenomena requires more examination.

5. Conclusions

This study revealed that toxic metal levels in the feathers and eggshells of a high Arctic breeder, the little auk, were generally low. However, in the case of Hg, we found that almost one third of adult birds had elevated Hg level in feathers grown during the pre-breeding period.

Concentrations of Hg, Se and Mn in adult feathers were significantly higher in pre-breeding period reflecting higher exposition of birds to contaminants in areas outside the High Arctic where they performed pre-breeding moult and lower risk in the High Arctic zone where they moult during the post-breeding period. Sex related differences were mostly non-significant, beside Ca and Zn.

Chick body feathers were characterized by relatively high levels of K and Cu, but concentrations of about half of analysed elements were below the analytical limits of detection. Chick down feathers (representing maternal input) were characterized by relatively high levels of several elements, including As and Cd. High levels of Se in chick down and high Se:Hg molar ratios suggest excretion of maternal Hg to the egg but with mitigation of Hg toxicity.

Post-hatching eggshells are not very effective biomonitoring tool, as they reflect only short and specific phase of female annual life cycle. The concentrations of several non-essential elements (Bi, Cd, Cr, Hg, Ni and Pb) in eggshells were below detection limits. It cannot be excluded that at least some of them have been resorbed from eggshell by the developing embryo. High level of Sr suggest a need for further studies, to understand its toxicological impact on developing embryo.

Despite some inherent limitations of the study, data presented here may serve as reference points for future research of Arctic seabird contamination. Understanding the impacts of human activities and future climate change on the exposure of polar species to contaminants is treated as a research priority by the Arctic Council (AMAP, 2011, 2018). Studies on avian species are becoming an effective way to understand global processes occurring in the marine ecosystem. There is still scarce information available concerning elemental exposure throughout the course of the year in seabirds along the coastal areas of the European Arctic, thus our study enhances our understanding of this issue.

CRediT authorship contribution statement

Aneta Dorota Pacyna-Kuchta: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing. **Dariusz Jakubas:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Marcin Frankowski:** Methodology, Validation, Writing - original draft, Writing - review & editing. **Żaneta Polkowska:** Conceptualization, Writing - original draft, Writing - review & editing. **Katarzyna Wojczulanis-Jakubas:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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