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Spatial ecotoxicology: migratory Arctic seabirds are exposed to mercury contamination while overwintering in the Northwest Atlantic

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Abstract

Arctic organisms are exposed to various levels of pollutants, among which mercury (Hg) has raised important environmental concerns. Previous studies examining Hg levels, trends and effects on Arctic marine top-predators have focused on the Arctic region. However, many of these top-predators, such as seabirds, migrate to spend a large part of their life-cycle far from the Arctic in areas where their exposure to contaminants is largely unknown. By combining biotelemetry, Hg and stable isotope analyses, we studied the seasonal Hg contamination of little auks (*Alle alle*; the most abundant Arctic seabird) in relation to their distribution and marine foraging habitat, as well as its potential impacts on bird reproduction. We show that little auks were about 3.5 times more contaminated when outside the breeding season, and that Hg accumulated during this non-breeding non-Arctic period was related to egg size the following season with females having more Hg laying smaller eggs. Our results highlight that ecotoxicological studies should be expanded to yield a comprehensive understanding of contamination risks and associated threats to top-predators over their entire annual cycle. Furthermore, we show that an important non-breeding area located in the northwest Atlantic was associated with higher Hg contamination and demonstrate the utility of bird-borne miniaturized technology to evaluate the contamination of marine systems at large spatial scales.
TOC/Abstract Art.
Introduction

Although lying far from major human industries, the Arctic region is threatened by pollution risks and pollutant levels. Transported over large distances by ocean currents and atmospheric circulation, many pollutants originating from industrialized or developing countries are deposited into the Arctic.\textsuperscript{1,2} More recently, sea-ice cover is much reduced in some areas, releasing pollutants trapped over decades and opening up new areas to human activities such as shipping and extractive industries, thereby increasing direct discharges of pollutants into Arctic ecosystems.\textsuperscript{3,4} As a consequence of all these activities, concentrations of pollutants such as trace metals or hydrocarbons have increased in some parts of the Arctic over the last decades.\textsuperscript{5-7} Once in the environment, pollutants can become bioavailable, enter the food-chain and can have major impacts on organisms and biodiversity. Among pollutants that are liable to affect Arctic wildlife, mercury (Hg) has raised important environmental concerns and drawn extensive attention.\textsuperscript{8} This non-essential metal is highly toxic, particularly in its main organic form of methylmercury, and even at low concentrations has been shown to be a powerful neurotoxin.\textsuperscript{9,10} In this context, defining concentrations, trends and ecotoxicological effects of Hg on Arctic organisms is important in order to develop strategies for conservation of vulnerable species and ecosystems. Recently, the Arctic Monitoring and Assessment Programme (AMAP) working group of the Arctic Council published an extensive review of the current knowledge about the Hg-contamination of Arctic species and the deleterious effects of Hg.\textsuperscript{8} This group emphasized the importance of focusing on marine top predators since they are expected to be exposed to high concentrations of Hg through bioaccumulation and biomagnification.\textsuperscript{11} Marine top-predators, such as seabirds, can be highly mobile during their annual cycle. For instance, many seabird species leave the Arctic after their breeding season and migrate
hundreds to thousands kilometres to winter areas, spending many months of the year outside of the Arctic, in boreal, temperate or even tropical regions.12-14 There, they could face environments equally or even more contaminated than the Arctic 15 and therefore accumulate high concentrations of Hg which could in turn have impacts on their physiology and behaviour and ultimately reproduction, survival and populations dynamics.16,17 Understanding exposure to pollutants, including Hg, during the season spent outside the Arctic is therefore essential to obtain a complete view of the risk faced by these organisms in a changing environment. Nevertheless, and to our knowledge, only one recent study, focused on persistent organic pollutants (POP), previously linked Arctic seabird contamination to their non-Arctic winter areas, highlighting the importance of considering the entire annual cycle in ecotoxicology studies.18 This focus on the annual cycle has yet to be considered for Hg in the Arctic seabirds. In this study, we examined and compared Hg concentrations measured in little auks (Alle alle), a small Arctic seabird, during different periods of the year and in relation to their winter distribution. We also investigated how Hg accumulated during the non-breeding period could affect their reproduction.

The little auk represents an ideal species to investigate Hg contamination of Arctic seabirds during the breeding and non-breeding periods. This species, like most of the alcids, moults twice per year; a complete feather moult soon after the breeding season (September) involves the replacement of the entire body plumage while a partial pre-breeding moult occurring during spring involves the replacement of throat and head feathers.19-21 The timing of this pre-breeding moult is not accurately known. However, little auks collected in March at their wintering site were all in winter plumage 22 while birds seen at breeding colonies in May are in breeding plumage. It is therefore likely that little auks moult their head and throat feathers in April, before arriving at their colony. The feather moult, along with egg production, is a mechanism by which seabirds excrete Hg accumulated in their body tissues,23 which makes
concentrations measured in feathers an indicator of Hg levels accumulated since the last moulting sequence.\textsuperscript{24-26} Hence, it can be assumed that Hg concentrations measured in little auk head feathers reflect contamination accumulated during the non-breeding period while cover feathers from other parts of the body reflect contamination accumulated during the breeding season.

By analysing Hg concentrations in blood and feathers and combining these results with data on bird movement and wintering areas, obtained with biotelemetry tracking and stable isotope analysis, we tested the following hypotheses: (1) little auk Hg contamination is higher during the non-breeding season spent outside of the Arctic. (2) Contamination of birds is related to their non-breeding distribution and foraging habitat. (3) Hg levels accumulated during the non-Arctic non-breeding period affect the reproduction of little auks.

**Materials and Methods**

*Study sites and sample collection*

In July 2009 and 2010, a total of 135 adult little auks breeding at Kap Höegh (East Greenland; 70°44’N, 21°35’W) were equipped with a miniature geolocator data-logger (GLS; Mk14 and Mk18L, British Antarctic Survey, mass = 1.5 g, \textasciitilde1\% of adult body mass) mounted on a conventional metal leg ring in order to track their non-breeding movements and distribution\textsuperscript{27,28}. The following years, birds were recaptured (2010: \textit{n}=47 (22 males, 24 females and one unknown sex), 2011: \textit{n}=35 (22 males and 13 females)), the data logger retrieved and downloaded, and blood and feather samples collected. Blood samples (\textasciitilde0.3 ml) were collected from the brachial vein, stored in seventy percent ethanol, and kept frozen at -20°C. Two batches of feathers were plucked from each bird: 1 from the body (back or belly)
and 1 from the head (cheek, neck or throat). These 2 batches are hereafter called ‘body feathers’ and ‘head feathers’, respectively. All feathers were kept at ambient temperature in sealed plastic bags until analysis. A few additional feathers were collected and stored at -20°C for subsequent molecular sexing.

Little auks nest in crevices in talus slopes and lay one single egg per breeding season. When accessible, eggs of tracked birds were measured (maximal length ($L$) and breadth ($B$)) and their volume ($V$) calculated following the equation $V = \pi/6 \times LB^2 / 1000$. Nests were then checked every two days until hatching to determine for each nest the hatching date. Evidence of hatching was provided by the direct sight of a chick or by the presence of egg shell fragments in the nest chamber.

Little auks were also collected in Placentia Bay (Newfoundland; 47°30’N, 54°00’W) in March 2011 and immediately kept frozen at -20°C until dissections. Newfoundland waters are known to be the main wintering quarter for little auks breeding in Greenland, including those from East Greenland. From these carcasses, blood samples were collected (from the cardiac clot) and kept frozen at -20°C until analysis.

**Hg and stable isotope analyses**

Prior to analyses, feathers were cleaned in order to remove any external contamination. Feathers were rinsed once in a 2:1 chloroform:methanol solution and twice in a methanol solution. Feathers were then dried for 48 h at 50°C. Blood samples were dried for 72h at ambient temperature to remove ethanol and then lyophilized for 48 h.

Total Hg (hereafter referred to as Hg) concentrations were measured in whole blood and feather samples collected from little auks breeding at Kap Hoëgh and in blood samples
collected from little auks wintering off Newfoundland. Hg analyses were performed at the Littoral Environnement et Sociétés laboratory (LIENSs, La Rochelle, France) on ca. 3 mg of homogenized whole blood or on 1 complete feather, using an advanced Hg analyzer spectrophotometer (Altec AMA 254). Analyses were repeated 2-3 times for each sample until the relative standard deviation for two samples was <10%, samples not meeting this criterion were excluded from the analysis. The mean T-Hg concentrations for those two measurements were then considered for statistical analyses. To ensure accuracy of measurements, a certified reference material was used (Lobster Hepatopancreas Tort-2; NRC, Canada; Hg concentration: 0.27 ± 0.06 μg.g⁻¹ dry weight (dw)) and measured every 10 samples. Average measured value was 0.26 ± 0.01 μg.g⁻¹ dw, n = 44. Additionally, blanks were run at the beginning of each sample set. The detection limit of the method was 0.005 μg.g⁻¹ dw.

Stable isotope carbon ratios were measured on non-lipid extracted whole blood samples, collected from little auks wintering off Newfoundland. Analyses were performed at LIENSs on ca. 0.5 mg subsamples of material loaded into tin cups, using an elemental analyser (Thermo Fisher, Flash EA 1112) coupled in continuous flow mode to an isotope ratio mass spectrometer (Thermo Fisher, Delta V Advantage, Bremen, Germany). Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation: δ¹³C = [(R_sample/R_standard)–1] × 1000, where R is the molar ratio ¹³C/¹²C. Standard values for C were Vienna-PeeDee Belemnite (VPDB). Replicate measurements of internal laboratory standards (acetanilide) indicated that measurement error was ±0.06.

The δ¹³C isotopic values mainly reflect the carbon source (i.e. foraging habitat) used by birds.
Spatial analyses

Light-level data were extracted from GLS loggers and processed following published procedures. Briefly, light-level data were converted into positions using the BASTrak software package (BAS, Cambridge, UK). We used threshold light intensity of 10, an angle of sun elevation of -3.0°, and applied compensation for movements. The angle of sun elevation was defined following the ‘Hill–Ekstrom calibration’ method. For each equipped bird, two positions were obtained per day (at local noon and midnight). From individual positions, we then calculated the median geographical position occupied by each tracked bird during the non-breeding period (15 October – 20 February). Since day and night durations are equal during equinoxes, bird non-breeding positions from 20 February to 1 April could not be accurately determined from light-level recordings and were therefore not included in the analysis. Spatial analyses were performed using ArcMap 10.1 (ESRI, Redlands, CA, USA).

Statistical analyses

Statistics were computed using R software, version 3.0.2 (R Development Core Team 2011). Differences of Hg concentrations between seasons, sexes and years were tested using Student’s t-tests or Mann-Whitney tests in cases where distributions were not close to normal. We used a multiple linear regression to test for a relationship between Hg concentrations accumulated during the non-breeding period (as reflected by T-Hg concentrations in head feathers) and the non-breeding distribution of birds, defined as their median longitude and latitude between 15 October and 20 February. Simple linear regressions were used to investigate whether Hg concentrations were linearly correlated to δ13C values and whether little auk egg volumes and hatching date were correlated to Hg concentrations measured in
head feathers. For these two latter relationships, only females were considered. Values are presented as means ± SD, and statistical significance was assumed at p < 0.05.

Results

Hg concentrations were slightly but significantly higher in 2011 than in 2010 in blood samples collected during the breeding season (t-test: t = 3.51, df = 61, p < 0.001) and in body feather samples (Mann-Whitney test, U = 245, p < 0.01) (see Supporting Information (SI) Figure S1). Hg concentrations in head feathers were similar in 2011 and in 2010 (t-test: t = 0.41, df = 61, p = 0.68; see SI Figure S1). No difference was found between male and female Hg concentration in body and head feathers (t = 0.62, df = 63, p = 0.54 and t = 0.20, df = 61, p = 0.84, respectively). However, breeding males had slightly but significantly higher blood Hg concentrations than females (t-test: t = 3.58, df = 61, p < 0.001; SI Figure S2). Importantly, differences observed between years and sexes did not affect the contrasting seasonal Hg contamination patterns observed in little auks (see below).

Seasonal Hg contamination

Hg concentrations measured on blood samples were higher in non-breeding (wintering) than in breeding little auks (Mann-Whitney test, U = 0, p < 0.001, n = 14 and 63, respectively; Figure 1). During the non-breeding season off Newfoundland, birds had an average Hg concentration in blood of 2.86 μg.g⁻¹ dw ± 0.78 (min = 1.72, max = 4.25) while this concentration was 0.84 μg.g⁻¹ dw ± 0.20 (min = 0.44, max = 1.25) for individuals breeding in East Greenland. A similar trend was found in feather samples, Hg concentrations in head feathers were significantly higher than in body feathers (t-test: t = 11.07, df = 126, p < 0.001;
Figure 1). Head feather concentrations (average = $3.17 \mu g g^{-1} dw \pm 0.83$, min = 1.53, max = 5.73, n = 81) reflect contamination during the non-breeding period while body feather concentrations (average = $1.53 \mu g g^{-1} dw \pm 0.84$, min = 0.71, max = 4.33, n = 78) reflect contamination during the breeding season (see Introduction for details).

**Hg contamination vs non-breeding distribution**

Hg concentrations were negatively and linearly correlated to $\delta^{13}C$ values in blood samples collected during winter ($F_{1,12} = 7.92$, $p = 0.02$, $R^2 = 0.35$; Figure 2), indicating that non-breeding Hg contamination of little auks is linked to their foraging habitat. The multiple regression analysis showed a significant relationship between bird median position during the non-breeding season and Hg concentrations measured in head feathers ($F_{2,46} = 3.78$, $p = 0.03$, $R^2 = 0.10$), with Hg concentrations were higher in birds wintering at more southerly ($p = 0.02$) and westerly ($p = 0.03$) positions. More specifically, little auks with a median non-breeding position located along the eastern slopes of the Grand Banks of Newfoundland and the Flemish Cap were globally more contaminated compared to other areas (Figure 3).

**Relationship between female Hg contamination and their reproduction**

A negative linear relationship between Hg concentrations in female head feathers (i.e. non-breeding Hg contamination) and their egg size was observed ($R^2 = 0.18$, $F_{1,16} = 4.83$, $p = 0.04$; Figure 4). There was no relationship between Hg concentrations in female head feathers and hatch dates ($R^2 = 0.0$, $F_{1,9} = 0.007$, $p = 0.94$). Furthermore, there was no relationship between female blood Hg concentrations and their body mass at recapture or between female head feather Hg concentrations and their median non-breeding position (all $p > 0.2$).
Discussion

Hg concentrations have previously been measured in a large variety of Arctic seabirds and other top-predators.\textsuperscript{8,34} These studies provided essential information about Hg concentrations in organisms,\textsuperscript{34} about their trends over past decades or centuries,\textsuperscript{6} as well as about the ecotoxicological effects of this contaminant.\textsuperscript{10} This work has greatly improved our understanding of the threat posed by mercury to the Arctic wildlife, and of the risks associated with increases of Hg in the marine environment. Nevertheless, all of this work focused on the Arctic region, mainly during summer when seabirds are breeding, which excludes a large part of the annual cycle. Consequently, and to our knowledge, no study previously investigated the intra-individual seasonal Hg contamination of Arctic seabirds. This is unfortunate since seabirds can travel over large distances and spend the non-breeding period far from their breeding site to non-Arctic areas where their exposure to contaminants remains largely unknown. Taking advantage of the specific moulting pattern of little auks and by combining biotelemetry, Hg and stable isotope analyses, our results demonstrate that ecotoxicological studies should be extended to yield a comprehensive understanding of contamination risks and associated threats to seabirds and other top-predators over their entire annual cycle. Indeed, we show by analysing blood samples that little auks breeding in East Greenland and wintering in the northwest Atlantic off Newfoundland were 3.5 times more contaminated during the winter period. This difference was lower in feather samples, but contamination over the non-breeding period was still twice as high. Hg concentrations measured in feathers reflect the amount accumulated in body tissues since the last moult\textsuperscript{24-26}, which corresponds to a period extending from approx. April to September for little auk body feathers.\textsuperscript{20,21} Although we considered this period as the breeding season, it also includes a period of post-breeding movements (between the breeding site in East Greenland and a moulting area in the
Greenland Sea\textsuperscript{21,28} as well as pre-breeding movements, as it is unlikely that little auks moult their summer plumage at their breeding site.\textsuperscript{19,28} During these short periods, little auks were in areas where their exposure to Hg was possibly different than at their breeding site,\textsuperscript{8,35} leading to slightly higher Hg concentrations in body feathers when compared to blood samples.

The AMAP 2011 Impact Assessment reviewed several studies that investigated the non-breeding distribution and Hg contamination of various seabird species.\textsuperscript{36-41} From their results, AMAP suggested that the lack of a significant temporal trend in Hg concentrations of Arctic seabirds that winter at low latitudes, outside of the Arctic, could be explained by a lower exposure to Hg during their non-breeding period, in contrast to species wintering at higher latitudes which showed increasing Hg levels.\textsuperscript{8} Here we demonstrate that this explanation should not be generalized to all species wintering in the northwest Atlantic, which may actually be exposed to higher Hg contamination when not breeding. An alternative hypothesis to explain the lack of trend reported by AMAP is that species and populations considered in their study did not winter in the northwest Atlantic as supposed, but in other regions where Hg concentrations are lower. Our results indeed confirm that the geographical position as well as the foraging habitat used by birds can affect their Hg contamination. In the northwest Atlantic, we found that little auk Hg contamination was higher in birds that had median non-breeding positions to the south and west. Within the Arctic, Mallory and Braune\textsuperscript{42} also highlighted a relationship between seabird breeding distribution in the Canadian Arctic and their Hg concentrations, with birds breeding in the High Arctic being more contaminated than those from the low Arctic.\textsuperscript{42} Isotopic results showed that wintering birds with enriched carbon ratios were less contaminated. The $\delta^{13}$C ratio is an indicator of the source of primary production in marine systems and can also be used to indicate inshore \textit{versus} offshore contribution to feeding habitats.\textsuperscript{43,44} Since enriched carbon values in marine predators have been showed to correspond to a more inshore diet,\textsuperscript{43,44} this suggests that little auks foraging
closer to the shore were less exposed to Hg contamination than birds that had recently foraged offshore. This difference could either reflect contrasting baseline Hg contamination of these habitats, or be the result of a different diet when foraging offshore and inshore with contrasting contamination levels of consumed prey items.\textsuperscript{45} Our results therefore highlight the need to extend seasonal contamination studies and focus on additional populations and species that spend the non-breeding season in different regions and habitats where Hg exposure is different.\textsuperscript{35} Studies combining contaminant analyses, biotelemetry and an isotopic approach will provide essential knowledge of the seasonal contamination of Arctic seabirds and confirm the pollution risk that regions south of the Arctic represent for this community (see also 18 for persistent organic pollutants). For instance, little auks breeding in Spitsbergen winter at higher latitudes than Greenlandic breeders.\textsuperscript{28} Other species, such as kittiwakes (\textit{Rissa tridactyla}), guillemots (\textit{Uria} spp.), and razorbill (\textit{Alca torda}) overwinter in different areas of the North Atlantic,\textsuperscript{13,46,47} whereas long-tailed skua (\textit{Stercorarius longicaudus}) and Sabine’s gulls (\textit{Larus sabini}) migrate to the South Atlantic Ocean along the West African coasts.\textsuperscript{14,48} Moreover, future studies could also help identify, at large spatial scales, sensitive areas where contamination risks are higher for Arctic wildlife. In this context, our results suggest that long-lived top-predators such as seabirds can be efficient indicators of the state of contamination of entire marine systems, by integrating contaminant concentrations at a large spatial scale and across marine food webs. Accordingly, our results indicate that the area located along the eastern slopes of the Grand Banks of Newfoundland and the Flemish Cap was associated with higher Hg contaminations and merits further attention.

As mentioned above, Hg contamination of organisms is also closely related to their diet and a seasonal change in diet could affect ingested Hg concentrations.\textsuperscript{45} Little auks breeding in East Greenland are known to mainly forage on copepods during the breeding season.\textsuperscript{49} However, isotopic data suggested a change of diet soon after breeding, towards krill and fish larvae.\textsuperscript{50}
Stomach content analyses performed on the same individuals as those used in the present study also showed a diet mainly composed of krill during late winter (March).\textsuperscript{22} Although we do not know if these different diets reflect different trophic positions, higher contamination observed during the non-breeding period could also partially result from a change in diet. Such a change could also be a second alternative explanation to trends interpreted by AMAP. Like Brünnich’s guillemots (\textit{Uria lomvia})\textsuperscript{51} or northern fulmars (\textit{Fulmarus glacialis}),\textsuperscript{52} which switch from a fish to a zooplankton-based diet during winter, other species could feed at lower trophic levels during the non-breeding period, resulting in lower Hg accumulations during the non-breeding period.

Moreover, our results suggested that female little auks that had higher head feather concentrations and so had accumulated more Hg during the non-breeding season laid smaller eggs. It is not possible from the present study to determine if Hg has a direct impact on little auk reproduction or if it rather reflects general contamination by trace metals or other contaminants. Hg concentrations measured in this study ranged from 0.4 to 5.7 μg.g\textsuperscript{-1} dw (1.5 to 5.7 μg.g\textsuperscript{-1} dw in head feathers). These values are similar or below concentrations measured in similar tissues in other seabird species\textsuperscript{45,53,54} so a direct relationship between Hg concentration and female investment in the reproduction may seem surprising. Hg contamination thresholds that lead to adverse effect are poorly known in seabirds, especially for feather and blood matrices (see\textsuperscript{55,56} for other matrices). However, studies performed on other aquatic birds suggested that Hg concentrations from 5 μg.g\textsuperscript{-1} dw in feathers can cause reproductive impairment.\textsuperscript{57} A recent Arctic study suggested that low Hg concentrations in blood samples (approx. 3 μg.g\textsuperscript{-1} dw) could affect the reproductive success of kittiwakes by disrupting their endocrine system.\textsuperscript{16} Therefore, further studies are required to confirm the adverse effect found in our study and to understand the direct or indirect physiological link
between Hg concentrations and egg synthesis in seabirds. Among others, it will be important to further consider selenium (Se) concentrations and the Hg:Se ratio. Indeed, Se interacts, through specific chemical mechanisms, with Hg to form nontoxic Hg-Se complexes, and therefore acts as a protection against methyl-Hg toxicity.\textsuperscript{58} Hence, high Hg levels would not necessarily induce adverse toxicological effects if Se is abundant compared to Hg and if the Hg:Se ratio is low. Many seabird species are known to overwinter in huge numbers in the Northwest Atlantic,\textsuperscript{13,59-61} and like little auks, could be exposed to high Hg contamination risks with associated threats to their reproduction. Such studies would improve our understanding of the threat that Hg exposure represents for these vulnerable species, within and outside of the Arctic.
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Supporting Information Available. Figures S1, S2. Annual and sex differences in Hg concentrations measured in little auk blood and feather samples. This information is available free of charge via the Internet at http://pubs.acs.org.
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Figure 1. Hg concentrations (in μg.g⁻¹ dry weight) measured in little auk blood and feather samples. Body feather concentrations indicate summer contamination; while head feather concentrations indicate winter contamination (see Introduction and Materials and Methods for details). Stars indicate significant differences between seasons in both blood and feathers (p < 0.001)
Figure 2. Blood Hg concentrations (in $\mu g.g^{-1}$ dry weight) in relation to the $\delta^{13}C$ isotopic values of little auks wintering in the northwest Atlantic. Higher (less negative) $\delta^{13}C$ values indicate a more inshore diet, and lower (more negative) a more offshore diet (Hobson & Welch 1992, Hobson et al. 1994).
Figure 3. Hg concentrations (in μg g^{-1} dry weight) measured in head feathers of little auks breeding in East Greenland, in relation to their non-breeding distribution in the Northwest Atlantic. Each point represents the median distribution of individually-tracked little auks during the non-breeding period (15 October–20 February). The blue star represents the breeding colony where birds were equipped.
Figure 4. Egg volume (cm$^3$) of female little auks breeding in East Greenland in relation the Hg concentrations (in μg.g$^{-1}$ dry weight) measured in their head feather (i.e. accumulated during the non-breeding period; see Introduction for details).