



## Arctic fox winter dietary response to damped lemming cycles estimated from fecal DNA

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Climate-caused changes in prey abundance may alter predator–prey dynamics in the Arctic food web. Lemmings (*Dicrostonyx* spp.) are important prey for Arctic foxes (*Vulpes lagopus*) and their annual population fluctuations drive fox reproduction, creating strongly linked predator–prey population cycles. Winter diet directly impacts Arctic fox reproductive success, but winter prey diversity on the tundra is low. Strategies such as using the marine environment to scavenge seals have allowed Arctic foxes to persist during years of low lemming abundance. However, warming winters have decreased snowpack quality, preventing lemmings from reaching their previous high abundances, which may reduce their impact on predator dynamics. We investigated Arctic fox dietary response to lemming abundance by reconstructing Arctic fox winter diet in the low Arctic. Next-generation sequencing of fecal DNA, from samples ( $n = 627$ ) collected at dens in winters of 2011–2018, identified prey both from terrestrial and marine environments. Despite lemming cycle damping, Arctic foxes still increased lemming consumption during years of higher lemming abundance. Alternative prey such as marine resources were consumed more during years of low lemming abundance, with up to 45% of samples containing marine resources in low lemming years. In addition, Arctic foxes consumed high proportions of meadow voles (*Microtus pennsylvanicus*), which may represent a new alternative prey, suggesting climate change may be creating new foraging opportunities. Changes in prey abundance illustrate how climate-caused disturbances are altering Arctic food-web dynamics. Dietary flexibility and availability of alternative prey may become increasingly important for Arctic predators as the Arctic continues to change.

Keywords: climate change, foraging ecology, metabarcoding, noninvasive genetic sampling, trophic dynamics

The strength of species interactions provides information on ecosystem function and may increase our ability to predict how disturbances will affect food-web structure (Paine 1980; Krebs 2011; Bowser et al. 2013). Arctic food webs are characterized by low species diversity, but strong predator–prey dynamics driven by seasonal and annual fluctuations in resources (Krebs 2011). Lemmings (*Dicrostonyx* spp., *Lemmus* spp.) are key components of the terrestrial Arctic food web and their population cycles drive predator population dynamics and habitat use, and indirectly affect alternative prey (Gilg et al. 2006; Julien et al. 2014; Lai et al. 2017). Many lemming populations fluctuate predictably in 3- to 4-year cycles (Krebs 2011; Ehrich et al. 2020), with the dramatic change in lemming abundance influencing terrestrial predator diet and reproduction. Years of high lemming abundance elicit both

functional responses (increase in number of prey consumed by each individual predator) and numerical responses (increase in litter size and number of breeding individuals) in Arctic foxes (*Vulpes lagopus*), snowy owls (*Bubo scandiacus*), short-tailed weasels (*Mustela erminea*), and long-tailed skuas (*Stercorarius longicaudus*; Angerbjörn et al. 1999; Krebs et al. 2003; Gilg et al. 2006; Krebs 2011).

Terrestrial Arctic predators may consume alternative prey or even leave the area entirely, using other ecosystems, during low lemming years (Roth 2003; Gilg et al. 2006; Julien et al. 2014). Tundra prey diversity is especially low during winter, when the many Arctic-breeding migratory birds have left (McDonald et al. 2017), and predators may be forced to forage in other habitats. For example, snowy owls might avoid settling on the tundra entirely in winters with low lemming abundance, or may

use the sea ice to forage for marine resources (Gilg et al. 2006; Therrien et al. 2011). In coastal areas, Arctic foxes may subsidize winter diet with marine resources by using sea ice to scavenge seals during low lemming years (Roth 2002, 2003; Samelius et al. 2007; Lai et al. 2017). Although alternative foraging strategies allow terrestrial Arctic predators to persist during years of low lemming abundance, predator populations still are particularly vulnerable to changes in lemming abundance (Gilg et al. 2012).

Arctic regions are experiencing an increase in surface air temperature at more than twice the global rate (Huang et al. 2017), spurring pronounced changes in the Arctic ecosystem and modifying species dynamics (Gilg et al. 2012). Throughout the Arctic, many lemming populations are no longer experiencing large fluctuations in abundance and data suggest cycles have damped, likely because warmer winters have reduced snow cover duration, quality, and quantity (Gilg et al. 2012; Fufachev et al. 2019; Ehrich et al. 2020). Changes in lemming abundance, with fewer lemmings on the tundra even in peak years, may have cascading effects on predator diet and reproduction. Consequences of lemming population cycle damping already have been detected in some terrestrial predators and responses have varied, but include increased alternative prey consumption and predator population declines (Ims and Fuglei 2005; Gilg et al. 2006; Schmidt et al. 2012).

Dietary flexibility, or the ability to modify diet in response to changing prey availability, may become increasingly important for Arctic predators as prey communities change (Gilg et al. 2012; Chaves and César Bicca-Marques 2013). Species diversity is increasing at the southern edge of the Arctic, as temperate species shift their range with changing climate conditions (Gilg et al. 2012). For example, a general trend of increasing vole (*Microtus* and *Myodes*) abundance has been noted in the low Arctic (Ehrich et al. 2020).

The Arctic fox is a circumpolar predator and, compared to other lemming specialists, is considered an opportunistic forager (Elmhagen et al. 2000; Eide et al. 2005; Schmidt et al. 2012; Pokrovsky et al. 2015). Consumption of migratory birds or marine resources can elevate Arctic fox breeding success, or even drive Arctic fox population dynamics, when lemmings are scarce (Roth 2003; Pokrovsky et al. 2015; Samelius and Alisauskas 2017). Although most populations of Arctic foxes exhibit annual fluctuations associated with lemming population cycles (Berteaux et al. 2017), in areas without rodents, Arctic fox reproduction is less variable (Tannerfeldt and Angerbjörn 1998; Dalerum et al. 2012; Eide et al. 2012). Arctic foxes in the low Arctic may have increased access to alternative prey as species composition changes. Increased prey diversity in the low Arctic will favor terrestrial Arctic predators with dietary flexibility (Gilg et al. 2012).

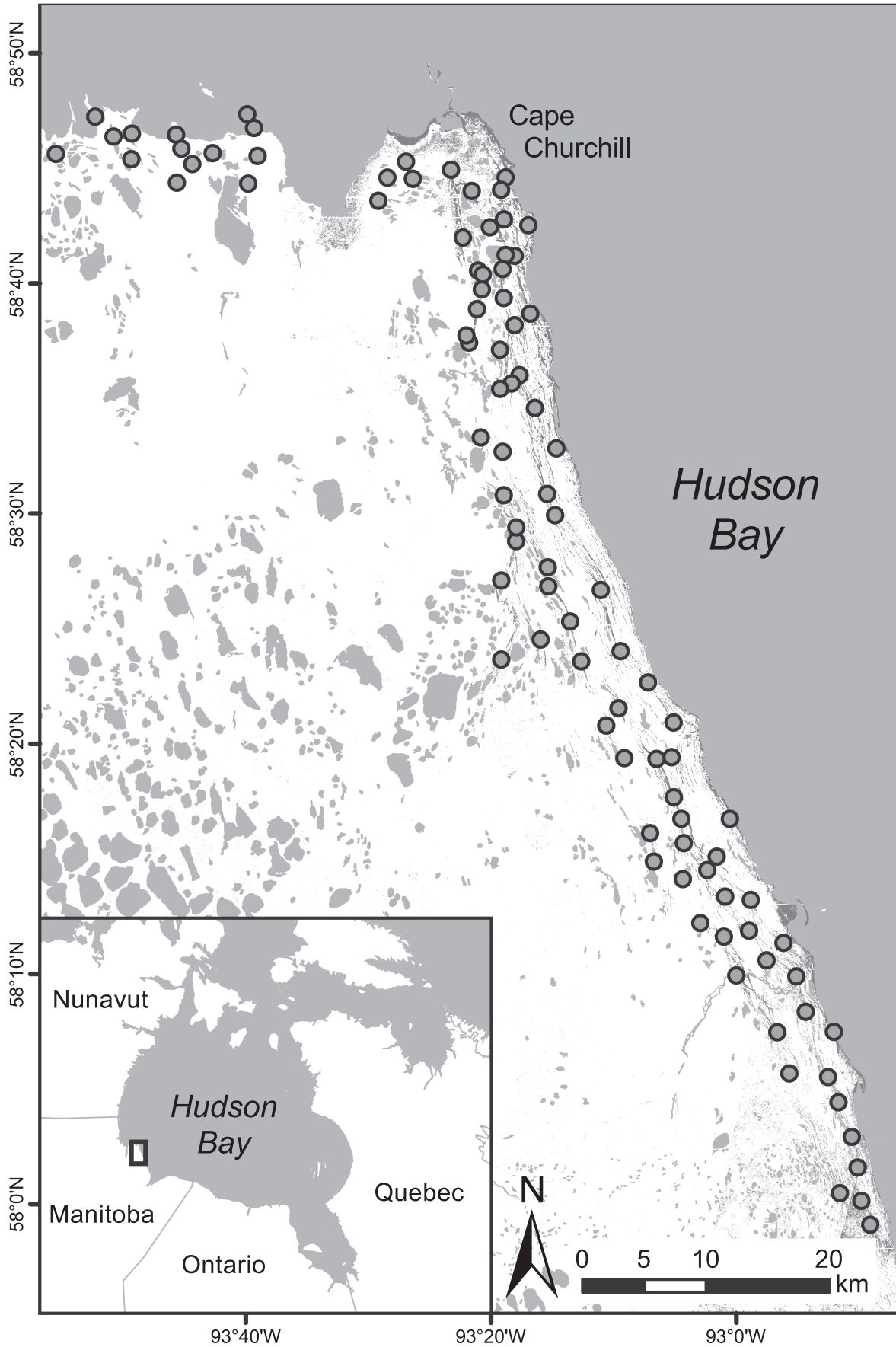
Our objective was to reconstruct the winter diet of Arctic fox from near Churchill, Manitoba, Canada, near the southern edge of species' distribution. The availability of prey during winter impacts Arctic fox reproductive success, but winter prey diversity on the tundra is low (McDonald et al. 2017). In this low-Arctic area, lemming population cycles are damped and

currently cycle at low densities, with peaks of only two lemmings per hectare, compared to peaks of 12 per hectare in the 1990s and 40 per hectare in the 1930s (Shelford 1943; Roth 2002; Ehrich et al. 2020). In addition, voles have increased in abundance on the tundra in this area and may represent a new alternative prey resource (Ehrich et al. 2020). We reconstructed Arctic fox winter diet over an 8-year period to identify prey consumed and to determine whether damped lemming populations still influenced Arctic fox diet. We predicted that (i) Arctic foxes would consume higher proportions of lemmings during high lemming years, and that (ii) Arctic foxes would consume higher proportions of alternative prey in low lemming years. We reconstructed Arctic fox winter diet using fecal DNA and next-generation sequencing, enabling higher taxonomic precision in prey identification than traditional diet analysis methods.

## MATERIALS AND METHODS

*Study area and sample collection.*—Our study area along the coast of Hudson Bay near Churchill, Manitoba (58°N, 94°W) is near the southern edge of the Arctic fox's distribution. Three biomes merge in this area: tundra, boreal forest, and marine. The primary year-round prey for Arctic foxes in this region is Richardson's collared lemming (*Dicrostonyx richardsoni*; Roth 2002). Other Arctic fox prey in the area include the meadow vole (*Microtus pennsylvanicus*) and western heather vole (*Phenacomys intermedius*; Shelford 1943; Smith and Foster 1957; Scott and Hansell 1989). When sea ice forms on Hudson Bay, Arctic foxes can access marine habitat and consume marine resources such as seals (Roth 2002, 2003). The 200 species of migratory birds that breed in the area in summer are absent in winter, but Arctic foxes may cache bird carcasses and eggs to consume in winter (Reiter and Andersen 2011; McDonald et al. 2017). Other winter prey include ptarmigan (*Lagopus* spp.), resident nonmigrating birds, and caribou (*Rangifer tarandus*). In recent years, small mammal trapping in the study area has detected only collared lemmings and meadow voles (Ehrich et al. 2020), although meadow voles are caught infrequently, compared to lemmings, on our research grids, and voles were not detected on the coastal tundra prior to 2010 (Roth 2002; Ehrich et al. 2020). In our study area, voles typically are trapped in wet graminoid habitat, while lemmings more likely are found in dry upland habitat (Scott and Hansell 1989; Morris et al. 2011). To examine annual variation in lemming abundance, we estimated annual lemming winter nest density (Krebs et al. 2012). In 2013–2018, lemming winter nests from the previous winter were counted in June using two permanent strip transects (2 km long, 10 m wide) along the east side of an upland dry gravel ridge in the middle of our study area (Gharajehdaghpour and Roth 2018).

We collected fox fecal samples opportunistically from 90 known dens surveyed in April from 2011 to 2018 (Fig. 1). Fecal samples were collected from on top of snow, reflecting recent deposition, and were placed in labeled plastic bags and stored at –20°C. We chose a subset of 959 samples for DNA extractions from the 1,800 archived fecal samples (we randomly chose at



**Fig. 1.**—Map of study area near Churchill, Manitoba, Canada (58°N, 94°W). Each point is a fox den on the tundra, visited annually during April 2011–2018 for fecal sample collection.

least one sample per den per year, plus up to three additional samples per den if available).

**Laboratory methods.**—We subsampled approximately 200 mg of fecal material from each sample, added 3.5 ml of ASL buffer (Qiagen Inc., Valencia, California), and vortexed until homogenized (Tsukada et al. 2014). The remaining DNA extraction procedure was conducted using the QIAamp DNA Stool Mini Kit and followed the manufacturer's instructions (Qiagen Inc., Valencia, California). One extraction negative control was included with each set of extractions.

Because Arctic foxes and red foxes (*V. vulpes*) occur sympatrically in our study area, we determined from which species each fecal sample originated using species-specific primers following the methods described in Dalén et al. (2004). One PCR negative control was included in each amplification to check for contamination. In addition, we used this step to eliminate any low-quality samples. Fecal DNA extractions that failed to amplify were repeated and any sample that failed species identification twice was excluded from further analysis.

Following species identification and quality screening, we amplified a ~135-bp region of the V5 variable region of the 12S rRNA gene using the primer pair 12SV5F/R described in Riaz et al. (2011) for subsequent diet analysis. We designed a blocking primer (5'-CTATGCTTAGCCCTAAACATAAATGTTCTACAACAAAACAATTCG-3') to reduce the amount of fox DNA that was amplified (Vestheim and Jarman 2008). The 12SV5F/R primers were modified by the addition of M13 sequences on the 5' end for high-throughput sequencing. The diet PCRs were carried out in a 20 µl reaction with 1× buffer (Promega GoTaq Flexi, Promega Corp., Madison, Wisconsin), 0.2 mM dNTP (Promega Corp., Madison, Wisconsin), 2.5 mM MgCl<sub>2</sub>, 0.2 µg/µl BSA, 0.2 µM of each primer (12SV5 F/R), 4 µM blocking primer, ddH<sub>2</sub>O, 1 U GoTaq Flexi DNA Polymerase (Promega Corp., Madison, Wisconsin), and 4 µl of DNA template. Amplification conditions were 95°C for 2 min, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, and 60 s at 72°C, and a final extension at 72°C for 10 min. PCR products were visualized using gel electrophoresis on a 1% agarose gel. Samples were run once and based on amplification success we selected 760 fox samples for sequencing. PCR product from the 760 individual fecal samples, and eight negative controls, were then sent to the Canadian Centre for DNA barcoding at University of Guelph, Biodiversity Institute of Ontario where they were tagged with universal molecular identifiers, then pooled for sequencing. Sequencing was carried out on an Ion Torrent S5 (Thermo Fisher Scientific, Waltham, Massachusetts) and produced sequence reads for each fecal sample. Each sequence read corresponds to a DNA fragment amplified from the fecal sample.

Initial bioinformatics was conducted by the Canadian Centre for DNA barcoding, where sequences were filtered to remove low-quality sequence reads and to remove primer and adaptor sequences. Trimmed reads were compared to a 12S region reference library assembled from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), along with sequenced prey not available on GenBank, specifically red-backed vole (*Myodes*

*gapperi*) and Richardson's collared lemming (*D. richardsoni*). Unique taxonomic identification of each sequence was done by the Canadian Centre for DNA barcoding using the Basic Local Alignment Search Tool algorithm (Altschul et al. 1990).

We undertook additional bioinformatics filtration to reduce background sequencing errors. Taxonomic identifications only were accepted as genuine if specific quality thresholds were met: at least 100 reads matched a reference sequence with at least 98% identity across at least a 100-bp length region and e-values less than 10<sup>-20</sup> (Vesterinen et al. 2013; Biffi et al. 2017). In addition, we removed sequences that occurred in <0.01% of reads per sample to avoid inflating the importance of rare species and background sequencing errors (Deagle et al. 2019). Finally, we manually checked sequences that were rare or identified species occurring outside of our study area to determine if sequences could be identified as a species occurring within our study area, or if sequences needed to be assigned to a higher taxonomic level (genus, family, etc.; De Barba et al. 2014; Biffi et al. 2017; Kerley et al. 2018). For example, due to a lack of sequence variation in the 12S V5 region, ducks, geese, and swans, only can be identified to family (Anatidae) and therefore all sequences were assigned to the taxonomic level of family.

**Statistical analysis.**—We categorized prey taxa into functional groups that represented distinct winter prey consumed by Arctic foxes. Our functional groups included small mammals, marine resources, migratory birds, ungulates, resident birds, and fish. Because lemmings are considered broadly the most important driver of Arctic fox dynamics, for statistical analyses we separated the small mammal category by genus: lemmings (*Dicrostonyx* spp.), voles (*Microtus* spp.), and shrews (*Sorex* spp.). We grouped all occurrences of marine resources (seals or whales), because Arctic foxes are known to use sea ice to scavenge polar bear kills (Roth 2003). We grouped migratory birds (Passeriformes, Charadriiformes, Anseriformes) separately from resident birds (Galliformes) because migratory birds are absent during winter and likely are consumed from caches of carcasses or eggs (Samelius et al. 2007; McDonald et al. 2017). Other sequences were excluded from analyses because percent reads were below the 0.01% threshold (Deagle et al. 2019).

We used den as the unit of analysis and combined diet information (presence/absence of a diet item) from all samples at a given den in a single year. We measured the relative importance of prey using frequency of occurrence (hereafter referred to as occurrence), calculated as the number of occurrences (presence) of each prey group divided by the number of dens for each year. We also calculated relative read abundance (RRA), or the proportion of unique reads representing a specific prey taxon divided by the total number of reads at a den. As RRA may not accurately reflect prey biomass and may be affected by differential recovery of taxa (Deagle et al. 2019; Lamb et al. 2019), we based subsequent diet analyses on occurrence results, although we report RRA diet estimates and trends (Supplementary Data SD1). We used species-accumulation curves to assess if sampling effort was sufficient to detect all prey taxa each year (Colwell et al. 2012; Kartzinel et al. 2015).

We used linear regression to assess the effect of lemming density (as measured by winter lemming nest density) on the proportions of different prey consumed. We tested for relationships between the occurrence of lemmings in Arctic fox winter diet and occurrence of other functional groups using Pearson’s correlation. We measured the distance of each den to the coast of Hudson Bay using ArcGIS (version 10.6.1; [Environmental Systems Research Institute 2018](#)) and used logistic regression with den as a random effect to test whether distance to the coast affected the presence of prey items at dens (prey were considered present if they occurred at least once across all years).

All statistical analyses were done in the program R version 4.0.0 ([R Core Team 2020](#)) using RStudio version 1.2.5042-1 ([RStudio Team 2020](#)). We used the R package Vegan to create species accumulation curves ([Oksanen et al. 2019](#)).

**RESULTS**

We extracted DNA from 959 fecal samples: 802 were identified as Arctic fox, 17 as red fox, and 140 samples failed species identification. We selected 743 Arctic fox samples based on amplification success for sequencing. Prey DNA was detected in 657 of these samples and the final sequence library had 33,448,304 reads prior to filtering. After filtering for high-quality sequences, 14,518,046 sequences remained. We then removed obvious contaminants such as environmental, pig (a known PCR kit contaminant; [Leonard et al. 2007](#)), and human DNA sequences. Of the remaining 13,310,021 sequences, 41.6% were Arctic fox sequences, indicating that our blocking primer reduced predator amplification but did not eliminate it. Samples with red fox DNA ( $n = 30$ ) were excluded from further analysis (see [Dudenhoeffer 2020](#)).

After sequence filtration and quality control, our final sequence library contained 5,532,707 sequences from 627 Arctic fox fecal samples. Reads per individual sample averaged  $8511.9 \pm 279.8 SE$ , with a range of 102–47,459. Across all

samples, sequences belonged to 28 prey taxa, with nine taxa identified to species level, 15 to genus, one to subfamily, and three to family ([Table 1](#)). Across all years, the number of unique prey taxa per sample ranged from 1 to 6, with an average of  $1.86 \pm 0.04$ . The number of unique prey taxa detected per den across years ranged from 1 to 8, with an average of  $4.13 \pm 0.16$ . The number of dens with samples varied across years with an average of  $39.13 \pm 7.52$  dens with fecal samples and the number of samples at each den averaged across all years was  $1.91 \pm 0.17$  fecal samples per den. Sample-based species accumulation curves for each year (with diet data pooled by den) approached asymptotes, suggesting adequate sampling for each year ([Supplementary Data SD2](#)).

Lemming nest density variation from 2013 to 2018 suggested continued population cycling. Small mammals were the primary prey consumed in all years and occurred in 82–100% of samples ([Table 2](#)). The lowest proportion of small mammals consumed was in 2015, the year of lowest lemming nest density. Other prey groups were relatively unimportant compared to small mammals ([Fig. 2](#)). Within the small mammal functional group, voles and lemmings were the most important species and their relative proportion varied across years based on changes in lemming abundance.

Arctic fox winter diet was strongly influenced by annual changes in lemming abundance, with a strong positive relationship between lemming abundance and occurrence in the diet ( $R^2 = 0.95$ ,  $\beta = 0.012$ ,  $F_{1,4} = 70.4$ ,  $P = 0.001$ ; [Fig. 3](#)). Vole occurrence also was positively related to lemming abundance ( $R^2 = 0.75$ ,  $\beta = 0.004$ ,  $F_{1,4} = 11.73$ ,  $P = 0.027$ ). Conversely, lemming abundance negatively influenced consumption of shrews ( $R^2 = 0.82$ ,  $\beta = -0.015$ ,  $F_{1,4} = 19.1$ ,  $P = 0.012$ ) and marine resources ( $R^2 = 0.74$ ,  $\beta = -0.009$ ,  $F_{1,4} = 11.59$ ,  $P = 0.027$ ). The relative proportion of prey types varied across years ([Table 2](#)), but lemming abundance did not affect the proportion of ungulates ( $R^2 = 0.02$ ,  $\beta = 0.001$ ,  $F_{1,4} = 0.10$ ,  $P = 0.76$ ), migratory birds ( $R^2 = 0.001$ ,  $\beta = -0.0002$ ,  $F_{1,4} = 0.01$ ,  $P = 0.96$ ), resident

**Table 1.**—Unique prey identified by next-generation sequencing of 627 Arctic fox fecal samples collected in April 2011–2018 from dens located near Churchill, Manitoba. Identification was based on species occurring in our study area and taxa were categorized into functional groups for analysis.

Functional group	Taxon	Common name	Number of samples	Number of reads	Percent of reads
Fish	<i>Pungitius</i>	Stickleback	25	9,742	0.18
Marine resources	<i>Cystophora</i>	Hooded seal	1	1,174	0.02
	<i>Erignathus</i>	Bearded seal	1	10,247	0.19
	Monodontidae	Beluga/narwhal	1	1,047	0.02
Migratory birds	Phocini	Harbor/ringed seal	52	91,872	1.67
	Anatidae	Geese, ducks, swans	149	186,499	3.39
	<i>Calcarius</i>	Longspur	2	758	0.01
	<i>Cephus</i>	Guillemot	1	304	0.01
	<i>Fringillidae</i>	Finch	27	26,876	0.49
	<i>Gallinago</i>	Snipe	1	5,637	0.10
	<i>Larus</i>	Gulls	10	4,688	0.09
	<i>Tringa</i>	Yellow legs	2	310	0.01
Resident bird	<i>Lagopus</i>	Ptarmigan	22	123,745	2.25
Small mammals	<i>Dicrostonyx</i>	Lemming	331	3,065,611	55.69
	<i>Microtus</i>	Vole	487	1,894,174	34.41
	<i>Sorex</i>	Shrew	47	43,960	0.80
Ungulate	<i>Alces</i>	Moose	1	5,920	0.11
	<i>Rangifer</i>	Caribou	33	24,934	0.45

**Table 2.**—Frequency of occurrence of the six most common prey functional groups identified using next-generation sequencing in Arctic fox fecal samples ( $n = 627$ ). Fecal samples were collected during annual visits to 90 dens in April from 2011 to 2018 near Churchill, Manitoba. Occurrence of genera in the small mammal functional group is reported in italics. See [Supplementary Data SD1](#) for corresponding relative read abundance tables.

	2011	2012	2013	2014	2015	2016	2017	2018
Small mammal	96.7%	82.4%	100%	94.8%	81.8%	96.7%	93.3%	100%
<i>Vole</i>	73.3%	23.5%	92.0%	82.8%	72.7%	83.3%	88.9%	93.8%
<i>Lemming</i>	76.7%	58.8%	81.3%	77.6%	36.4%	46.7%	71.1%	79.2%
<i>Shrew</i>	23.3%	17.6%	2.7%	3.4%	36.4%	60.0%	0.0%	0.0%
Migratory birds	13.3%	11.8%	65.3%	34.5%	45.5%	46.7%	46.7%	35.4%
Marine resources	16.7%	11.8%	18.7%	13.8%	45.5%	23.3%	8.9%	0.0%
Ungulate	13.3%	29.4%	4.0%	3.4%	0.0%	13.3%	20.0%	8.3%
Fish	0.0%	0.0%	14.7%	6.9%	0.0%	23.3%	0.0%	2.1%
Resident bird	10.0%	0.0%	6.7%	3.4%	0.0%	10.0%	13.3%	2.1%
Lemming nest density (ha <sup>-1</sup> )	NA	NA	41.4	36.1	9.8	13.1	37.1	46.6
Dens with samples	30	17	75	57	11	30	45	48
Mean number of samples per den	1.63	1.18	2.04	2.28	1.27	2.2	2.31	2.38

birds ( $R^2 = 0.001$ ,  $\beta = 0.0001$ ,  $F_{1,4} = 0.03$ ,  $P = 0.87$ ), and fish ( $R^2 = 0.07$ ,  $\beta = -0.002$ ,  $F_{1,4} = 0.28$ ,  $P = 0.63$ ) in Arctic fox diet. The proportion of lemming in winter diet was negatively correlated to the proportion of marine resources (Pearson's  $r = -0.77$ ,  $P = 0.03$ ) and shrew ( $r = -0.80$ ,  $P = 0.02$ ), but no other functional groups were correlated with each other ([Supplementary Data SD3](#)).

Across all years, voles and lemmings were the most widely distributed prey, with voles occurring at 89 of the 90 dens sampled and lemmings occurring at 81 dens. Migratory birds were detected at 74 dens and all other prey types occurred at < 34 dens. Distance of den to the coast of Hudson Bay ranged from 0.16 to 11.35 km. As the distance of a den to the coast increased, the presence of marine resources in Arctic fox diet decreased ( $z = -2.501$ ,  $P = 0.012$ ), the presence of migratory birds decreased ( $z = -2.270$ ,  $P = 0.023$ ), and the presence of lemmings increased ( $z = 2.673$ ,  $P = 0.008$ ). No other significant trends in prey presence were detected ([Supplementary Data SD4](#)).

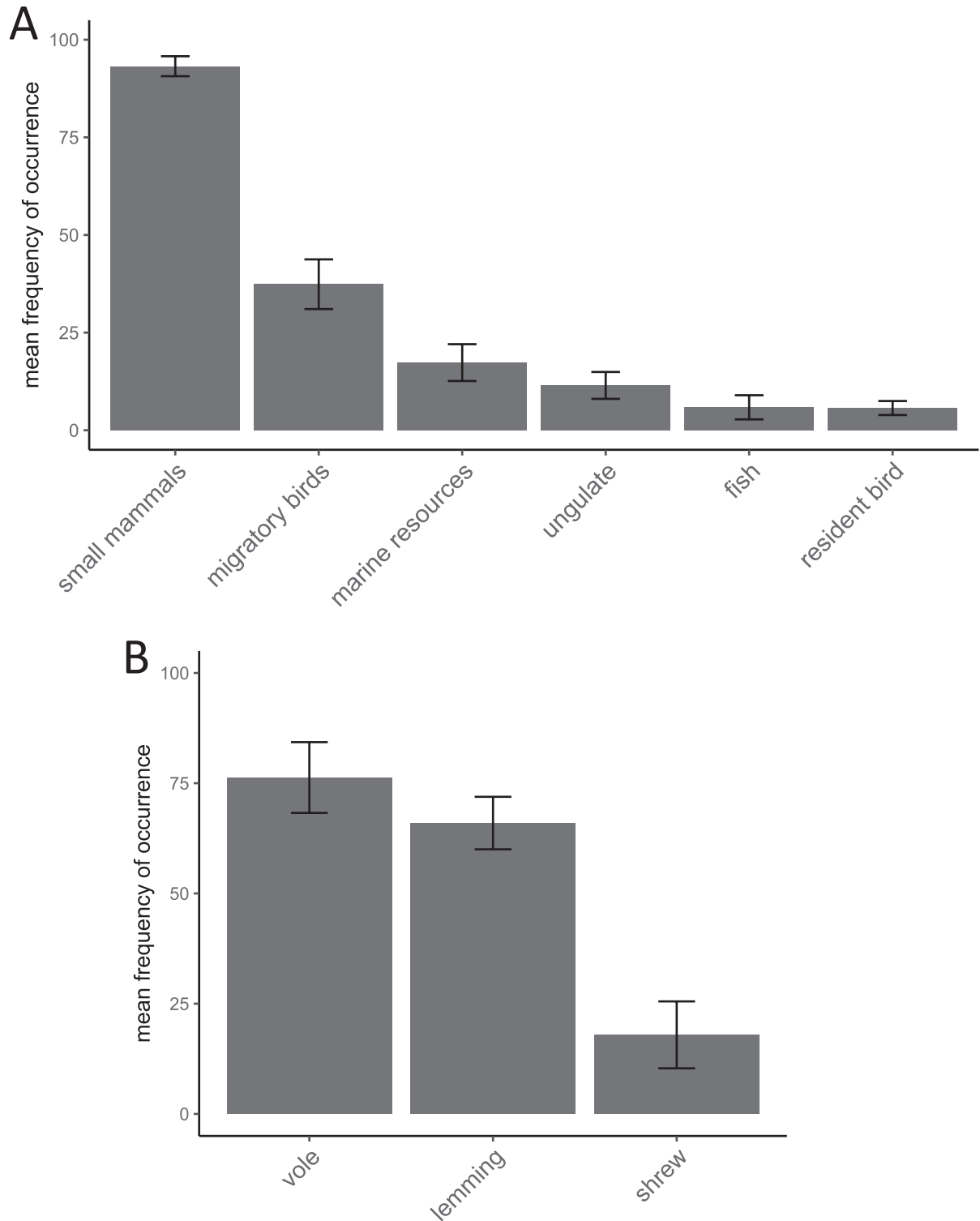
## DISCUSSION

Molecular methods revealed that Arctic foxes consumed a variety of prey, and consumption varied based on annual changes in lemming abundance, suggesting dietary flexibility. Arctic fox diet still is strongly influenced by annual changes in lemming abundance despite lemming population cycle damping ([Ehrich et al. 2020](#)), because they consumed more alternative prey, such as marine resources and shrews, when lemming abundance was low. Yet the consumption of high proportions of temperate prey such as meadow voles, which previously had not been detected in these foxes' diet, suggests changes in the prey community that may impact these predators.

Arctic foxes consumed meadow voles in high proportions in most years, indicating that voles are important prey and accessible on the tundra during winter as an established population. Yet meadow voles were infrequently live-trapped in this area during the years our samples were collected (2011–2018; [Ehrich et al. 2020](#)), and previously (1994–1997) only collared lemmings were captured ([Roth 2002](#); [McDonald et al. 2017](#)). Our observations are similar to other low-Arctic regions

where the dominant species in small mammal assemblages are shifting from lemmings (*Dicrostonyx* and *Lemmus*) to voles (*Microtus* and *Myodes*) ([Fufachev et al. 2019](#); [Ehrich et al. 2020](#)). Increasing vole populations could provide Arctic foxes an alternative prey resource if peak lemming densities continue to decline. While some studies suggest voles could subsidize Arctic foxes, possibly replacing lemmings as a resource ([Ehrich et al. 2017](#)), others suggest Arctic foxes will respond numerically only to lemming population peaks regardless of lemming cycle damping or vole presence ([Samelius et al. 2011](#); [Ims et al. 2017](#)). In our study, the dietary response to variation in vole and lemming consumption differed. As lemming consumption increased, the proportion of alternative prey in diet declined, specifically marine resources and shrews. Conversely, consumption of voles was unrelated to the proportion of alternative prey in diet. The small mammal community in the low Arctic might be changing, but further research is needed to understand these changes and how the presence of voles impacts the low-Arctic food web.

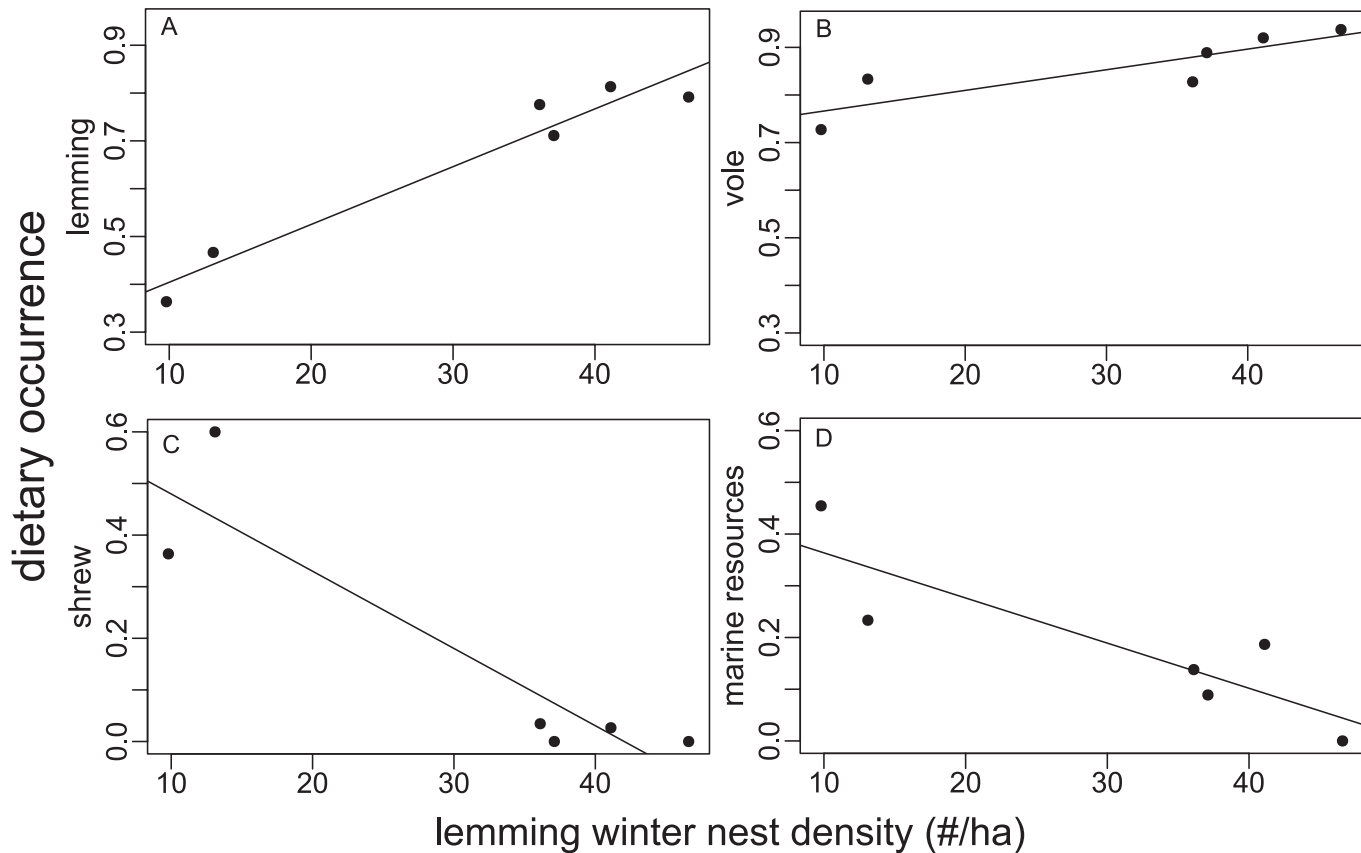
Alternative resources and access to adjacent ecosystems are especially important for terrestrial Arctic predators ([Gilg et al. 2006](#); [Dalerum et al. 2012](#); [Julien et al. 2014](#)). Arctic foxes used both the terrestrial and marine ecosystems, but facultatively consumed marine resources in higher proportions during years of low lemming density. Our results confirm that marine resources are an important alternative resource for Arctic foxes when lemmings are scarce ([Roth 2002, 2003](#)), especially for foxes that reside in dens closer to the coast, suggesting the spatial distribution of alternative prey resources can influence fox denning patterns. However, our estimated proportion of marine resources consumed was lower than previous diet estimates using other methods. Stable-isotope analysis of fox tissues collected from the Canadian tundra have found that marine resources accounted for up to two-thirds of Arctic fox winter diet ([Roth 2002, 2003](#)). This technique reflects long-term diet (a few months) while fecal DNA analysis reflect prey consumed 24–48 h prior to defecation, providing a short-term diet estimate ([Deagle et al. 2019](#)). Arctic foxes can go on short commuter trips or longer trips onto the sea ice ([Lai et al. 2017](#)) and likely defecate on sea ice, whereas the



**Fig. 2.**—Mean frequency of occurrence of A) prey functional groups and B) genera within the small mammal functional group (*Microtus*, *Dicrostonyx*, *Sorex*) in Arctic fox winter diet near Churchill, Manitoba. Frequency of occurrence ( $\pm$  SE) for each prey functional group was averaged over the 8-year study period from 2011 to 2018.

fecal samples used for our analysis were collected from dens on land. Therefore, our sampling strategy likely underestimated the proportion of marine resources consumed by Arctic foxes. In our study area, marine resources are becoming less accessible to foxes because sea ice duration is declining in

Hudson Bay (Florko et al. 2018). Climate-caused changes in the marine food web, such as projected major declines in ringed seal and polar bear populations, may have cascading effects on the terrestrial food web because these habitats are highly linked (Reimer et al. 2019).



**Fig. 3.**—Relationship between lemming winter nest density (#/ha, data collected during June from 2013 to 2018) and the frequency of occurrence of A) lemming, B) vole, C) shrew, and D) marine resources in Arctic fox winter diet near Churchill, Manitoba, Canada. Each point represents the mean annual frequency of occurrence of the prey type in Arctic fox winter diet.

Next-generation sequencing of Arctic fox fecal DNA allowed us to reconstruct Arctic fox winter diet by identifying prey with higher taxonomic precision and detecting under-represented prey. Traditional methods of diet analysis such as visual identification of prey remains may underestimate prey without hard identifiable undigested material, while stable-isotope analysis relies on different prey signatures and may not have high taxonomic precision (Deagle et al. 2019). For example, a previous study of Arctic fox winter diet in our study area identified prey from fecal samples using bones, hair, and feathers. Rodents were found in similar proportions as our molecular methods, but visual inspection failed to detect marine resources in 2011 or 2012, likely due to a lack of hard identifiable material (McDonald et al. 2017). Using the same fecal samples, we found marine resources made up 16.7% of Arctic fox winter diet in 2011, and 11.8% in 2012. In addition, metabarcoding revealed four different taxa of marine mammals consumed by Arctic foxes. Molecular methods are becoming increasingly useful as technology advances and can reveal a wider diversity in diet than expected (Seah et al. 2020).

Arctic foxes consumed a relatively diverse assemblage of prey species given low species diversity characteristic of tundra ecosystem. Alternative prey identified by sequencing included a range of taxa in Arctic fox winter diet including 28 unique prey taxa, with most of the species diversity being due to the presence

of migratory birds such as geese, Lapland longspurs, gulls, and shorebirds (Table 1). Our results are similar to those of other studies that found Arctic foxes primarily consumed rodents, but opportunistically consumed alternative prey (Angerbjörn et al. 1999; Elmhagen et al. 2000; Pokrovsky et al. 2015). One interesting prey item detected by sequencing was the presence of shrews (*Sorex*) in Arctic fox diet, and the negative relationship between lemming abundance and the occurrence of shrews in diet. Other literature suggests that shrews were unimportant in Arctic fox diet and that shrews even may be avoided by foxes (Elmhagen et al. 2000). The negative relationship between lemming abundance and the occurrence of shrew and marine resources suggests some alternative prey may experience increased predation pressure when lemming abundance is low. In some regions, migratory birds are consumed as alternative prey, specifically during low lemming years (Samelius et al. 2007). We found no relationship between lemming abundance and migratory bird occurrence in the diet. Migratory birds are not cached by all foxes equally, because juvenile foxes do not have the opportunity to kill and stash migratory birds when they are available during summer (McDonald et al. 2017). Differences in caching behavior due to demography may explain the absence of a relationship between consumption of migratory birds and lemming abundance. Other alternative prey, such as ungulates and fish, were consumed in low proportions and likely are



consumed opportunistically; we therefore expect some alternative prey groups to be unrelated to lemming abundance.

In the Western Hudson Bay region, Arctic foxes continue to respond to damped lemming cycles. Our study highlights continued annual changes in Arctic fox winter diet related to lemming abundance, but also the increased importance of alternative small mammals such as meadow voles and shrews. Despite the high proportions of lemmings in Arctic fox winter diet, a long-term decline in the local Arctic fox population has been related to winter snow conditions that affect lemmings (Verstege 2016). Other studies have suggested Arctic fox reproductive output may be more stable if alternative prey is available (Pokrovsky et al. 2015). Voles are expected to continue expanding on the tundra as climate change increases the amount of preferred vole habitat and decreases the amount of suitable lemming habitat (Morris et al. 2011). As the community composition of the Arctic changes, predators with dietary flexibility may better be able to adapt to local changes in prey composition, abundance, and distribution if alternative prey are available.

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#### SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Mammalogy* online.

**Supplementary Data SD1.**—Arctic fox winter diet estimated with relative read abundance.

**Supplementary Data SD2.**—Sample-based species accumulation curves for each year.

**Supplementary Data SD3.**—Correlation table of prey groups in Arctic fox winter diet.

**Supplementary Data SD4.**—Spatial analysis results.

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