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Baseline

Trace elements and ingested plastic debris in wintering dovekies (*Alle alle*)

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ABSTRACT

We provide the first report on winter concentrations of 32 trace metals from dovekies (*Alle alle*), a small, Arctic seabird that has a seasonal shift in diet from small zooplankton in the breeding season to larger zooplankton and small fish in the non-breeding season. Concentrations of selected trace elements, as well as stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope concentrations for a sample of 25 dovekies, were similar between adult males and females, and there was evidence that dovekies feeding at higher trophic levels had higher hepatic Hg. We also found plastic debris in nine of 65 (14%) gizzards examined. Our study helps provide a more complete picture of the foraging ecology and contaminant profile of dovekies, an important species in Arctic marine food webs.

Contamination of the Arctic marine environment by long-range transport of anthropogenically-derived pollutants has been a research focus for several decades (Mallory and Braune, 2012). Seabirds have been valuable indicators of levels and trends in these pollutants, in part because different species forage across different trophic levels, so sampling among seabird species can provide a robust picture of the health of marine ecosystems (Montevecchi, 2001; Mallory and Braune, 2012). Although seabird tissues serve as useful tools to monitor contaminant levels, concentrations of these pollutants may also be sufficient to cause sublethal or lethal effects in birds (i.e., pose a wildlife health issue; Sagerup et al., 2000), or may rise to levels warranting concern for the indigenous peoples that live in the Arctic and consume wild foods as a substantial part of their diet (Kuhnlein and Receveur, 2007).

Many seabird species have been sampled in eastern Canada to examine levels of trace elements (Mallory and Braune, 2012) or ingested plastics (Provencher et al., 2014). Some trace elements and plastic debris are increasing in many marine birds of this region (Provencher et al., 2014), but one species that has received little attention in relation to these contaminant threats is the dovekie (*Alle alle*). This is surprising because it is the most numerous seabird in the North Atlantic, and millions forage in Canadian Arctic waters and overwinter along the coast of Newfoundland and Labrador (Egevang et al., 2003; Mosbech et al., 2012; Fort et al., 2010, 2014). The dovekie is a small diving seabird that feeds primarily on zooplankton but has seasonal shifts in diet, likely in response to changing food availability (Harding et al., 2008; Karnovsky et al., 2008; Fort et al., 2010; Rosing-Asvid et al., 2013). They feed at the lowest trophic level relative to other Arctic seabirds, as evidenced by dietary and stable isotope studies (Campbell et al., 2005; Harding et al., 2008; Karnovsky et al., 2008; Fort et al., 2010; Rosing-Asvid et al., 2013). As a consequence, the limited data available suggest that mercury and other trace elements tend to be lower in dovekies than in other seabirds during the breeding season (Hobson et al., 2002; Braune et al., 2005; Campbell et al., 2005) and except for Hg (Fort et al., 2014), there are no studies on winter contaminants for this species.

We examined trace element concentrations, stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$), and ingested plastic debris in tissues from dovekies recovered along the coast of Newfoundland in late winter. Based on recent tracking studies (Mosbech et al., 2012; Fort et al., 2013) and morphometrics of our sample (Fife, unpubl. data) in relation to those of other dovekies (Robertson et al., 2006; Wojczulanis-Jakubas et al., 2011), we believe our birds originated from Greenland and were wintering off the coasts of Newfoundland and Labrador. We assessed how winter trace element levels compared to those known from the breeding season and we examined the influences of sex, age, and tissue type on carbon and nitrogen isotope ratios, and the influence of sex on several non-essential trace elements in adults. We predicted that: (1)

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isotope ratios from this study would fall within the range reported for dovekies and that they would reflect the dietary shift observed from fall to winter as seen in dovekies from West Greenland (Fort et al., 2010); (2) given their low trophic position, trace elements would generally be lower compared to other seabirds that feed higher up in the food web; and (3) mercury (Hg) would be higher in dovekies with higher $\delta^{15}N$ (an index of trophic position) because this element biomagnifies in food webs (e.g., Campbell et al., 2005).

We collected 65 freshly-dead, complete dovekie carcasses found on beaches in January 2013 near White Bay, Newfoundland (~49°78′N, 56°63′W), presumably shortly after they washed ashore (i.e., they were not significantly decomposed or degraded). Birds were dissected to determine sex and age at Environment Canada offices in Newfoundland before being shipped to Acadia University, Nova Scotia and stored in a −20 °C freezer. They were fully thawed for 24 h for sampling described below.

From each bird, we collected the P10 feather from one wing to sample the late autumn/early winter foraging period (i.e., when nutrients are shunted to feathers during growth; Montevecchi and Stenhouse, 2002). We also took ~1 g of pectoralis muscle from each bird to sample the mid-winter foraging period, which is assumed to reflect diet approximately four weeks before birds were recovered (based on muscle turnover rates; Hobson, 1993). Feather and muscle samples were sent to the Stable Isotopes in Nature Laboratory (SINLAB) in Fredericton, New Brunswick, Canada for stable carbon and nitrogen isotope analyses. Dried samples were homogenized, freeze-dried, weighed in clean tin cups, combusted using a Carlo Erba NC2500 Elemental Analyzer, and analyzed for $\delta^{13}C$ and $\delta^{15}N$ values using a Thermo-Finnigan Delta XP isotope-ratio mass spectrometer (Bremen, Germany). Carbon and nitrogen isotopes are expressed in parts per thousand (‰) and represent $^{13}C/^{12}C$ or $^{15}N/^{14}N$, calibrated to the International Atomic Energy Association (IAEA) scale using IAEA Vienna Pee Dee Belemnite carbonate and atmospheric nitrogen as standards for carbon and nitrogen, respectively. For 25 of the dovekies (10 adult female, 10 adult male, 5 juveniles), we removed ~2 g of liver tissue and homogenized each sample using a small hand blender. All instruments used to extract and process samples were cleaned in 70% ethanol, followed by distilled water between each sample. Each sample was sealed individually in a sterilized vial. These samples were sent to RPC Science and Engineering Inc. in Fredericton, New Brunswick, for analysis of trace elements. Portions of samples were prepared by microwave-assisted digestion in nitric acid and resulting solutions analyzed for trace elements by inductively coupled plasma mass spectrometry (ICP-MS). Mercury was analyzed by cold vapor atomic absorption (CVASS). Quality assurance and quality control measures included the analyses of two method blanks (purified water), two certified biological reference tissues (DORM-2 and DOLT-2; National Research Council, Canada), and three randomly assigned duplicate samples. All QA/QC measures were in compliance with the normal laboratory operating procedures at the time of analysis. Concentrations of each element are presented as $\mu g\ g^{-1}$ wet weight (moisture in tissues was 69 ± 7%).

Gizzard contents from birds used in trace element analyses as well as from the other 40 dovekies were examined to quantify the proportion of birds that ingested plastic. Intestines in many of the birds were in poor condition, so only gizzards (all of which were completely intact) were examined. Each gizzard was severed at the base of the proventriculus and the start of the small intestine and a lengthwise incision was made using scissors. Gizzard contents were then flushed into a petri dish using distilled water and searched manually for plastic and other debris visible under a dissecting scope at 10× magnification. If plastic was found, pieces were weighed to the nearest 0.0001 ± SD g with a Mettler AE 260 DeltaRange® analytical scale and measured to the nearest 0.01 ± SD mm using dial calipers (as in Bond et al., 2013).

All statistical analyses were performed in R version 2.15.1 (R Core Team, 2012). For isotopic data, $\delta^{13}C$ and $\delta^{15}N$ data distributions approximated normality within tissue type (Shapiro–Wilks tests, $p \geqslant 0.2$), and thus we used generalized linear models (GLMs; with family as 'Gaussian') to test which variables best predicted ratios of stable carbon and nitrogen isotopes. Initial GLMs included sex, age class, and tissue types as predictors of stable carbon and nitrogen isotope ratios but interactions were not included because of small sample sizes. We sequentially removed non-significant associations; if only two explanatory variables remained, we then tested whether the interaction between them was significant (Crawley, 2005). In contrast to isotopic data, the distributions of trace element data did not approximate normality even after various transformations, and consequently we used non-parametric Kruskal–Wallis tests to compare trace element values among sexes or age groups of dovekies. Finally, we compared incidence of plastic in dovekies by age and gender using Fisher Exact tests.

Differences in concentrations of trace elements between sexes were compared using Kruskal–Wallis tests for adults only, as were relationships between selected trace elements and values of $\delta^{15}N$. Concentrations that were below the detection limit for a given element (Al – $n = 17$; B – $n = 2$; Cr – $n = 14$) were set to 50% below that limit, as in Mallory et al. (2004), to be included in analyses.

Male and female dovekies had similar $\delta^{13}C$ and $\delta^{15}N$ (GLMs; $t = -0.4$, $p = 0.69$ and $t = -0.03$, $p = 0.98$, respectively; Fig. 1). The best GLMs for both isotopes included an interaction between age and tissue type ($t = -5.3$, $p < 0.001$). Among adults, feathers were significantly lower in $\delta^{13}C$ ($t = 3.9$, $p < 0.001$) and higher in $\delta^{15}N$ ($t = -10.74$, $p < 0.001$) compared to muscle. In contrast, among juveniles, $\delta^{13}C$ was higher in feathers than in muscle ($t = -5.0$, $p = 0.001$), and there was a trend of higher $\delta^{15}N$ in feather than muscle tissue ($t = -2.0$, $p = 0.08$). Feather $\delta^{13}C$ values were lower and $\delta^{15}N$ values were higher in adults compared to juveniles ($t = 5.8$, $p < 0.001$, and $t = -4.0$, $p < 0.001$, respectively), whereas for muscle, no significant differences were seen between age classes for $\delta^{13}C$ ($t = -0.3$, $p = 0.79$), but there was a trend of higher $\delta^{15}N$ in juvenile tissue ($t = 1.8$, $p = 0.08$; Fig. 1).

Analyses of all 25 dovekie livers found the following trace elements were below detection limits if present: antimony (detection limit 0.005 $\mu g\ g^{-1}$), barium (0.05 $\mu g\ g^{-1}$), beryllium (0.005 $\mu g\ g^{-1}$), bismuth (0.05 $\mu g\ g^{-1}$), nickel (0.05 $\mu g\ g^{-1}$), tellurium (0.005 $\mu g\ g^{-1}$), thallium (0.005 $\mu g\ g^{-1}$), tin (0.01 $\mu g\ g^{-1}$), uranium (0.005 $\mu g\ g^{-1}$), vanadium (0.05 $\mu g\ g^{-1}$). Adult female and male

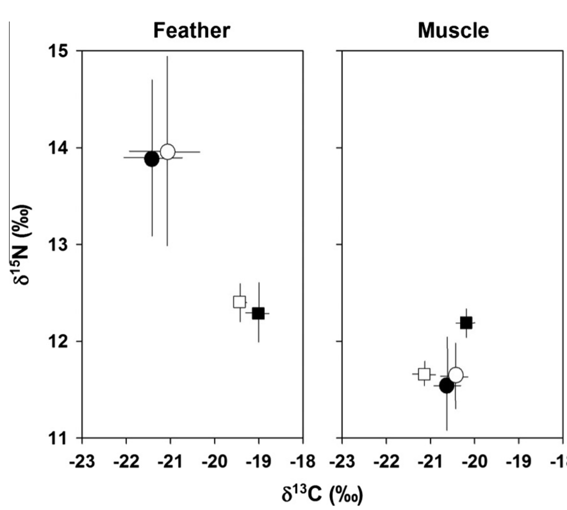

Fig. 1. Mean (±SD) stable carbon and nitrogen values (‰) in P10 wing feather and pectoralis muscle tissue of adult male ($n = 10$, filled circle), adult female ($n = 10$, open circle), juvenile male ($n = 3$, filled square) and juvenile female ($n = 2$, open square) dovekies.

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dovekies had similar concentrations for trace elements of toxicological interest (Table 1, all $p \geqslant 0.47$; additional trace element data are in Table 2). Similarly, adult and juvenile dovekies had similar concentrations in most trace elements (Table 1, $p > 0.05$), except for Cu ($\chi^2 = 5.0$, df = 1, $p = 0.02$) and Hg ($\chi^2 = 9.8$, df = 1, $p = 0.002$), both of which were higher in juvenile dovekies. There were no statistically significant relationships between $\delta^{15}N$ (i.e., index of trophic level) and any of the selected trace elements in adult dovekies (Spearman rank correlations; all $|r_s| < 0.38$, all $p > 0.10$). When data for juveniles and adults were pooled, Hg was higher in birds feeding at higher trophic levels (as indexed by muscle $\delta^{15}N$; $r_{s25} = 0.47$, $p = 0.02$).

Gizzard contents, for the most part, did not include any distinguishable food material, consistent with the near absence of visible fat reserves, suggesting that the birds were emaciated. Of 65 gizzards, nine contained plastic debris (14%; eight birds with one piece, one bird with two pieces). Debris consisted of either small, unidentifiable fragments, or bits of nylon fishing line, all of which appeared to be user plastic. Mean mass of plastic debris was 0.0183 ± 0.0205 g, while mean length, width, and height were 6.3 ± 3.0 mm, 2.2 ± 1.4 mm, and 1.2 ± 1.0 mm, respectively. The proportion of adults that ingested plastic was 4/21 for females and 4/26 for males, whereas for juveniles it was 0/6 for females and 1/9 for males. Consequently, prevalence of plastic in dovekies did not differ significantly according to age or gender (Fisher Exact tests, all $p > 0.4$).

Our isotopic data for dovekies help complete the picture of the annual foraging pattern of this species across its range. There do not appear to be sex-specific differences in diet in either the fall or winter, as indexed by isotopes, and in the winter there were no differences in diet between adults and juveniles (Fig. 1). Fort et al. (2010) examined summer, winter, and fall isotopic ratios in dovekies from five colonies, and found that birds from Svalbard colonies tended to have high $\delta^{15}N$ in the fall and winter and lower in the summer, but birds from East and West Greenland had low winter values and high fall $\delta^{15}N$. Karnovsky et al. (2008) measured isotopes in dovekies from West Greenland (the source of many Newfoundland wintering birds; Fort et al., 2010), and found a dietary shift that resulted in an increase in $\delta^{15}N$ between spring/summer and fall samples (~11.25 to 13.25‰), when birds shifted from a diet dominated by calanoid copepods to higher trophic level prey such as amphipods and fish (confirmed by fatty acid analysis; Karnovsky et al., 2008). Burke et al. (2014) sampled dovekies in early winter near Newfoundland and found $\delta^{15}N$ of 12.1 ± 0.3‰, a decline from fall samples. We found that fall birds had a slightly higher ratio (~13.9; Table 1) than Karnovsky et al. (2008), but that this declined to ~11.6‰ in the mid-winter. All studies found that mean $\delta^{13}C$ remained between −20‰ and −22‰; that is, dovekies appear to forage all year on offshore, pelagic, rather than near shore, benthic prey. Collectively, these studies suggest that dovekies forage relatively higher in the marine food web post-breeding than during the rest of their annual cycle, but differ in their foraging patterns in the winter depending on whether the birds winter at high latitudes near Greenland and Iceland, or at lower latitudes near Newfoundland.

Table 2
Mean (SD) wet weight (μg g^{-1}) of other trace elements not generally of toxicological interest in dovekie liver tissue.

Element	Detection limit	Adult (n = 20)		Juvenile (n = 5)	
Boron	0.05	0.14	(0.08)	0.14	(0.09)
Calcium	2	71.10	(29.99)	97.20	(72.63)
Cobalt	0.005	0.02	(0.00)	0.02	(0.01)
Iron	1	137.20	(67.49)	99.40	(42.24)
Lithium	0.005	0.09	(0.12)	0.07	(0.07)
Magnesium	0.5	109.98	(29.64)	106.44	(13.43)
Manganese	0.05	0.93	(0.22)	0.97	(0.24)
Molybdenum	0.005	0.26	(0.06)	0.36	(0.08)
Potassium	1	1054.2	(198.30)	1235.0	(308.30)
Rubidium	0.005	0.62	(0.11)	0.76	(0.19)
Silver	0.005	0.01	(< 0.01)	0.02	(0.01)
Sodium	2	817.10	(204.72)	918.80	(279.80)
Strontium	0.05	0.47	(0.29)	0.44	(0.28)
Zinc	0.5	14.50	(3.02)	15.44	(4.09)

Dovekies occupy a low trophic position among seabirds in marine food webs (e.g., Campbell et al., 2005), thus we expected low hepatic concentration values for biomagnifying elements like Hg. The available, published data from trace elements in dovekies are limited, and come from studies during the spring or breeding season. Atwell et al. (1998) sampled one dovekie among eight marine bird species in the Canadian Arctic and found it had the lowest $\delta^{15}N$ (12.5) and the lowest hepatic Hg (0.07 μg/g ww), both lower than what we found in this study (although Hg has increased in the Arctic since that study; e.g., Braune et al., 2005). Campbell et al. (2005) analyzed livers from nine dovekies (Table 1) and found that most trace elements were at lower concentrations in dovekies than in seven other marine bird species in the Northwater Polynya. Fort et al. (2014) showed that Hg in dovekies was higher in the winter than in the summer, presumably due to geographic differences in Hg in marine food webs and the segregation of winter and summer habitats for these birds. Our data showed that male and female dovekies had similar trace element concentrations, consistent with isotopic analyses, and that most hepatic trace element concentrations were lower than in other Arctic marine birds (e.g., Campbell et al., 2005). However, compared to the adult birds collected in the Northwater Polynya (presumably from the same breeding population), birds wintering in Newfoundland had lower As and Cd and higher Hg (Table 1). We also found a predictable trend within dovekies: birds foraging at higher trophic levels had

Table 1
Mean (SD) wet weight (μg g^{-1}) of selected trace elements of toxicological interest in dovekie liver tissue. Values for adult males and females are provided separately as well as pooled. NWP adults refer to data from a previously published study of birds collected in the Northwater Polynya between Greenland and Canada in May 1998 (Campbell et al., 2005).

Trace element	DL	Adults			Juveniles (n = 5)	NWP adults (n = 9)
		Male (n = 10)	Female (n = 10)	Pooled (n = 20)		
Aluminum	0.5	0.73 (0.87)	0.79 (0.83)	0.79 (0.82)	0.25 (0.00)	
Arsenic	0.05	0.40 (0.08)	0.36 (0.14)	0.38 (0.12)	0.47 (0.21)	1.51 (0.37)**
Cadmium	0.0005	2.04 (0.91)	1.93 (0.90)	1.98 (0.88)	1.42 (0.57)	5.78 (1.19)**
Chromium	0.05	0.07 (0.09)	0.09 (0.13)	0.08 (0.11)	0.05 (0.03)	
Copper	0.05	4.41 (0.77)	4.21 (0.77)	4.32 (0.76)	5.75 (1.58)	8.18 (1.70)
Lead	0.005	0.01 (0.01)	0.02 (0.01)	0.01 (0.01)	0.01 (0.00)	0.27 (0.61)
Mercury	0.01	0.54 (0.25)	0.49 (0.13)	0.52 (0.22)	1.06 (0.23)	0.27 (0.08)**
Selenium	0.05	2.08 (0.38)	2.53 (1.25)	2.31 (0.93)	2.45 (0.75)	3.85 (0.83)

** Post hoc t-tests on summary data comparing values from Newfoundland winter pooled adult birds to NWP pre-breeding birds, $p < 0.001$.

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higher hepatic Hg, consistent with overall patterns among Arctic marine birds (e.g., Campbell et al., 2005). Some trace elements, including Cd (Lovvorn et al., 2013) and Hg (e.g., Braune and Gaskin, 1987) can vary considerably in tissue concentrations through the annual cycle, and thus further investigation is required to determine whether these patterns reflect a long-term trend (e.g., Mallory et al., 2014) or are snapshots of annual variation.

Despite the abundance of dovekies in Arctic waters and concerns over increasing plastic debris in our oceans (Provencher et al., 2014), we found only one report of plastic debris ingestion in this species: Falk and Durinck (unpublished, in Provencher et al., 2014) reported no debris in 19 birds in 1988/89 in Davis Strait. This is not statistically different from our finding of 14% incidence (Fisher Exact test, $p = 0.11$), but mirrors increases observed in murres and fulmars from this region (Provencher et al., 2009, 2010; Bond et al., 2013). Consequently, it is unclear whether our observation of 14% incidence represents a true increase in plastic ingestion in the species over 24 years, or whether recent samples near Newfoundland come from birds with greater exposure to plastic than birds along West Greenland. In either case, this new information adds compelling evidence that Arctic waters are polluted with plastic debris, which is consumed even by small, diving planktivores.

Collectively, this study provided three important, baseline results on dovekies: (a) in the winter, their Hg and other trace element levels are generally low compared to other Arctic marine birds; (b) based on isotopes, they appear to forage at relatively low trophic levels in the winter, somewhat different than is the case for wintering dovekies from Svalbard; and (c) although the incidence of plastic debris in wintering dovekies was low, it may be increasing. Arctic seabirds are continuing to face threats from a rapidly changing marine environment, including higher levels of contaminants and marine pollution, as well as regional changes in prey availability, all of which may alter their foraging behavior, and nutritional health. Thus, a numerous and widespread species in the North Atlantic, the dovekie, is an important indicator of marine contaminants that are potentially toxic to other marine wildlife, as well as the indigenous peoples that rely on these species as a staple in their diet (Kuhnlein and Receveur, 2007).

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