

*Environmental Toxicology*

## REGIONAL AND SPECIES SPECIFIC BIOACCUMULATION OF MAJOR AND TRACE ELEMENTS IN ARCTIC SEABIRDS

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**Abstract**—Twenty-five essential and nonessential elements were analyzed in Arctic seabirds to study the influence of phylogeny, tissue, Arctic region, and diet on avian element accumulation and to identify co-occurrence among metals. Muscle and liver concentrations were positively correlated, generally being higher in liver than in muscle, and generally did not differ by sex. Zinc showed the highest absolute concentrations in all samples (mean, 11.2–26.7  $\mu\text{g/g}$  in muscle, depending on species and area), followed by copper (5.2–7.5  $\mu\text{g/g}$ ), arsenic (0.5–5.4  $\mu\text{g/g}$ ), selenium (1.0–5.8  $\mu\text{g/g}$ ), rubidium (1.4–2.2  $\mu\text{g/g}$ ), and cadmium (0.04–1.2  $\mu\text{g/g}$ ). Mercury levels ranged from 0.05 to 0.8  $\mu\text{g/g}$  in muscle. The concentrations varied among species (dovekie [*Alle alle*], black guillemot [*Cephus grylle*], thick-billed murre [*Uria lomvia*], black-legged kittiwake [*Rissa tridactyla*], northern fulmar [*Fulmar glacialis*], ivory gull [*Pagophila eburnean*], Thayer's gull [*Larus thayeri*], and glaucous gull [*Larus hyperboreus*]), and between the northern Baffin Bay (Canada) and the Barents Sea, depending on the element. Whereas some elements (e.g., mercury and zinc) increased in absolute and standardized concentrations with trophic level in the northern Baffin Bay, most elements showed no relationship with trophic level or other dietary descriptors. In absolute concentrations, nonessential elements differed between regions, whereas essential elements differed among species but not within a species across the two regions. Standardized concentrations (element pattern) of both essential elements and nonessential elements generally did not differ between regions but was highly species specific and, thus, determined by the phylogenetic element regulation capacity. The usefulness of multivariate ordination in element wildlife studies is illustrated, which provides additional insight regarding element co-occurrence in wildlife, allows inclusion of species with low sample number, and reduces the possibility of type II errors created by low sample size.

**Keywords**—Auks Gulls Metals Mercury Minerals

## INTRODUCTION

Elevated levels of elements such as metals and metalloids in wildlife are of concern even in remote areas with limited anthropogenic activity, such as Arctic marine ecosystems [1] (<http://www.amap.no/documents/index.cfm?dirsub=/AMAP%20Assessment%20Report:%20Arctic%20Pollution%20Issues&sort=default>). For example, levels of Hg, Pb, and Cd in ringed seals (*Phoca hispida*), Atlantic cod (*Gadus morhua*), and seabirds from some Arctic regions have exceeded Health Canada and World Health Organization guidelines for human consumption [2]. In fact, concentrations of many contaminants in Arctic biota are similar to those measured in analogous temperate species sampled closer to primary contaminant sources [2]. Concentrations of elements in biota vary across the Arctic, in large part because of different global and regional sources and pathways of contaminants [1]. Element concentrations also may vary among species from the same area, which is related to the biological characteristics of the species and the biochemical characteristics of each element [3,4]. For example, concentrations of Hg in ringed seals vary across the Canadian Arctic [5] and are greater than levels observed in lower-trophic-level fish and zooplankton, because Hg biomagnifies in the food web [6].

In contrast to synthetic organic chemicals, several elements

occur naturally in the environment because of geological sources. Some elements are important components in animals and their biochemical processes (essential elements [EEs]) and are found at high levels in wildlife (major elements). Other elements occur at low levels (trace elements) and are either EEs, in that they are necessary even at low concentrations for processes such as growth and metabolism, or nonessential elements (NEEs), in that they have no known biological function [7]. The bioaccumulation of elements depends not only on the exposure via respiration but also on the exposure and bio-availability from the diet and the respective animal's ability to regulate the element. Even though NEEs do not have any known function in the animal, they may accumulate or be regulated in a fashion similar to that of EEs, or they may display a contrary behavior. For example, based on accumulation studies, it was suggested that Zn, an EE, is more actively regulated by aquatic invertebrates compared with Pb and other NEEs [8].

With inductively coupled plasma–mass spectrometry as a common tool in environmental element research, it is usual for laboratory analyses to produce data for more than 20 elements within a single sample. This potentially provides insights regarding the co-occurrence and behavior of elements and, subsequently, the mechanisms that regulate their bioaccumulation. Despite quantification of several elements in each sample, most studies of element accumulation in biota inves-

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tigate the elements individually using univariate statistics, such as analyses of variance or regression (see, e.g., [4,6]). Most studies of elements in Arctic biota have been limited to Hg, Pb, Cd, and Se because of widespread concerns about the toxicity and concentrations of these elements. However, several elements may be co-occurring in environmental samples because of similar origin, exposure, or behavior in the environment and biota. When the presence of several individual elements is analyzed simultaneously using multivariate ordination, such as factor analyses, an axis (the perfect explanatory variable) is extracted by minimizing the total residual sum of squares among all the elements. This axis accounts for the largest part of the element variance among the samples [9]. Thereafter, another axis can be extracted similarly from the remaining variance. This way, the information represented by a large number of variables (e.g., all elements) is condensed into a few axes representing the variance among samples with a minimal loss of information [9]. In addition, because of restricted sample sizes, repeated univariate analyses of individual response variables may not be advantageous, because the power is low (high risk of type II error [to fail in rejection of a false-null hypothesis]) [9]. In studies of organic contaminants, such as halogenated hydrocarbons, it has been common in recent years to apply multivariate statistics to extract and to analyze the underlying structure in absolute and standardized concentrations among several compounds (see, e.g., [10]). Even though it has been used in abiotic element studies (see, e.g., [11]), this approach has been applied only recently in biotic studies of element accumulation [12] and rarely in wildlife studies. In the same way, that ratios of different halogenated synthetic organics have been used to illustrate distance to sources (e.g.,  $\alpha$ -/ $\gamma$ -hexachlorocyclohexane), the relationship among elements (both absolute and standardized concentrations) illustrate the similarities and differences in the behavior of elements in wildlife.

To date, few direct statistical comparisons of element concentrations in Arctic wildlife from different regions have been performed, and even fewer have considered the wide range of elements analyzed. The present study therefore focuses on how different elements bioaccumulate in Arctic avian wildlife. More specifically, differences among elements are emphasized: Does the element bioaccumulation vary depending on the element being essential or nonessential, by geographic region (European or North American Arctic), dietary choice, or phylogenetic related factors (seven seabird species) such as species-specific element regulation? In the present study, both uni- and multivariate statistical techniques were used to illustrate the usefulness of multivariate statistics in studies of element accumulation in wildlife. The study regions (central Barents Sea [CBS] in the European Arctic and northern Baffin Bay [NBB] in the Canadian Arctic) support ecologically, culturally, and economically important marine ecosystems that share common species and food-web interactions [13,14]. The present study is, to our knowledge, the first to compare multiple element levels and patterns simultaneously in the same seabird species from two Arctic regions using multivariate techniques.

## MATERIALS AND METHODS

### Sampling

The seabirds were collected in NBB (75–78°N, 74–76°W) and in CBS (north-central, 76°08'–76°96'N, 32°52'–33°31'E; north-western, 76°46'–77°45'N, 27°00'–28°13'E). Details regarding sample collection from NBB (May 1998 and June

1998) and CBS (May 1999) have been summarized previously [15,16]. Seabirds from NBB included dovekie (*Alle alle*), black guillemot (*Cepphus grylle*), thick-billed murre (*Uria lomvia*), black-legged kittiwake (*Rissa tridactyla*), northern fulmar (*Fulmaris glacialis*), ivory gull (*Pagophila eburnean*), Thayer's gull (*Larus thayeri*), and glaucous gull (*Larus hyperboreus*). Of these, dovekie, black guillemot, and thick-billed murre are auks (Alcidae), and kittiwake, ivory gulls, Thayer's gull, and glaucous gull are gulls (Laridae). Northern fulmar is a petrel (Procellariidae). Seabirds from CBS included dovekie, thick-billed murre, and black guillemot. The seabirds were collected opportunistically from a zodiac using a shotgun with pellets of stainless steel. They were measured and sexed, then dissected for liver (NBB) and muscle (NBB and CBS) samples that were stored frozen (–20°C) until element analysis.

### Element analysis

All samples were analyzed for element contents at the National Laboratory for Environmental Testing (NLET) of the National Water Research Institute (Burlington, ON, Canada). The elements were classified as EE or NEE according to lists in Puls [17] and Greenwood and Earnshaw [18]. Total Hg (NEE) in liver and muscle tissue (NLET Method 02-2802) was analyzed by cold-vapor atomic absorption spectrometry. Analyses of 24 elements in liver and muscle samples (NLET Method 02-2705) were analyzed by inductively coupled plasma-sector field spectrometry, with 22 elements analyzed at low resolution (EEs: Co, Cu, Li, Mn, Mo, Ni, Cr, V, and Zn; NEEs: Ag, Ba, Cd, Ga, Pd, Pt, La, Pb, Rb, Sr, Sb, Tl, and U) and two elements at high resolution (NEE: As; EE: Se). Instrumental detection limits (DLs) for most elements was 0.001  $\mu\text{g/g}$  except for Sr (0.05  $\mu\text{g/g}$ ), Pt (0.01  $\mu\text{g/g}$ ), Pd (0.1  $\mu\text{g/g}$ ), Li (0.1  $\mu\text{g/g}$ ), and Sb (0.01  $\mu\text{g/g}$ ). All elements were analyzed in wet tissue. Values are expressed as wet-weight concentrations. The NLET is certified by the Canadian Environmental Analytical Laboratory program of the Canadian Standards Association and participates in the Quality Assurance Program for the Northern Contaminants Program, with good results [19].

### Data analyses

Concentrations of the elements Ag, Ba, Cr, V, La, Pt, Pd, U, and Tl were less than the DL in more than 30% of samples and were excluded from the statistical analyses. For the remaining 16 elements, a few values were less than the DL for some elements (5% for Li and Pb, 9% for Ni, and 8% for Sb). To avoid missing values distorting the statistical outcomes, those values were replaced by randomly generated numbers (StatPlus V2.5 Excel Addin 2002 for Windows®) using 0.5·DL for the respective element as the mean and assuming 30% standard deviation based on the mean variation of estimated means in the present dataset. Absolute element concentrations were logarithmically transformed to reduce skewness and heterogeneity before the parametric statistical analyses. Whereas univariate analyses (general linear methods [GLM] and type III sum of squares in SAS® V8 for Windows® [20]) were performed only on absolute element concentrations, multivariate ordination (principal component analyses [PCA] in Canoco 4.5 for Windows [21]) was performed both on absolute concentrations and standardized concentrations (sample-standardized by norm, which is similar to the metal pattern in which each element is a proportion of the total). Both GLM

and PCA were performed on absolute element concentrations, which is rarely seen in wildlife studies and illustrates the usefulness of multivariate ordination methods in element accumulation studies.

Muscle and liver element concentrations in NBB seabirds were compared by Spearman's rank-order correlation ( $R_s$ ; SAS V8 for Windows). Because of the elements' divergent uptake, storage, depuration, and biomagnification properties, selected correlations among elements were carried out on a molar unit: Between Hg and Se, because of their known biochemical relationship [22]; among Hg, Zn, and Rb, because of their potential biomagnification capacity [6,23]; and between Cd and Pb, because of their similar biochemical affinity for calcium and storage in bones [24].

The initial GLM (element = species + sex) revealed no difference between males and females; thus, sex was excluded from the final analyses. When element = species was significant, postcomparison of means (Tukey's test) was performed to contrast seabird species. Thayer's gull was not included in the GLM, because  $n = 1$ .

The PCA extracts ordination axes that minimize the total residual sum of squares among all the response variables (in this case, elements), and it assigns scores to the individual samples that are linear combinations of the elements (Figs. 1–3). Principal component 1 (PC1; x-axis) accounts for the largest part of the element variance among the samples, whereas principal component 2 (PC2; y-axis) is uncorrelated to PC1 and accounts for the largest part of the remaining element variance among the samples. Elements are presented as arrows pointing to the direction of increasing absolute or standardized value in the PCA of concentrations or pattern, respectively. Elements with shorter arrows vary little among the samples, whereas longer arrows illustrate elements with high variation among samples that thereby contribute more to the separation of samples in the ordination space. Only elements that correlated more than 20% are shown in the ordination plot, because the others do not contribute much to sample separation in the ordination diagram. The angles between arrows indicate correlations (or covariance) between the elements: A small angle means a high correlation, whereas an angle of  $90^\circ$  means that the occurrence of elements in the samples is not correlated. Trophic position based on stable isotopes of nitrogen in NBB seabirds ( $\delta^{15}\text{N}$  from Fisk et al. [15]) was entered as a passive variable to investigate the element behavior in relation to trophic position of the seabirds. Further details for diagram interpretation are described elsewhere [25,26].

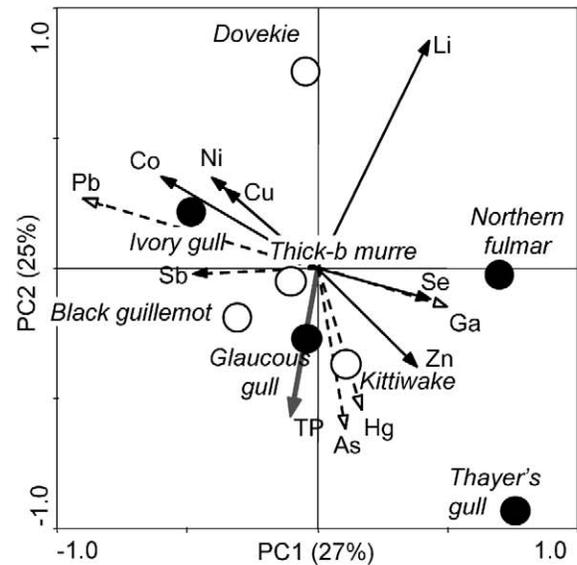
To compare element bioaccumulation between the two Arctic regions, PCA and GLM were performed by including only species collected from both regions: Black guillemot, dovekie, and thick-billed murre (GLM: element = region + species + sex). Again, sex was not a significant covariate except for Sr ( $F_{1,44} = 4.4$ ,  $p = 0.0408$ ) and Pb ( $F_{1,44} = 6.02$ ,  $p = 0.0182$ ), and it was excluded from the final GLM and the PCA except for the GLM of Sr and Pb.

## RESULTS

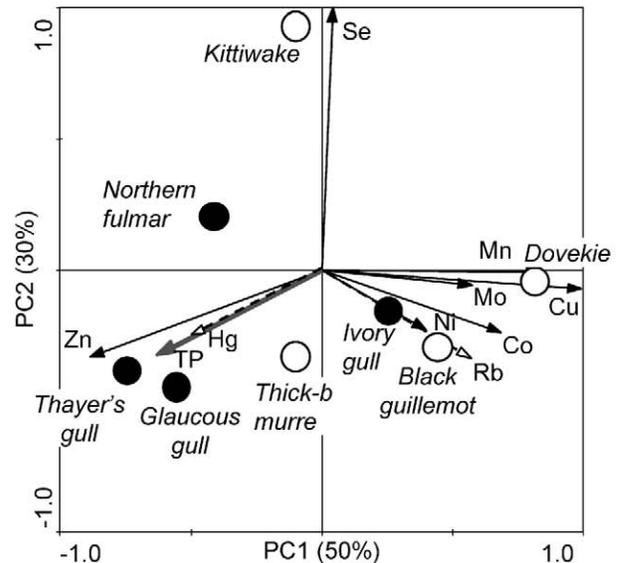
### Elements quantified and specific element relationships

Of the 25 elements analyzed, Ag, Ba, Cr, V, La, Pt, Pd, U, and Tl were less than the DL in more than 30% of samples. Palladium was detected only in one sample (NBB black-legged kittiwake liver, 0.01 mg/kg wet wt) and Pt in five samples (NBB thick-billed murre liver,  $0.001 \pm 0.000$  mg/kg wet wt; mean  $\pm$  CI, 95% confidence interval adjusted for sample size).

### a) Concentration



### b) Pattern

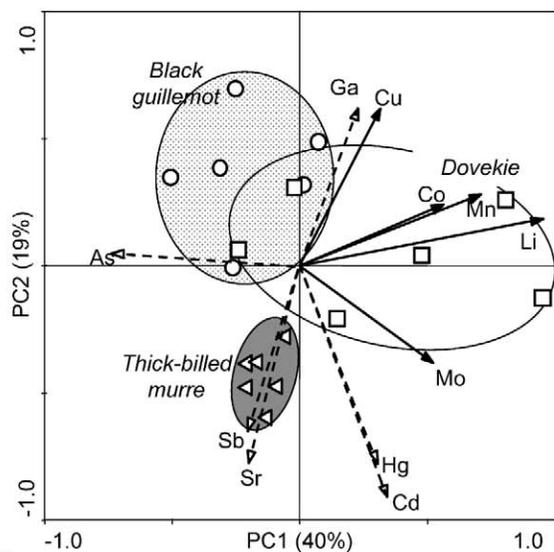


→ Essential element      .....▶ Non-essential element

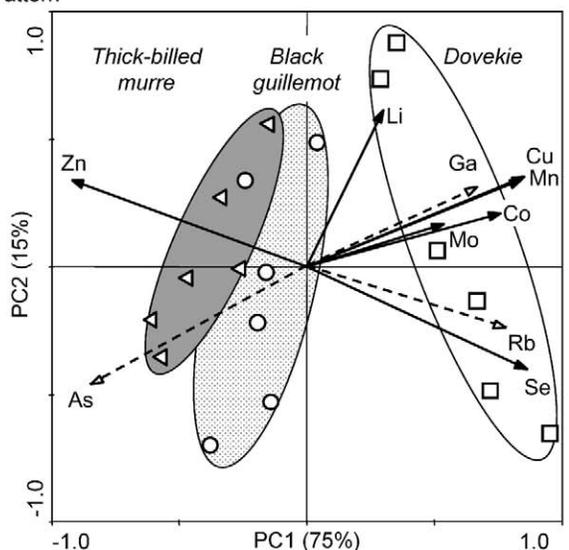
Fig. 1. Biplot of Northern Baffin Bay seabird species mean scores (circle) on the principal components (PC) extracted by principal component analyses (PCA) and the element loadings on the PCs. Absolute element concentrations (a) (log mg/kg) and standardized element concentrations (b) (pattern) in muscle of seven seabird species. Black circles are scavengers, white are non-scavengers, and black solid and stippled arrows are the respective element pointing in the direction of increasing value. The direction of increasing trophic position (TP) is indicated by the gray arrow TP, based on the  $\delta^{15}\text{N}$  according to Fisk et al. [15]. (See *Data analyses* for the description of diagram interpretation.)

Zinc had the highest concentrations in both liver and muscle in all species and from both regions (Table 1). Other prominent elements in all species and both tissues were Cu, As, Se, Rb, and Cd (Tables 1 and 2). The seabird species with the highest element concentration depended on the specific element (Tables 1–3). Lead was the element showing the largest variation

## a) Concentration



## b) Pattern



→ Essential element    - - - → Non-essential element

○ Black guillemot    □ Dovekie    ◁ Thick-billed murre

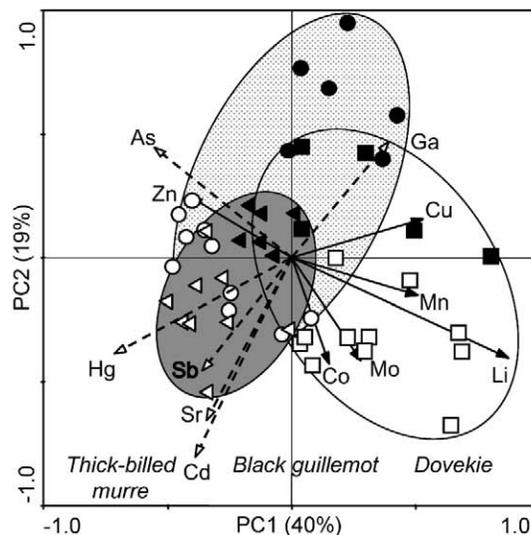
Fig. 2. Biplot of Central Barents Sea seabird scores (symbols) on the principal components (PC) extracted by principal component analyses (PCA) and the element loadings (arrows) on the PCs. Absolute element concentrations (a) (log mg/kg) and standardized element concentrations (b) (pattern) in muscle of three seabird species. Envelopes are drawn around each species' scores to simplify the plot. (See *Data analyses* for the description of diagram interpretation.)

among the seabird species, whereas Rb and Mn showed the lowest variations (Tables 1 and 2).

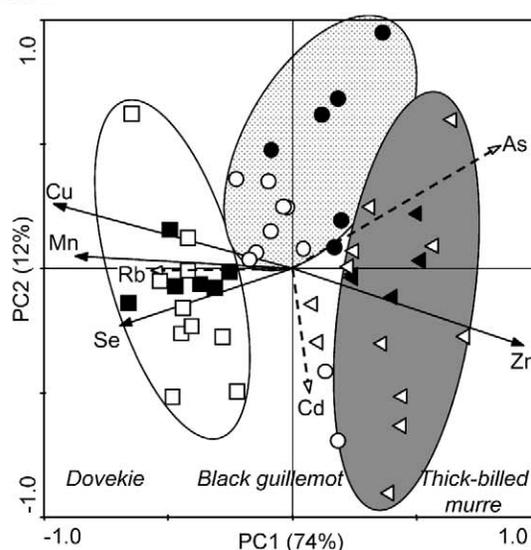
Concentrations of most elements were higher in liver than in muscle (Tables 1 and 2). Most elements were positively correlated between liver and muscle (Spearman's rank order,  $n = 48$ ,  $R_s = 0.40-0.84$ ,  $p$  from  $<0.0001$  to  $0.0049$ ) except for Mn, Mo, and Ni (all EEs) as well as Sr (NEE). Because most elements correlated between muscle and liver, and because muscle was analyzed in most species from both CBS and NBB, the present study focused on element data in muscle.

The molar units of Hg (NEE) in muscle correlated positively with Se (EE,  $n = 48$ ,  $R_s = 0.22$ ,  $p = 0.0373$ ), Zn (EE,

## a) Concentration



## b) Pattern



→ Essential element    - - - → Non-essential element

○ Black guillemot    □ Dovekie    ◁ Thick-billed murre

Fig. 3. Biplot of Central Barents Sea (CBS; black) and Northern Baffin Bay (NBB; white) seabird scores (symbols) on the principal components (PC) extracted by principal component analyses (PCA) and the element loadings (arrows) on the PCs. Absolute element concentrations (a) (log mg/kg) and standardized element concentrations (b) (pattern) in muscle of three seabird species. Envelopes are drawn around each species' scores to simplify the plot. (See *Data analyses* for description of diagram interpretation.)

$R_s = 0.63$ ,  $p < 0.0001$ ), and Rb (NEE,  $R_s = 0.31$ ,  $p = 0.0035$ ), whereas Cd and Pb (both NEE) did not correlate ( $R_s = -0.06$ ,  $p = 0.5827$ ).

#### Species-specific element accumulation

When analyzed separately using univariate statistics (GLM followed by Tukey's test), concentrations of most elements differed among seabird species from NBB and CBS, even though the interspecies relationships differed between the regions (Table 3). In NBB, Ni, Ga, and Sr did not differ among the species, whereas in CBS, Li, Ni, Sb, and Pb did not differ among the species (Table 3).

Table 1. Essential element concentrations (ng/g wet wt) in Arctic marine seabirds<sup>a</sup>

	<i>n</i>	$\delta^{15}\text{N}$ (mean $\pm$ SE)	Co (mean $\pm$ CI)	Cu (mean $\pm$ CI)	Li (mean $\pm$ CI)	Mn (mean $\pm$ CI)	Mo (mean $\pm$ CI)	Ni (mean $\pm$ CI)	Se (mean $\pm$ CI)	Zn (mean $\pm$ CI)
Barents Sea										
Dovekie										
Muscle	6	10.5 $\pm$ 0.1	21 $\pm$ 3	7,468 $\pm$ 303	271 $\pm$ 364	717 $\pm$ 72	43 $\pm$ 7	45 $\pm$ 21	2,293 $\pm$ 1,100	11,917 $\pm$ 1,008
Thick-billed murre										
Muscle	6	13.1 $\pm$ 0.1	13 $\pm$ 4	5,620 $\pm$ 495	18 $\pm$ 4	461 $\pm$ 32	34 $\pm$ 7	33 $\pm$ 11	957 $\pm$ 74	14,000 $\pm$ 1,681
Black guillemot										
Muscle	6	14.2 $\pm$ 0.1	16 $\pm$ 3	7,257 $\pm$ 1,008	34 $\pm$ 33	542 $\pm$ 68	28 $\pm$ 7	38 $\pm$ 18	1,135 $\pm$ 280	14,633 $\pm$ 1,848
Northern Baffin Bay										
Dovekie										
Muscle	10	10.8 $\pm$ 0.1	21 $\pm$ 6	6,973 $\pm$ 403	182 $\pm$ 161	650 $\pm$ 51	42 $\pm$ 8	34 $\pm$ 23	1,976 $\pm$ 436	11,172 $\pm$ 774
Liver	9		70 $\pm$ 43	8,181 $\pm$ 1,309	283 $\pm$ 274	3,324 $\pm$ 237	695 $\pm$ 59	90 $\pm$ 48	3,853 $\pm$ 636	32,267 $\pm$ 3,705
Kittiwake										
Muscle	10	13.3 $\pm$ 0.1	14 $\pm$ 2	5,749 $\pm$ 382	19 $\pm$ 13	520 $\pm$ 54	37 $\pm$ 3	19 $\pm$ 10	5,792 $\pm$ 1,062	16,030 $\pm$ 1,041
Liver	10		34 $\pm$ 6	6,048 $\pm$ 648	87 $\pm$ 127	3,515 $\pm$ 478	623 $\pm$ 53	60 $\pm$ 32	11,220 $\pm$ 3,215	35,550 $\pm$ 5,961
Thick-billed murre										
Muscle	11	13.5 $\pm$ 0.1	23 $\pm$ 5	5,205 $\pm$ 464	38 $\pm$ 3	486 $\pm$ 35	41 $\pm$ 4	35 $\pm$ 24	1,295 $\pm$ 201	14,245 $\pm$ 1,066
Liver	11		44 $\pm$ 9	7,155 $\pm$ 862	61 $\pm$ 2	3,487 $\pm$ 504	756 $\pm$ 80	43 $\pm$ 31	2,812 $\pm$ 528	45,218 $\pm$ 3,299
Black guillemot										
Muscle	10	13.7 $\pm$ 0.0	20 $\pm$ 2	6,896 $\pm$ 393	23 $\pm$ 26	607 $\pm$ 64	27 $\pm$ 4	35 $\pm$ 21	1,459 $\pm$ 182	13,350 $\pm$ 1,206
Liver	10		30 $\pm$ 4	8,073 $\pm$ 1,042	43 $\pm$ 42	2,666 $\pm$ 302	713 $\pm$ 98	32 $\pm$ 15	4,762 $\pm$ 1,283	35,800 $\pm$ 2,980
Northern fulmar										
Muscle	10	14.2 $\pm$ 0.1	10 $\pm$ 2	5,334 $\pm$ 287	157 $\pm$ 169	677 $\pm$ 80	36 $\pm$ 8	25 $\pm$ 10	4,093 $\pm$ 941	20,550 $\pm$ 1,998
Liver	10		28 $\pm$ 5	6,244 $\pm$ 696	202 $\pm$ 204	4,293 $\pm$ 396	535 $\pm$ 53	30 $\pm$ 14	10,027 $\pm$ 1,795	63,210 $\pm$ 10,753
Ivory gull										
Muscle	5	14.1 $\pm$ 0.1	22 $\pm$ 6	7,434 $\pm$ 1,470	81 $\pm$ 160	496 $\pm$ 148	34 $\pm$ 9	52 $\pm$ 27	2,190 $\pm$ 668	16,080 $\pm$ 2,384
Glaucous gull										
Muscle	10	16.5 $\pm$ 0.1	14 $\pm$ 2	5,179 $\pm$ 268	33 $\pm$ 26	539 $\pm$ 37	39 $\pm$ 2	34 $\pm$ 29	1,508 $\pm$ 187	23,290 $\pm$ 1,795
Thayer's gull										
Muscle	1	17.2	15	5,330	10	492	44	20	1,790	26,700
Liver	1		52	7,310	10	4,310	863	90	4,740	44,300

<sup>a</sup> Seabirds are listed with increasing trophic position (based on  $\delta^{15}\text{N}$ ) according to Fisk et al. [15] and Borgå et al. [16]. CI = 95% confidence interval adjusted for sample size; SE = standard error.

Table 2. Nonessential element concentrations (ng/g wet wt) and elements less than the detection limit (DL) in more than 30% of samples of Arctic marine seabirds<sup>a</sup>

	Cd (mean ± CI)	Pb (mean ± CI)	Rb (mean ± CI)	Sr (mean ± CI)	Ga (mean ± CI)	As (mean ± CI)	Sb (mean ± CI)	Hg (mean ± CI)
<b>Barents Sea</b>								
Dovekie								
Muscle	152 ± 96	10 ± 3	1,897 ± 262	46 ± 15	16 ± 1	698 ± 202	1 ± 0	118 ± 104
Thick-billed murre								
Muscle	285 ± 126	20 ± 11	1,422 ± 218	60 ± 7	13 ± 1	2,368 ± 862	2 ± 2	121 ± 36
Black guillemot								
Muscle	37 ± 25	16 ± 18	1,553 ± 75	34 ± 11	16 ± 2	2,898 ± 1,176	1 ± 0	48 ± 15
<b>Northern Baffin Bay</b>								
Dovekie								
Muscle	414 ± 133	93 ± 160	1,727 ± 118	133 ± 64	14 ± 7	476 ± 74	2 ± 1	77 ± 16
Liver	5,781 ± 914	273 ± 470	1,954 ± 222	124 ± 52	17 ± 7	1,511 ± 283	2 ± 2	274 ± 61
Kittiwake								
Muscle	464 ± 171	16 ± 6	1,392 ± 54	67 ± 28	14 ± 7	3,023 ± 1,568	1 ± 1	299 ± 66
Liver	8,617 ± 4,052	17 ± 9	1,890 ± 193	580 ± 545	17 ± 5	8,834 ± 4,981	3 ± 2	1,047 ± 309
Thick-billed murre								
Muscle	554 ± 134	22 ± 10	1,688 ± 114	195 ± 168	6 ± 3	2,498 ± 3	2 ± 1	333 ± 54
Liver	13,831 ± 3,720	91 ± 132	1,958 ± 135	405 ± 233	11 ± 2	10,421 ± 2	6 ± 2	1,114 ± 195
Black guillemot								
Muscle	417 ± 108	39 ± 12	1,693 ± 155	93 ± 31	14 ± 7	1,572 ± 258	1 ± 0	340 ± 71
Liver	6,828 ± 1,186	33 ± 13	1,957 ± 202	250 ± 85	17 ± 6	6,646 ± 1,731	3 ± 1	1,172 ± 320
Northern fulmar								
Muscle	1,168 ± 829	6 ± 4	2,186 ± 286	137 ± 111	12 ± 6	2,716 ± 1,072	1 ± 1	391 ± 102
Liver	21,836 ± 9,453	14 ± 12	2,738 ± 287	254 ± 82	16 ± 7	6,937 ± 4,983	2 ± 1	3,412 ± 1,162
Ivory gull								
Muscle	191 ± 145	118 ± 244	2,036 ± 377	99 ± 88	4 ± 1	2,176 ± 1,800	1 ± 1	214 ± 76
Glaucous gull								
Muscle	213 ± 117	36 ± 35	2,233 ± 284	91 ± 42	13 ± 7	1,861 ± 859	1 ± 0	801 ± 191
Thayer's gull								
Muscle	82	1	1,650	21	24	5,370	1	483
Liver	1,770	65	1,850	145	11	19,600	10	1,940
Less than the DL in more than 30% of samples								
	Tl (mean ± CI)	U (mean ± CI)	V (mean ± CI)	La (mean ± CI)	Cr (mean ± CI)	Ag (mean ± CI)		
<b>Barents Sea</b>								
Dovekie								
Muscle	2 ± 1		8 ± 1		50	2 ± 2		
Thick-billed murre								
Muscle	1		4 ± 2			1		
Black guillemot								
Muscle	1		8 ± 5		57 ± 12			
<b>Northern Baffin Bay</b>								
Dovekie								
Muscle	1 ±	1	6 ± 2		150	1 ± 0		
Liver	2 ± 0	2 ± 0	10 ± 3	1	75 ± 16	10 ± 4		
Kittiwake								
Muscle	1		10 ± 5		70	3 ± 1		
Liver	1 ± 1	2 ± 1	16 ± 10	1	63 ± 8	21 ± 12		
Thick-billed murre								
Muscle	1 ± 0	3	7 ± 3		1,122 ± 231	3 ± 2		
Liver	2 ± 0	2 ± 1	8 ± 3	1	189 ± 61	26 ± 7		
Black guillemot								
Muscle	1		2		55 ± 5	1		
Liver	2 ± 1	1		1	65 ± 15	19 ± 4		
Northern fulmar								
Muscle	1	1	5 ± 2		65 ± 15	2 ± 1		
Liver	1	4 ± 2	44 ± 19	2 ± 1	60 ± 10	17 ± 2		
Ivory gull								
Muscle	1		4 ± 1			4 ± 3		
Glaucous gull								
Muscle	1		10 ± 6		70	2 ± 1		
Thayer's gull								
Muscle	1							
Liver	1			6		3		

<sup>a</sup> CI = 95% confidence interval adjusted for sample size.

Table 3. Tukey's test results from means comparison among species of muscle concentrations of essential and nonessential elements<sup>a</sup>

	Essentials											Nonessentials						
	Co	Cu	Li	Mn	Mo	Ni	Se	Zn	Pb	Sb	Rb	Ga	Sr	As	Cd	Hg		
Barents Sea																		
Dovekie	B	B	NS	C	B	NS	B	A	NS	NS	B	B	AB	A	B	AB		
Thick-billed murre	A	A	NS	A	AB	NS	A	AB	NS	A	A	A	B	B	B	B		
Black guillemot	AB	B	NS	B	A	NS	A	B	NS	A	B	A	A	B	A	A		
Northwater Polynya																		
Dovekie	CD	B	B	CD	B	NS	B	A	B	B	NS	NS	NS	A	BC	A		
Kittiwake	B	A	A	AB	B	NS	D	C	AB	A	NS	NS	NS	B	BCD	BC		
Thick-billed murre	CD	A	AB	A	B	NS	A	BC	B	B	NS	NS	NS	B	CD	BC		
Black guillemot	BC	B	A	BD	A	NS	AB	B	A	B	NS	NS	NS	B	BC	BC		
Northern fulmar	A	A	A	D	AB	NS	C	D	A	C	NS	NS	NS	B	CD	C		
Ivory gull	CD	B	AB	AB	AB	NS	B	BC	B	BC	NS	NS	NS	B	AB	B		
Glaucous gull	B	A	A	ABC	B	NS	AB	D	B	C	NS	NS	NS	B	A	D		

<sup>a</sup> Classification based on lists in Puls [17] and Greenwood and Earnshaw [18]. Species with similar letters are not statistically different ( $\alpha = 0.05$ ); increasing letter indicates increasing element concentration. The seabirds are listed with increasing trophic position according to the methods described by Fuk et al. [15] and Borgå et al. [16]. NS = no significant difference was found among the species.

The multivariate ordination (PCA) of absolute element concentrations within both NBB and CBS (Figs. 1 and 2) provided results that were relatively similar to those found in the univariate analysis (Table 3). Some differences at the specific element or species level were found, both because one sample from Thayer's gull was included in the NBB PCA and because both PCAs considered all the elements simultaneously rather than one at the time. However, the main trend regarding species differences in element concentrations from the GLM was reflected by the PCA. The two first components extracted in the PCAs (PC1 and PC2) accounted for 52 and 59% of the total variability among the samples in NBB and CBS, respectively. In NBB, only Hg and As increased with trophic level, whereas Li decreased because of the high Li concentrations in some of the dovekie samples (Fig. 1a). The other elements were correlated to trophic level only weakly or not at all. No difference was found in element concentrations between scavengers and non-scavengers or any apparent grouping of EEs and NEEs. Dovekie separated from the other species because of higher Li concentrations, whereas ivory gull had higher concentrations of Pb, Co, Ni, and Cu than the other species (Fig. 1a). Thayer's gull and northern fulmar separated from the other species because of low concentrations of Pb and Cu and high concentrations of Cd and As in fulmar and Ga in Thayer's gull (Fig. 1a).

In the Barents Sea, thick-billed murre separated from dovekie and black guillemot because of higher concentrations of Pb, Sr, and Sb and lower concentrations of Ga, Cu, and Rb (Fig. 2a). Furthermore, dovekie had lower As and higher Co, Mn, Li, and Mo levels than thick billed murre and black guillemot, even though dovekie overlapped with black guillemot because of high variation among the dovekie samples.

The PCA of element pattern displayed a difference in the pattern of element accumulation among the NBB seabirds (Fig. 1b). In NBB, scavenging species generally separated from non-scavenging species, with the exception of ivory gull, because of higher relative contributions of Hg, Zn, and Rb. The auks grouped because of a higher relative contribution of Cu, Ni, Co, and Mo, and the black-legged kittiwakes separated from all other species because of higher relative contribution of Se (Fig. 1b). The relative contribution of Zn and Hg to the element pattern increased with trophic level. Within CBS (Fig. 2b), the PCA of element pattern separated the three auk species because of higher relative contributions of Zn and As in thick-billed murre and black guillemot than in dovekie and because of higher relative contributions of Mn, Cu, Co Mo, and Se (all EEs) as well as of Ga and Rb (both NEEs) in dovekie. Black guillemot had an intermediate element pattern and grouped between the two other species, slightly overlapping with thick-billed murre (Fig. 2b).

#### Comparison of elements in seabirds between Arctic regions

All regional comparisons were performed including only black guillemot, dovekie, and thick-billed murre. Both the univariate (GLM) and multivariate (PCA) statistical analyses yielded the same results. Based on GLM (with species [and sex for Pb and Sr], as covariate), concentrations of Sr, Pb, Cd, Sb, and Hg (all NEEs) as well as Co (EE) were higher in NBB than in CBS seabirds (GLM:  $F_{1,48} = 5.24-58.45$ ,  $p$  from  $<0.00001$  to  $0.0258$ ), whereas Ga and As (both NEEs) and Cu (EE) were higher in CBS seabirds (GLM:  $F_{1,48} = 5.41-9.98$ ,  $p = 0.0028-0.0245$ ). The rest of the elements (Li, Mn, Mo, Ni, Rb, Se, and Zn, all of which are EEs except for Rb)

did not differ between the regions (GLM:  $F_{1,48} = 0.07\text{--}3.43$ ,  $p = 0.0704\text{--}0.7925$ ). For Hg, Sr, Pb, Ga, and Cd (all NEEs) as well as Co (EE), region explained more of the variance in concentration compared with species, whereas species explained most of the variance for the other elements (EEs: Cu, Li, Mn, Mo, Sb, Se, and Zn; NEEs: As and Rb). Thus, region explained differences in NEE concentrations, whereas concentrations of EEs were species specific.

Both PC1 and PC2 explained 59% of the total variability in element concentrations among samples (Fig. 3a), and the PCA results generally were similar to those of the GLM. Most Barents Sea samples separated from the NBB samples because of higher Ga (NEE) and Cu (EE) concentrations in the CBS seabirds and higher Hg, Sr, Sb, Cd, and Pb (NEEs) concentrations in the NBB seabirds (Fig. 3a). Zinc, As, Li, and the rest of the elements contributed to the sample ordination, mainly because of the species identity rather than sampling region (Fig. 3a). When species was included as a covariable in the PCA, the sample separation based on PCA was similar to that of the GLM (results not shown); however, it was of interest to keep the species identity in the PCA.

Regional comparison of element patterns (standardized concentrations) resulted in a different ordination of the samples than the one obtained from absolute concentrations (Fig. 3b). For element pattern, the species were better separated, with less variability within species along PC1, whereas no clear separation was found because of Arctic region (Fig. 3b). Thick-billed murre had higher relative contributions from Zn and As, and dovekie had higher relative contributions from Mn, Cu, Rb, and Se. However, black guillemot samples had an intermediate element pattern, with a low relative contribution from Cd, resulting in a position between dovekie and thick-billed murre in the ordination diagram. In black guillemot, samples from NBB had a higher relative contribution of Cd compared with samples from CBS.

Thus, the two regions differed in absolute concentrations of NEEs, whereas absolute concentrations of EEs depended more on species than on geographic region. On the other hand, the pattern of both EEs and NEEs did not differ by region but by species identity.

## DISCUSSION

Earlier studies have considered element accumulation in several seabird species (see, e.g., [3,4,27–30]), but few have simultaneously included as many elements (25 elements), species (eight species), tissues (muscle and liver), and different regions (CBS and NBB) as the present study. For example, a previous study in the CBS region included 13 seabird species but only six elements [4]. Similarly, from the Canadian Arctic, 23 elements were studied in three seabird species, but the element levels in the seabirds were investigated separately [30]. The present study therefore is valuable because it analyzes the co-occurrence of several elements simultaneously using multivariate techniques as well as including several species from two different Arctic regions. This is particularly important to identify the factors controlling element bioaccumulation across trophic levels and physiological adaptations because of phylogeny.

Because PCA and GLM yielded generally similar results regarding differences between species in absolute element concentration, multivariate statistics also seem to be useful in studies of element accumulation in wildlife. Whereas the PCA of concentrations is helpful to evaluate exposure differences,

the PCA of pattern (standardized concentrations) looks beyond exposure and reveals features of element accumulation, such as species-related element regulation. Additionally, multivariate ordination was helpful, because it allowed inclusion of species with low sample size, such as Thayer's gull ( $n = 1$ ), which had to be removed from the univariate analyses. The multivariate ordination of all the elements helped to investigate any difference in EE and NEE bioaccumulation and how they co-occurred in the seabirds.

### *Species-specific element accumulation*

*Northern Baffin Bay.* Only a few elements were largely influenced by the seabirds' diet and trophic position. Arsenic and Hg (both NEEs) increased with trophic position, but Zn also was particularly high in glaucous and Thayer's gulls and low in dovekie. Increasing Zn concentrations with higher trophic levels (and correlation with Hg) may suggest biomagnification, even though Zn is an EE. Mercury is known to biomagnify in terrestrial, freshwater, and marine food webs worldwide (see, e.g., [6,28]). As such, the positive correlation between Hg, Rb, and Zn may suggest biomagnification not only for Hg but also for Rb and Zn, even though the correlation with Rb was weak. When enrichment of stable nitrogen isotopes was used to determine trophic biomagnification in NBB for the food web, Rb, Zn, and Hg were found to biomagnify from algae to seabirds [6]. The high Li levels in dovekie compared to those in the other species may result from accumulation from calanoid copepods, the main prey of dovekies [31]. Marine crustaceans have high Li levels as an important component of their carapace (calcium carbonate) [32]. Lithium is an EE, so the high levels in the dovekie show that diet may, in some cases, also influence the observed bioaccumulation of EEs.

With the exception of ivory gull, scavenging species had a element pattern different from that of nonscavenging species because of higher relative contribution of Zn and Hg in scavengers and higher relative contributions of Cu, Mn, Ni, Mo, Rb, and Co in nonscavengers (mainly auks). This is consistent with biomagnification of Hg, Zn, and Rb in the NBB food web [6]. Kittiwake differed from the other species because of the high relative contribution of Se. Thayer's gull and glaucous gull are more similar in pattern than in absolute concentrations, mainly because of the high relative contribution of Hg and Zn. It is not clear if the scavengers and nonscavengers differ in pattern mainly as a result of exposure from food (high relative contribution of Zn and Hg in Thayer's and glaucous gulls) or if the separation is related to differences in element regulation between auks and gulls. A different bioaccumulation pattern has been found between auks and gulls for chlorinated hydrocarbons, such as chlordanes [15] and polychlorinated biphenyls [16]. Fulmars, which are petrels, had element concentrations and patterns that differed from those of both gulls and auks, in accordance with their dietary choice and metabolism [33]. Because most elements do not show concentrations related to the species' diets, the present results support earlier studies pointing toward other factors, especially element regulation, as being important for avian element accumulation.

*Central Barents Sea.* Surprisingly, Hg concentrations in the Barents Sea seabirds were lowest in black guillemot, the auk with the highest trophic level in the present study [16]. The lack of an increase in Hg with trophic level probably related to the narrow trophic range analyzed in the Barents Sea, because Hg increased with trophic level in the NBB samples,

which also included high-trophic-level seabirds, such as glaucous gull. Previous studies have suggested that the accumulation of Cr and Pb increases with increasing invertebrate proportion in the diet [34]. In the present study, however, Cr was less than the DL in more than 30% of the samples, and the Pb concentrations did not differ among the seabirds.

The element patterns of the auks were consistent with those seen in the NBB seabirds, with higher Mn, Cu, Mo, and Co in dovekie, high relative contribution of Zn in thick-billed murre, and an intermediate position for black guillemot. This further suggests that physiological requirements and element regulation are important determinants in observed element patterns in Arctic seabirds, even when comparing species from the same family.

#### Regional element accumulation

Whereas the absolute concentrations of NEEs differed between the Arctic regions, the EEs, which differed between species, were comparable across the Arctic regions. This supports the contention that metabolic regulation of elements is more efficient toward EEs than toward NEEs, and that in the present study, EEs are at the levels required for the seabirds' biological functions. The similar EE levels within a species across the two regions also suggest that the element regulatory mechanisms of the seabirds have not been altered or impaired by the observed element levels.

The finding that the As concentrations were higher in CBS than in NBB seabirds (in addition to differences among the species) corresponds to previously elevated levels in seabirds from the eastern CBS close to Novaya Zemlya, where nuclear tests were carried out in the 1960s [4]. However, the finding that species is more important to determine the levels in seabirds (both GLM and PCA) suggests that this NEE is regulated through the same mechanisms as other elements, especially Zn, with which it co-occurs. Whereas Hg concentrations have been shown to increase with latitude [35], seabird surveys have reported no clear geographic pattern in element accumulation among the Arctic regions [1,36]. More regional-specific studies generally have found higher Cd and lower Hg levels in western compared to eastern Greenland [37]. In the present study, concentrations of not only Cd but also Hg were higher in NBB than in CBS seabirds. Seasonal effect on element accumulation can be excluded, because all birds from both NBB and CBS were collected in May to June of subsequent years.

The element pattern, which generally did not differ between the two areas, clearly separated the samples because of species identity. Thick-billed murre had higher relative contribution of Zn and As than dovekie and black guillemot. The relative contributions of most other elements were highest in dovekie, with an intermediate pattern in black guillemot. Again, this emphasized the importance of species-specific element regulation for overall element accumulation.

#### Specific element relationships

As expected from earlier studies [4,27], element concentrations in Arctic seabirds were greater in liver than in muscle and were positively correlated across all samples. Gender did not influence element concentrations in the seabirds of the present study. However, the influence of gender on element accumulation is inconclusive based on the results of earlier studies. Whereas some report that generally no difference exists between the sexes [37], others have found sex-specific

element accumulation in birds, though dependent on species, element, and tissue (see, e.g., [38]).

Similar to the results of past studies [27,29], Se and Hg were positively correlated in the NBB and CBS seabirds of the present study, with a surplus of Se compared to Hg. Positive correlation between Se and Hg has been suggested to reflect an antagonistic interaction between their toxicity [27]. The ratio of Se to Hg in marine mammals is reported to be 1:1, but Arctic seabirds have been shown to have a surplus of Se [29], which may be beneficial, because Se reduces certain toxic effects of Hg [22].

According to the United Nation Economic Commission for Europe Convention on long-range transboundary air pollution [39], Hg, Cd, and Pb are the toxic elements of highest concern because of environmental and human toxicity. Based on the guidelines of the Arctic Monitoring and Assessment Programme [1], Cd and Hg levels in the NBB seabirds were less than the hepatic threshold levels for biological effects. Hepatic Se levels were near threshold levels for deformities in embryos. In general, little evidence is available for biological effects of elements in Arctic seabirds [2].

#### CONCLUSION

The present study has shown that bioaccumulation of most elements cannot be related to diet or trophic levels, with some exceptions (e.g., Hg and Zn). Whereas the absolute concentrations of NEEs differed between the regions, the essential elements differed between species but were comparable across the Arctic regions. On the other hand, the pattern of both NEEs and EEs differed between species but not regions. This suggests that NEEs are regulated through similar mechanisms as EEs are, but that the EE regulation is more efficient.

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