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Spatio-temporal patterns in arctic fox (*Vulpes alopex*) diets revealed by molecular analysis of scats from Northeast Greenland

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ABSTRACT

The arctic fox (*Vulpes lagopus*) is endemic to the Arctic where it holds a central position in the trophic interactions. The diet of the species has previously been described as being highly flexible, but whether this flexibility is a constant trait through time, or merely reflects fast temporal changes in abundance among prey taxa, has so far been poorly resolved. Using molecular analyses of arctic fox scats from Northeast Greenland, we first examined the temporal dynamics of arctic fox diets during the short snow-free season, and then examined whether local food availability at different sites affected arctic fox dependence on lemmings. Arctic fox diets included most terrestrial vertebrate species found in the region, and exhibited substantial temporal changes, generally reflecting the dynamic changes in prey availability from late winter through autumn. This dietary flexibility was also reflected geographically, with arctic foxes consuming a variety of local prey (mainly waterfowl and lemmings) in summer. Moreover, the dietary response of arctic foxes to changes in lemming abundance depended on access to non-lemming prey. Based on these findings, we discuss whether varying degrees of lemming-dependency, combined with geographical differences in winter food availability, may explain previously published differences in arctic fox breeding patterns in high arctic Greenland.

1. Introduction

The arctic fox (*Vulpes lagopus*) is the only endemic terrestrial predator in the Arctic (Fuglei and Ims, 2008). Holding a central position in the terrestrial trophic interactions on the tundra (Ehrich et al., 2015; Schmidt et al., 2017), the arctic fox is regarded as a flagship species by the IUCN, and has been identified as an indicator of climate-induced changes in biotic interactions in arctic and alpine regions (Siller-o-Zubiri and Angerbjörn, 2009). The species is therefore a focal species of many long-term research and monitoring efforts across the circum-arctic region (Berteaux et al., 2017). Throughout their distributional range, arctic foxes are regarded as opportunistic generalist predators, feeding on a variety of prey species (Angerbjörn et al., 1999; Giroux

et al., 2012; Samelius et al., 2007), which has also been reported from high arctic Greenland (Dalerum and Angerbjörn, 2000; Gilg et al., 2006). Arctic fox diets vary markedly geographically, and in some areas, such as Scandinavia and some sites in Greenland, lemmings constitute the major part of arctic fox diets (Elmhagen et al., 2000; Gilg et al., 2006). In areas where lemmings are absent, carcasses of large mammals and avian prey may dominate their diets (Eide et al., 2005), and where several alternative prey species occur, these species may form the largest proportions of arctic fox diets (Bantle and Alisauskas, 1998; Tarroux et al., 2012).

Traditionally, the diet of the arctic fox has been inferred from hard part analyses (Dalerum and Angerbjörn, 2000; Eide et al., 2005; Gilg et al., 2006). More recently, these estimates have been supplemented by

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analyses of stable isotopes of arctic fox tissues (Carbonell Ellgutter et al., 2020; Ehrich et al., 2015; Giroux et al., 2012). While such methods provide valuable information about fox diets, the taxonomic resolution is often low. Additionally, larger prey which is not consumed whole may leave no identifiable hard parts.

During the last few decades, molecular methods have been introduced as efficient tools for overcoming taxonomic issues (Monterroso et al., 2019). As a potential drawback, they provide less quantitative or biased dietary information (Clare, 2014). Recent studies, however, suggest that the general diet composition can be inferred from the relative read abundances of the various taxa consumed, recovered from for instance scat samples (Deagle et al., 2019).

In this study we use molecular analysis of arctic fox scats collected in high arctic Greenland to document the diet of arctic foxes. First we ask whether the previously reported dietary flexibility of the arctic fox also reflects the rapidly changing prey availabilities during the short snow-free season in the high Arctic. During the snow season, we expect arctic foxes to rely mainly on resident vertebrate prey, and in particular on muskox (*Ovibos moschatus*) carcasses (Schmidt et al., 2008), as well as cached prey (Fay and Stephenson, 1989; Samelius et al., 2007). During the snow-free season, we expect lemmings and migratory birds to provide the main diet (Reneerkens et al., 2016; Schmidt et al., 2008). We then ask whether regional variation in both lemming prey and alternative food sources across high arctic Greenland is directly reflected into different arctic fox diets. To this aim, we compare arctic fox diet composition at three sites in Northeast Greenland with contrasting prey compositions and availabilities. Finally, we discuss how geographical differences in lemming and non-lemming prey availability might translate into arctic fox breeding patterns in Greenland.

2. Methods and data

2.1. Field sampling

For examining the seasonal patterns in arctic fox diet, we collected arctic fox scats at Zackenberg in high arctic Greenland (74°28′N, 20°34′W) from the months April through October. The study area consists of a broad valley close to a fjord, and covered with a mosaic of typical tundra vegetation types, mainly heaths and graminoid-dominated vegetation (Elberling et al., 2008). The vertebrate food web is centred around the lemming, the only rodent in Greenland. In addition to arctic fox, the lemming-predator guild consists of mainly long-tailed skua (*Stercorarius longicaudus*), snowy owl (*Bubo scandiacus*) and stoat (*Mustela erminea*) (Schmidt et al., 2012). The availability of non-lemming prey is given in Table 1. Upon arrival to the area in late winter, we cleared the seven known arctic fox breeding dens at Zackenberg for scats. Hereafter we collected fresh scats on the dens at weekly visits. Fresh scats encountered by chance away from dens were also collected. In the summer months, only scats from adult foxes were collected (based on scat size). Scats were placed in individual tubes and either stored frozen or in ethanol until processing. Most scats were collected in the 2012 season, but to increase our sample size we also included scats from other years (see below). During the years 2010–2015, fresh scats from the summer months (June through August) were collected at Zackenberg to allow for inter-site comparisons of arctic fox diets.

To assess differences in diets between sites in Northeast Greenland, we collected arctic fox scats at two additional sites, Hochstetter Forland (75°15′N, 19°70′W) and Karupelv (72°50′N, 24°00′W) (Fig. 1), characterized by contrasting prey availability in summer and winter (Table 1). Hochstetter Forland is a large lowland, dominated by graminoid-dominated vegetation types (Meltofte et al., 1981), while Karupelv is a broad valley close to a fjord, covered with mainly heath vegetation types (Büntgen et al., 2018). As at Zackenberg, the lemming is key for the vertebrate predator-prey dynamics and dominated by the same predators, though snowy owls are more frequent at Karupelv (Schmidt et al., 2012). Non-lemming prey is also available at both sites, but mainly at Hochstetter Forland (Table 1). At both sites fresh scats from adult foxes were collected opportunistically at known breeding dens (six dens at Hochstetter Forland and five at Karupelv) and when encountered in the field during the summer months (June through August) during the years 2010–2015. Scats from first visits to the dens were disregarded. At Karupelv and Hochstetter Forland, samples were air-dried and stored dry until processing. Unfortunately, small sample sizes prevented us from examining the inter-annual variability in diets at these sites. We therefore divided the years into lemming "high" years (more than 2 lemmings per hectare) or lemming "low" years at each site (Gilg et al., 2003; Therrien et al., 2014). We calculated the density of lemmings in spring based on counts of winter nests following Gilg et al. (2019, 2009). Lemming high years for Karupelv were 2011 and 2012, 2012 for Zackenberg, and 2013 for Hochstetter Forland. At all three sites we also classified the relative availability of non-lemming prey in the area into three categories based on field observations: low (limited availability, rare occurrence), intermediate (intermediate availability, occasional occurrence) or high (high availability, frequent occurrence).

2.2. Molecular analyses

We excluded scats collected at fox dens at first visit to the dens as these could not be adequately assigned to a month, and ended up extracting DNA from 380 arctic fox scats. For each scat, we retained only information concerning the target prey taxa: Birds, mammals, and fish. We removed all reads assigned to Canidae (i.e. arctic fox, potentially wolf *Canis lupus*) as we do not know whether these stem from cannibalism, grooming or from sloughed cells. Our final dataset thus consisted of 3,361,848 prey reads assigned to prey taxa. We were able to retrieve meaningful prey data from 319 out of the original 380 (84%) scat samples. The rest of the samples only contained bacterial or other non-target reads. Hence, for the examination of temporal changes in diet, a total of 139 scat samples were included (April: n = 14, May: n = 14, June: n = 31, July: n = 19, August: n = 29, September: n = 23, October: n = 9). Ca. 80% of these scats were from 2012. For the inter-site comparison of arctic fox summer diets, a total of 225 scats collected in June through August were included (Karupelv: n = 53; Hochstetter Forland: n = 47, and Zackenberg: n = 125).

Details on the extraction and PCR methodologies and library preparation can be found in Appendix A, but a short summary is included here. DNA was extracted from 380 (4 × 95) Arctic fox scat samples plus four negative controls (4 × 1) using ZR 96 Kits (cat nr D6011, Zymo Research Corp., Irvine, CA, USA) following kit manual. The purified DNA was then amplified using three different primer pairs, as follows. First primer was meant to amplify mammalian mtDNA *cytochrome oxidase subunit I* (COI), and we used a blocking primer in the reaction to prevent fox DNA to be co-amplified. A blocking primer is a specific oligo, that binds to the non-wanted DNA, here fox DNA, and is modified so that amplification in the PCR is not possible or is significantly reduced.

Table 1
Prey availability at the three study sites during the period 2010–2015. Lemming availabilities are given as mean densities (range) per km^2, while the availability of non-lemming prey is divided into categories: low (limited availability, rare occurrence), intermediate (intermediate availability, occasional occurrence) or high (high availability, frequent occurrence).

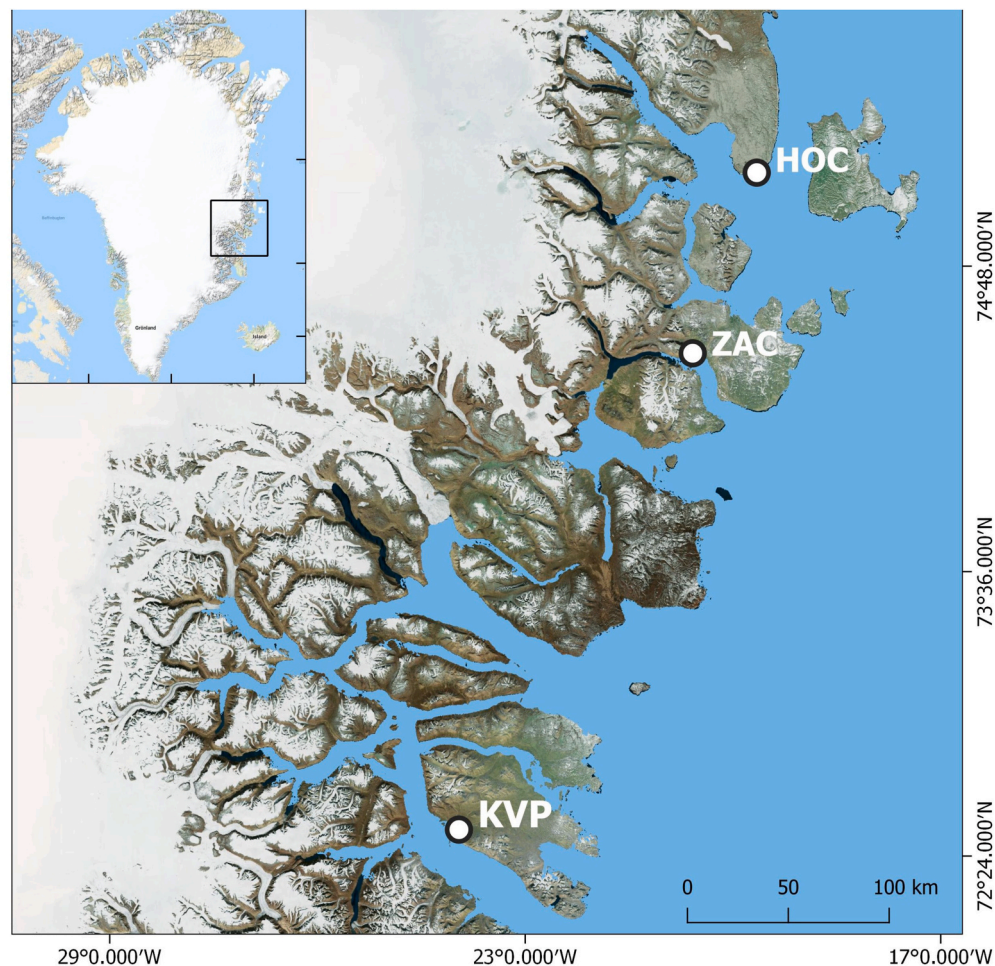| Prey taxa	Karupelv	Zackenberg	Hochstetter Forland
Lemming spring density	93.23 (1.37–284.21)	66.05 (5.03–233.55)	84.28 (6.40–331.89)
Waterfowl (summer)	Low	Intermediate	High
Waders (summer)	Intermediate	High	High
Muskox carcasses (winter)	Low	High	Low

Fig. 1. The location of the three study sites in Northeast Greenland, Karupelv (KVP), Zackenberg (ZAC) and Hochstetter Forland (HOC).

Another primer pair was targeting bird mitochondrial *cytochrome b* gene (CytB), and no blocking primer was used in this reaction. The third primer pair was designed based on all Greenland animal sequences, targeting vertebrate mtDNA COI gene, and a new blocking primer was designed against fox DNA amplification. After thorough testing, our final PCR and library preparation followed Vesterinen et al. (2018), with primer-specific PCR conditions. For each primer pair, the first PCR phase amplified the desired locus, and the subsequent second PCR stage was used to attach Illumina specific adapters and sample-specific index combinations. All the indexed samples were pooled per primer pair and purified using magnetic SPRI beads (Vesterinen et al., 2016). Sequencing was performed on two distinct runs. The first run included Fox-Mam + Fox-Bird pool (together with another library from an insect study) and was sequenced at the facilities of Macrogen Inc. (Seoul, South Korea) on one lane of an Illumina HiSeq4000 flow cell using 2×150bp run. The second run included the COI-Prey pool on Illumina NextSeq500 platform at the Functional Genomics Unit of the University of Helsinki, Finland, using Mid Output with 300 cycles and 2×150 bp paired-end read length.

2.3. Bioinformatics

The raw sequence output was uploaded to CSC servers (IT Center for Science, www.csc.fi) for trimming and further analysis. The platform-specific steps varied between primer pair outputs, and the details of each step have been collected into the supplements. In short, we identified 306 operational taxonomic units (OTUs) for Fox-Mam, 526 OTUs for Fox-Bird, and 6392 ZOTUs for COI-Prey. ZOTUs (zero-radius OTUs)

do not practically differ from traditional clustering of OTUs (which are based on pre-set percentage threshold), but the UNOISE algorithm performs better in (i) removing chimeras, (ii) PhiX sequences and (iii) Illumina artefacts according to Edgar and Flyvbjerg (2015). The OTUs from *COI* region were assigned to taxa based on two different resources: 1) the Barcode of Life Data systems API engine (Ratnasingham and Hebert, 2007), which we accessed using custom-made scripts (see Vesterinen et al., 2020), and 2) the GenBank nt database, against which we BLASTed (Altschul et al., 1990) our sequences before importing the results to software Megan for lowest common ancestor (LCA) analysis (Huson et al., 2007). *CytB* reads were assigned solely by BLAST followed by the LCA approach. The final dataset consisted of 500,644 reads in the Fox-Mam OTU table, 8,419,225 reads for Fox-Bird OTU table, and 11,955,842 reads for COI-Prey.

2.4. Data treatment

We used the number of taxa-specific DNA reads as indicator of the relative contribution of each taxon to arctic fox diets. To account for varying number of reads per scat, we first normalized the number of reads of prey types within each scat (i.e. number of reads per prey taxon divided by total number of reads in the scat). This metric has been reported to yield an adequate representation of diet composition (Deagle et al., 2019). To characterize general temporal (monthly) variation in arctic fox diet at Zackenberg, we normalized the number of reads from each prey type within each month. Similarly, to examine diets in high lemming and low lemming years at the three sites, we calculated the normalized read fraction for each prey taxon within each site and

lemming density (lemming high or low years). Following Angerbjörn et al. (1999), dietary fractions were converted into "whole scat equivalents" (WSE), which take into account the relative contributions from the various prey items whilst having the number of scats as sample sizes as in ordinary frequency of occurrence analyses. We then compared statistically the dietary composition between months, sites and between high and low lemming density years at the three sites using Likelihood Ratio Tests (G-test). Taxa with low WSE numbers were lumped together to meet the requirements of the test.

Finally, we calculated the dietary niche breadth for each month and for each site in the two lemming densities using Levin's measure (*B*) (Krebs, 1998):

$$B = \frac{1}{\sum P_j^2}$$

where P_j is the proportion (based on the normalized reads in the scats) of prey taxa *j* in each month or site. Levin's measure ranges from 1 (only one prey taxa consumed) to the maximum number of prey taxa consumed (i.e. all prey taxa consumed in equal amounts).

3. Results

The three sites examined here varied markedly with respect to the relative availability of both lemming prey and alternative food sources (Table 1). Mean lemming spring density was highest at Karupelv, intermediate at Hochstetter Forland and lowest at Zackenberg. Moreover, Hochstetter Forland and Zackenberg had similar amplitude in lemming fluctuations, whereas Karupelv lemmings exhibited an amplitude 5–6 times higher than the two other sites (Table 1).

We grouped prey taxa encountered into nine prey groups based on taxonomy or, in the case of "Others", their low frequencies in scats (Table 2). Most scats held between 1 and 3 prey taxa (n = 255), but up to nine prey taxa were found (n = 1).

At Zackenberg, the temporal changes in arctic fox diet from late winter (April) to late autumn (October) were considerable (Fig. 2). Not all monthly diet compositions differed significantly, but generally diets in the months April through August were more similar, whereas diets in the months September and October were more similar to each other (Appendix A Table S1). Hence, in late winter diet consisted mainly of muskox, but waterfowl, ptarmigans, lemmings and fish were also consumed. In the following months, muskox was still consumed but were less frequent, and diets were dominated by waterfowl, fish and lemmings. Hares and small passerines also constituted substantial fractions of diet during the snow-free season, whereas remaining prey taxa were only consumed in small amounts. In September, muskox, waders and fish constituted the main parts of diet. In October, muskox and fish made up the major fraction of the diet, but waterfowl were still relatively important (Fig. 2). The dietary niche breadth (Levin's (*B*)) varied

markedly between months (Fig. 2). Niche breadth was highest in late winter/spring (May–June), after which it decreased steadily as waterfowl became increasingly important as prey during summer. In early autumn (September), niche breadth increased sharply again, likely because most waterfowl have migrated South and foxes thus have to rely on other prey species. Thereafter, niche breadth declined again as muskox carcasses became the dominant prey in winter (Fig. 2).

Although arctic foxes consumed a variety of prey taxa in summer at all three sites examined, the general composition of summer diets differed significantly between sites (Fig. 3; G between 19.971 and 29.309, df = 8, and p between 0.000 and 0.010). Hence, arctic foxes mainly consumed waterfowl at Hochstetter Forland, lemmings at Karupelv, while at Zackenberg they consumed a broader variety of taxa dominated by waterfowl, muskoxen and lemmings. The impact of lemming abundance on arctic fox diet was most pronounced at Karupelv (i.e. where alternative prey is lowest in summer; Table 1), and the dietary composition in high versus low lemming years differed significantly (G = 18.887, df = 4, p = 0.001). Hence, arctic foxes responded to low availability of lemmings by consuming fewer lemmings and more small passerines as compared to years with high lemming abundance (Fig. 3). At Hochstetter Forland, lemmings were less important than at Karupelv. Nonetheless, arctic foxes still responded to changes in lemming abundance, and less waterfowl were consumed in lemming high years as compared to lemming low years. Nonetheless, dietary composition in lemming high and low years did not differ significantly (G = 4.625, df = 4, p = 0.328). At Zackenberg, the contributions of waterfowl and lemmings were similar in lemming high and lemming low years (Fig. 3). Small passerines were consumed more in lemming low years, whereas muskox was recovered only from scats in lemming high years. Remaining prey groups did not differ notably between lemming low and lemming high years at Zackenberg. The significantly different dietary composition between lemming high and low years at Zackenberg (G = 23.838, df = 6, p = 0.001) thus appears mainly attributable to differences in consumption of muskox. The site-specific dietary responses to lemming abundance were also reflected in the dietary niche breadth (Fig. 3). Hence, at Hochstetter Forland arctic fox diets was broader in lemming high years, whereas the diet in lemming low years was dominated by waterfowl. At Karupelv, dietary width was slightly increased in lemming low years, mainly due to a more equal contribution of lemmings, small passerines and waterfowl. At Zackenberg, the dietary width remained stable despite the fluctuations in lemming abundance (Fig. 3).

4. Discussion

The diets of arctic foxes in Northeast Greenland included most terrestrial vertebrate species found in high arctic Greenland. This observation confirms previous findings from other techniques suggesting that the species relies on a broad diet (Angerbjörn et al., 1999; Dalerum and Angerbjörn, 2000; Fay and Stephenson, 1989; Garrott et al., 1983; Gilg et al., 2006; Giroux et al., 2012; Samelius et al., 2007; Tarroux et al., 2012). Yet, arctic fox dietary composition changed markedly during the course of the year. In late winter, the main resources for arctic foxes are either cached food or carcasses when available. Finally, we speculate whether the observed geographical differences in arctic fox diets reflecting the local food availability may have repercussions for arctic fox breeding patterns. Below, we will discuss each finding in turn.
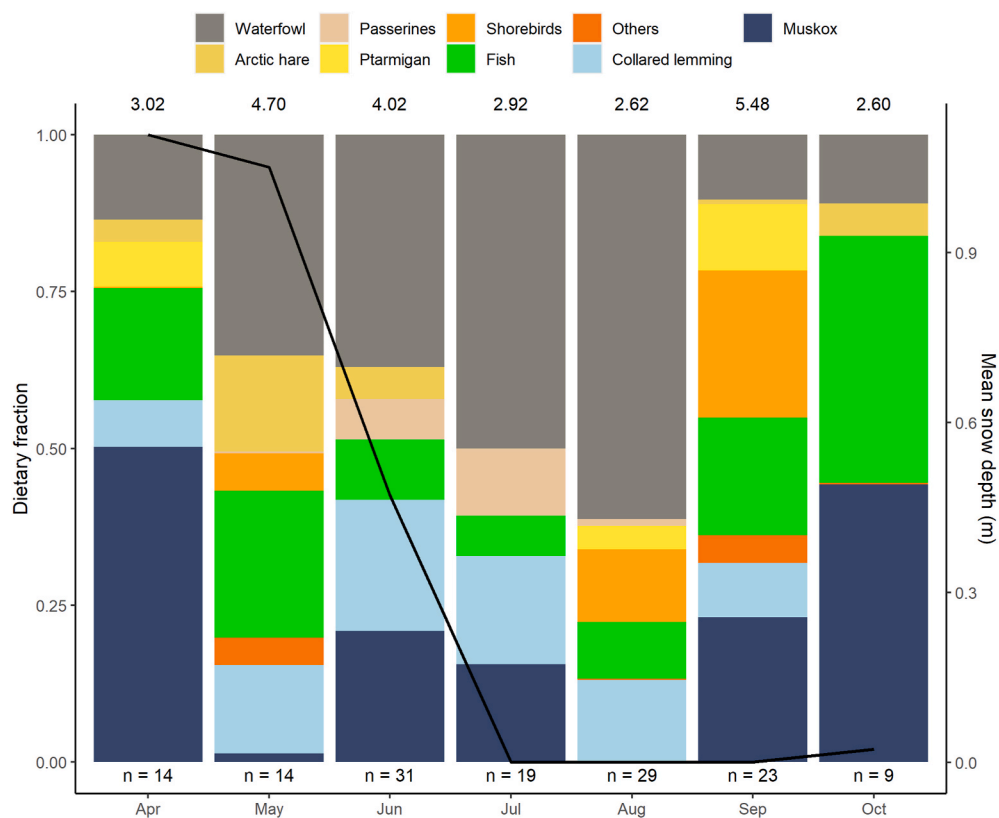
4.1. Seasonal changes in arctic fox diet

In addition to arctic foxes, only few other terrestrial vertebrate species remain in the high arctic year-round. In Northeast Greenland these include muskoxen, Northern collared lemmings, arctic hares, stoat and rock ptarmigans (Schmidt et al., 2012, 2018). When winter comes to an end, migratory birds such as small passerines (mainly snow
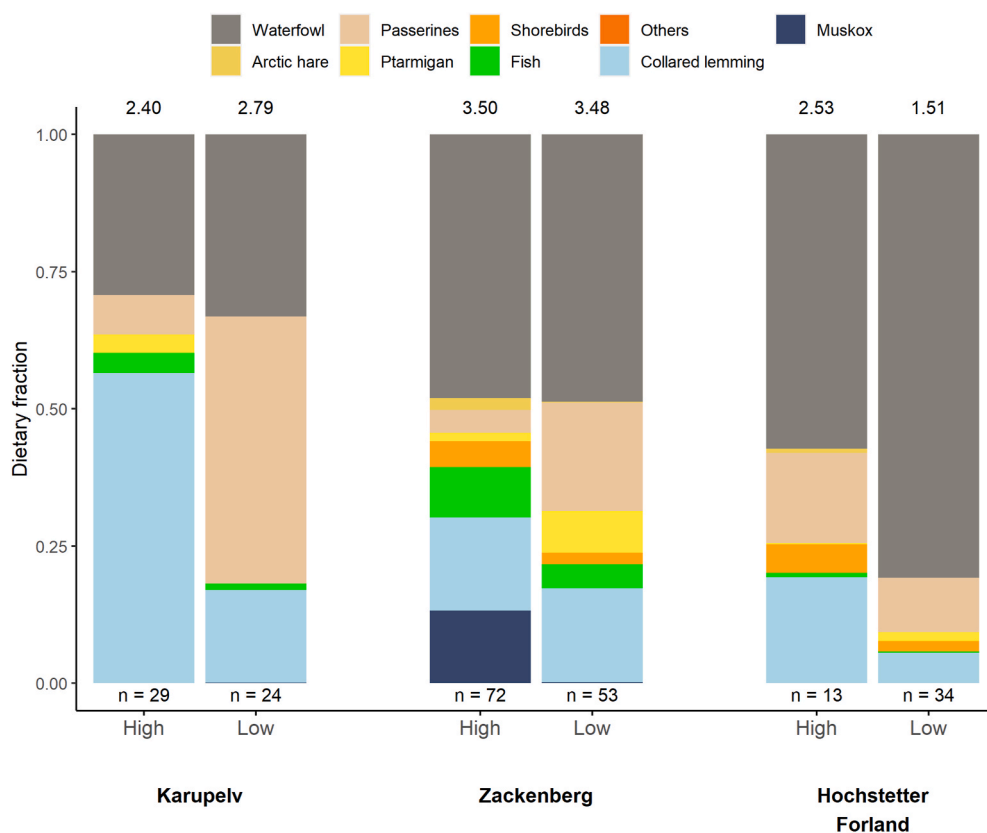
Table 2
Prey taxa detected by molecular analyses of arctic fox scats from Northeast Greenland.

Prey group	Prey taxa detected
Fish	Arctic char (*Salvelinus alpinus*), fish sp.
Waterfowl	Long-tailed duck (*Clangula hyemalis*), ducks (*Anas* sp.), geese (*Anser* sp.), red-throated diver (*Gavia stellata*)
Waders	Ringed plover (*Charadrius hiaticula*), ruddy turnstone (*Arenaria interpres*), sanderling (*Calidris alba*), dunlin (*Calidris alpina*), red knot (*Calidris canutus*)
Ptarmigans	Rock ptarmigan (*Lagopus muta*)
Passerines (small)	Snow bunting (*Plectrophenax nivalis*), redpolls (*Acanthis* sp.)
Muskoxen	Muskox (*Ovibos moschatus*)
Hares	Arctic hare (*Lepus arcticus*)
Lemmings	Northern collared lemming (*Dicrostonyx groenlandicus*)
Others	Long-tailed skua (*Stercorarius longicaudus*), common raven (*Corvus corax*), gulls (*Larus* sp.)

Fig. 2. Temporal dynamics of arctic fox dietary composition from late winter to the end of the snow-free season at Zackenberg as inferred from molecular analyses of fox scats. The black line depicts the development of mean snow depth centrally in the study area during the study period. Numbers above bars indicate the dietary width (Levin's *B*), while numbers below bars indicate sample size. G-test summary statistics are available in Appendix A Table S1. For monthly means and standard errors for each prey taxon, please see Appendix A Table S2.

Fig. 3. Arctic fox diets in summer (June through August) at three sites in Northeast Greenland (2010–2015; inferred from molecular analyses of fox scats) in lemming high and low years. Numbers above bars indicate the dietary width (Levin's *B*), while numbers below bars indicate sample size. For monthly means and standard errors for each prey taxon see Appendix A, Table S3.

buntings), multiple species of waders and waterfowl start arriving at their breeding grounds at Zackenberg. After the breeding season, most migratory birds start migrating south in late summer (August). These marked, temporal changes in the prey base of the resident arctic foxes were clearly reflected in their diet, and we found large dietary changes from late winter and into the autumn.

In late winter (April) arctic fox diet at Zackenberg mainly consisted of resident prey, in particular muskox (carcasses), but lemmings, ptarmigans and hares were also consumed. Remains of these prey species are also often found on arctic fox dens in the area in spring. Interestingly, migratory waterfowl and waders constituted a substantial proportion of the diet in late winter; a time of year before most birds have start arriving at their high arctic breeding grounds. Hence, in addition to early-arriving migratory birds, arctic foxes at Zackenberg may rely on prey depots of birds and/or eggs cached the year before. Caching-behaviour by arctic foxes is well-documented in other arctic regions, allowing them to feed on migratory birds even in winter (Fay and Stephenson, 1989) or to rely on cached eggs when collared lemmings are scarce (Samelius et al., 2007).

When the snow starts to melt, lemmings moving from their protected sub-nivean winter habitat to their summer habitat may be easy prey for predators (Schmidt et al., 2002). This is supported by our observation that lemmings formed an increasing part of the diet from late winter until the snow had disappeared. Hereafter their importance declined, likely as a consequence of predation-induced declining abundances as observed previously at Zackenberg (Schmidt et al., 2008) and Karupelv (Gilg, 2002). In part, this likely also reflected the arrival of migratory prey taxa, and in particular waterfowl. After snow melt, such birds became the dominant prey base for arctic foxes at Zackenberg and remained so until their southbound migration in autumn. While actual predation on waterfowl is rarely observed at Zackenberg, Meltofte (2006) reported that predation pressure on for instance goslings appears to be high, testifying to the importance of waterfowl as arctic fox prey.

The lack of waders in arctic fox diets in June and July was surprising, given that arctic fox predation appears to be the main cause of wader breeding failure at Zackenberg (Reneerkens et al., 2016). The perceived low importance of waders at Zackenberg may perhaps be attributable to the consumption of eggs in particular. Early in the incubation period, the egg will contain only limited amounts of DNA, as it contains only few cells and thus few nuclei. As a result, the proportion of eggs in the foxes' diet may be underestimated by DNA-based techniques. The higher contribution of waders to arctic fox diet in other months may then stem from consumption of young (especially in august before they fledge) and adult waders, rich in DNA. Nonetheless, breeding densities of waders in high arctic Greenland are low compared to other arctic sites in for instance arctic Alaska, Canada, and Siberia, which may contribute to the observed low importance of waders for arctic foxes at Zackenberg. At Zackenberg, arctic foxes thus appear important for wader breeding success, whereas waders on their part seem to contribute only little to fox diets.

Towards the end of the snow-free season (September), many taxa still contributed to arctic fox diet, including waders, waterfowl and muskox. Some, predominantly juvenile, migratory birds are still found in the area in early September, but usually only the snow buntings stay in the area until October. Waterfowl and waders found in scats in September and October therefore likely stem from prey cached earlier in the season. Interestingly, fish constituted substantial parts of diets early and late in the season. Zackenberg is located close to the coast, and arctic foxes are often observed foraging along the coast in the ice-free season. In late winter, however, ice still covers the fjords, and fish in the diet at that time likely stem from cached fish. Alternatively, fish in arctic fox diet in late winter may reflect consumption of stomach content from seal carcasses left behind by polar bears on the sea ice. However, as no seal (*Phoca* sp.) DNA was recovered from the arctic fox scats, this seems unlikely. Additionally, the Zackenberg river holds many arctic char (Schmidt et al., 2008), constituting a food source for arctic foxes there.

We also found other vertebrate predators (long-tailed skua, gulls; Table 2) in the scats of arctic foxes. While we do not know whether these were actually preyed or scavenged upon, we do know that intra-guild predation occurs (Gilg et al., 2006), and that at Zackenberg, arctic foxes prey heavily on long-tailed skua nests and young (Schmidt et al., 2008).

4.2. Regional variation in arctic fox diet

The large plasticity in arctic fox diet observed at Zackenberg over the course of the season was also reflected in diets of arctic foxes between the three sites examined. Hence, waterfowl dominated at Hochstetter Forland, lemmings at Karupelv, while arctic foxes at Zackenberg had broader diets consisting mainly of waterfowl and lemmings. However, both the number of dens, and thus arctic fox families, and the number of scats examined from Zackenberg was higher than for Hochstetter Forland and Karupelv, which may have contributed to the broader dietary niche observed. Similarly, air drying of scat samples at Hochstetter Forland and Karupelv may have resulted in lower prey recovery as compared to Zackenberg, where samples were either stored frozen or in ethanol until processing.

The splitting of samples into lemming high versus lemming low years also allowed us to assess the importance of the abundance of lemming prey at the three sites. As expected, the response to lemming density was most pronounced at Karupelv, where the lemming population fluctuates the most and where the availability of alternative prey is low. When lemmings were scarce there, arctic foxes mainly switched to small passerines. At Hochstetter Forland, the lemming fluctuations are less pronounced and at the same time pink-footed geese (*Anser brachyrhynchus*; both breeding birds and moulting flocks) constitute a large, rather stable alternative prey base for the arctic foxes in summer. Compared to Karupelv, fox diet at Hochstetter Forland included a smaller fraction of lemmings, but the dietary response of arctic fox to fluctuations in lemming densities were still similar: in lemming low years, less lemmings and more waterfowl were consumed than in lemming high years (ca. 3-fold difference in lemming reads between high and low years; Table S2). Nonetheless, the dietary composition was not significantly different between lemming high and lemming low years, possibly attributable to the limited number of scat samples from lemming high years. At Zackenberg, where the diet of the arctic fox was broadest, a shift towards more small passerines being consumed in lemming low years was the most pronounced dietary change in the significant dietary response to changes in lemming density. The substantial consumption of muskox during high lemming abundance at Zackenberg likely reflects a high availability of muskox carcasses (Schmidt et al., 2015) in the particular year included as the lemming high year, and the significant change in arctic fox dietary composition found between lemming high and low years should thus be taken with some caution. Still, these site-specific dietary responses to changes in lemming abundance stress that, when available, lemmings are important components of arctic fox diets in high arctic Greenland. However, they also show that the strength of the trophic link between arctic foxes and lemmings differs between sites.

4.3. Lemmings, alternative food sources and arctic fox breeding

The observed site-specific dietary compositions and responses to lemming density, combined with the site-specific availability and stability of alternative food source, point to marked geographical differences in the importance of lemmings as food source for arctic foxes. We are tempted to speculate that these differences may explain the marked differences in arctic fox breeding patterns previously observed for two of the sites (Schmidt et al., 2012). At Karupelv where lemmings constitute the main prey base for arctic foxes, the production of fox cubs is closely linked to lemming density, and thus highly variable between years (Gilg et al., 2006; Schmidt et al., 2012). At Zackenberg on the other hand,

arctic foxes have broader diets and are less dependent on lemmings. As a likely result, cub production at Zackenberg is not so tightly linked to lemming density as at Karupelv (Schmidt et al., 2012). Also, the density of breeding foxes is generally higher and more stable at Zackenberg as compared to Karupelv (Schmidt et al., 2012). Our knowledge about the arctic fox population at Hochstetter Forland is more limited, but our surveys this far suggest that breeding densities there are low but cub production rather stable and high (O. Gilg, unpubl. data). The latter is likely due to the easy access to waterfowl in summer, as also observed under similar prey conditions in other parts of the Arctic (Giroux et al., 2012; Pokrovsky et al., 2015).

While the differences in arctic fox cub production appear linked to availabilities of both lemming and non-lemming prey during the snow-free season at the three sites, the differences in arctic fox breeding densities are likely to be found in site-specific differences in the availability of winter food. As observed in this study, arctic foxes may rely on muskox carcasses for their overwinter survival, supplemented with cached prey. The three sites examined here differ markedly with respect to availability of muskox carcasses and thus of winter food. This may be an important parameter determining the overwinter survival of resident adult arctic foxes (Gilg et al., 2009), and thus a major determinant of the geographical differences in arctic fox breeding densities. The situation in Northeast Greenland appears to be somewhat different from the situation in Svalbard, where it is the predictable access to migratory geese in summer and reindeer (*Rangifer tarandus platyrhynchus*) carcasses in winter that determines arctic fox abundance and population dynamics (Eide et al., 2012). Given the generally high density of muskoxen at Zackenberg, new muskox carcasses appear in the valley every year (Schmidt et al., 2015). Here, muskox carcasses may constitute a relatively stable, albeit not necessarily spatially predictable, food source in winter. Judging from the differences in breeding densities of arctic foxes among the three sites examined here, such carcasses may also provide a better resource than cached waterfowl or lemmings for winter survival.

5. Methodological considerations

While faecal DNA approaches have been identified as an underutilised tool for carnivore diet analysis (Monterroso et al., 2019), the current study is, to our knowledge, the first one to apply molecular tools to directly quantify the diet of the arctic fox (but see Dudenhoeffer et al., 2021). Here, a few methodological caveats may be in place. It is well established that every method used for analysing scats comes with biases (Gosselin et al., 2017; Klare et al., 2011), and the molecular approach is no exception (e.g. Alberdi et al., 2019). Scat analyses are explicitly aimed at reconstructing the original diet contents *after* the carnivore has done its best to digest the prey. This results in uncertainties intrinsic to the process of reverse inference from what is left of food items passing through a complex digestive system. Though our approach resolves a wealth of dietary items from fox scats collected in high arctic Greenland, the dietary fractions presented are still associated with substantial uncertainties. Thus, while we were able to identify prey taxa in substantial detail using existing reference libraries, any inference regarding their relative importance in the diet of arctic foxes hinges on a link between the sequence reads in the scat and the quantitative composition of the original diet. Though normalized read numbers have been shown to provide good proxies of actual consumption (Deagle et al., 2019), the correlation is of course less than perfect. Hence, while we are confident that we have mapped out the various prey taxa adequately, their actual dietary contributions may be biased. Studies specifically examining the link between arctic fox consumption (and in particular eggs) and DNA reads recovered from scats are needed, and so are studies comparing various methods to estimate arctic fox diets, thereby uncovering the potential biases attached to each methodology. As the next step, we suggest well-controlled feeding trials using arctic fox individuals bred at fur farms or zoological gardens, and analyses of resulting scats subject to different environmental conditions. Such additional data may refine our quantification of links between diet consumed and scat contents observed. At the same time, we stress that they are unlikely to upset the ecological inference here reported. Our conclusions are built on relative differences, not absolute values, and we see no reason why comparisons between e.g. regions or years of different lemming densities should be biased.

6. Conclusions

Though lemming prey constituted an important component of arctic fox diets in high arctic Greenland, their dietary compositions also reflected the dynamic changes in prey availability from late winter to autumn. Across sites, local variation in availability of lemmings and alternative prey resulted in geographical differences in arctic fox diets, and to different dietary responses to changes in lemming density depending on the availability of alternative prey. With the environmental changes already observed in the Arctic (Box et al., 2019), the living conditions faced by arctic animals are changing. Extreme climatic events may add to this variation, by impacting the entire ecosystem simultaneously (Schmidt et al., 2019). However, the outcome of environmental change hinges on the structure of the trophic system (Schmidt et al., 2017). Our findings suggest that the availability of both key prey (lemming) and alternative prey in summer interact with the availability of winter food (cached or carcasses) to determine not only current patterns in arctic fox breeding but also how arctic fox populations will respond to altered environmental conditions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.polar.2022.100838.

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