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ORIGINAL ARTICLE

Predator–prey interactions in the Arctic: DNA metabarcoding reveals that nestling diet of snow buntings reflects arthropod seasonality

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Abstract

Tundra arthropods are of considerable ecological importance as a seasonal food source for many arctic-breeding birds. Dietary composition and food preferences are rarely known, complicating assessments of ecological interactions in a changing environment. In our field study, we investigated the nestling diet of snow buntings (*Plectrophenax nivalis* (L., 1758)) breeding in Svalbard. We collected fecal samples from 8-day-old nestlings and assessed dietary composition by DNA metabarcoding. Simultaneously, the availability of potential prey arthropods was measured by pitfall trapping. Molecular analyses of nestling feces identified 31 arthropod taxa in the diet, whose proportions changed throughout the brood-rearing period. Changes in nestling diet matched varying abundances and emergence patterns of the tundra arthropod community. Snow buntings provisioned their offspring mainly with Diptera (true flies) based on both presence/absence and relative read abundance of diet items. At the beginning of the season in June, Chironomidae (nonbiting midges) and the scathophagid fly *Scathophaga furcata* (Say, 1823) dominated the diet, whereas the muscid fly *Spilogona dorsata* (Zetterstedt, 1845) dominated the diet later in July. When accounted for availability, muscid flies were selected positively among the most often provisioned food taxa. Our study demonstrates the ecological role of the snow bunting as a generalist arthropod predator and highlights DNA metabarcoding as a noninvasive technique for diet analyses with high taxonomical precision if sufficient DNA-sequence libraries are available.

KEYWORDS
Arctic food web, diet analysis, insectivore, Passeriformes, pitfall trap, scatology, Spitsbergen, tundra ecology

1 | INTRODUCTION

Species in seasonal environments have evolved critical periods of growth, reproduction, energy storage, and migration to exploit seasonal pulses in resource availability (Varpe, 2017). Climate warming impacts the seasonal timing of key ecosystem processes, such as the onset of spring (Parmesan, 2006; Schwartz et al., 2006) and the phenological traits of animals (Radchuk et al., 2019). When phenological changes occur at different rates among trophic levels, there can be increasing phenological asynchrony and mismatches between resource availability and demand (Durant et al., 2007; Samplonius et al., 2021; Visser & Both, 2005). In migratory birds, the timing of migration and breeding can be constrained, so that adjustments of annual routines to match phenological shifts of resource availability are not possible or will not evolve rapidly enough (Simmonds et al., 2020; Visser et al., 2012).

The rapid warming of the Arctic has led to phenology changes in terrestrial, freshwater, and marine ecosystems (Post, 2017; Wrona et al., 2016). In tundra ecosystems, increasing temperatures can advance the emergence and flight times of arthropods, which are often the main food source for terrestrial bird species (Gilg et al., 2012; Høye & Forchhammer, 2008; Tulp & Schekkerman, 2008). Changes in arthropod phenology could lead to trophic asynchrony with the birds' food demand, but taxonomically detailed diets are not available for many arctic insectivorous birds (Gillespie et al., 2020; Schmidt et al., 2017; Shaftel et al., 2021). Studies assessing potential mismatches between insectivorous birds and their prey have often measured the availability of arthropods without assessing the actual diet of the surveyed species itself (Kwon et al., 2019; Leung et al., 2018; McKinnon et al., 2012; Saalfeld et al., 2019; Zhemchuzhnikov et al., 2021).

Diet studies on birds have traditionally used invasive or lethal methods to obtain crop or stomach samples, which have the disadvantage that the bird must be sacrificed. Crop samples can be collected with noninvasive procedures but usually involve the use of neck collars or emetics which can be stressful procedures (Carlisle & Holberton, 2006; Moreby & Stoate, 2000). Morphological identification of prey remains in fecal samples reduces the handling stress of the animals, but differential digestibility among prey species can lead to the overrepresentation of hard-bodied taxa in classical identification methods (Moreby & Stoate, 2000). Molecular identification of prey DNA in fecal samples with DNA metabarcoding improves morphological techniques because it allows the detection of otherwise unidentifiable soft-bodied prey but requires libraries of reference DNA sequences (Ando et al., 2020; Yoccoz, 2012). Comprehensive libraries of reference DNA sequences for arctic arthropods have been created in recent years and are now readily available in the Barcode of Life Data System (BOLD, Ratnasingham & Hebert, 2007) for diet studies (Stur & Ekrem, 2020; Wirta et al., 2016).

Snow buntings (*Plectrophenax nivalis* (L., 1758)) are long-distance migrants that spend the summer breeding season in the Arctic where they feed on a seasonal pulse of food resources. The species is a cavity breeder that nests in protected locations. During development, the altricial young require an energy-rich diet for rapid growth and early development of thermoregulation (Lyon & Montgomerie, 1987). The diet of chicks is almost entirely based on arthropods, but they switch to a diet consisting largely of seeds and other plant material as adults. In the high arctic archipelago of Svalbard, snow buntings are potentially experiencing a phenological mismatch between the seasonal availability of arthropods and the dietary needs of the offspring (Fossøy et al., 2015), but the diet composition of the nestlings remains unknown (Espmark, 2016).

The diet of nestlings has been described for populations of snow buntings in Arctic Canada (Hussell, 1972), eastern Greenland (Asbirk & Franzmann, 1978), and southern Norway (Hågvar et al., 2009). Although the three studies have revealed considerable differences in prey composition, lepidopteran (butterfly and moth) larvae and flies in the family Tipulidae have been important food items at all three sites. Svalbard is an archipelago with considerably fewer resident insect and spider species than mainland sites in the Arctic (Vernon et al., 1998); Tipulids are completely absent and lepidopterans are scarce (Coulson et al., 2014). It is therefore unclear which prey taxa are important in Svalbard and if lower prey species diversity could increase the snow buntings' vulnerability toward a phenological mismatch (Miller-Rushing et al., 2010).

Our study objectives were to determine the key food resources and seasonal changes in the nestling diet of a population of Svalbard snow buntings using DNA metabarcoding of fecal samples. Furthermore, we used pitfall trapping of tundra arthropods to assess prey availability for snow buntings and to evaluate the potential selection and avoidance of different species. We predicted that arthropod availability would change over the breeding season with an early peak abundance of Araneae (spiders) followed by abundance peaks of Diptera (true flies) and finally parasitoid wasps in the order Hymenoptera, based on seasonal patterns at other arctic sites (Bolduc et al., 2013; Høye & Forchhammer, 2008; MacLean & Pitelka, 1971). If parents used food resources in proportion to their availability, we expected to observe concurrent changes in the snow bunting nestling diet over time. For specific prey taxa in the diet, we expected frequent detections of Araneae and flies in the family Chironomidae because those taxa were frequently found in other diet analyses (Asbirk & Franzmann, 1978; Hussell, 1972) and are widespread in Svalbard (Coulson et al., 2003; Dahl et al., 2018; Gillespie & Cooper, 2021). Last, assuming that factors such as detection rate and prey handling time are similar among the local taxa, we expected that snow buntings would select prey taxa with high digestibility, nutritional value, and/or large biomass as preferred food for developing nestlings (Razeng & Watson, 2014; Schwagmeyer & Mock, 2008).

2 | MATERIALS AND METHODS

2.1 | Study area and species

Our field site was located in Adventdalen adjacent to Longyearbyen (15.38° E, 78.13° N; Figure 1a) on central Spitsbergen, the largest island of the high arctic archipelago of Svalbard. Adventdalen is characterized by moss-rich mire and marsh plant communities on the valley floor and snowbed vegetation dominated by heaths along the slopes. In the 30-year period of 1986–2015, the mean summer air

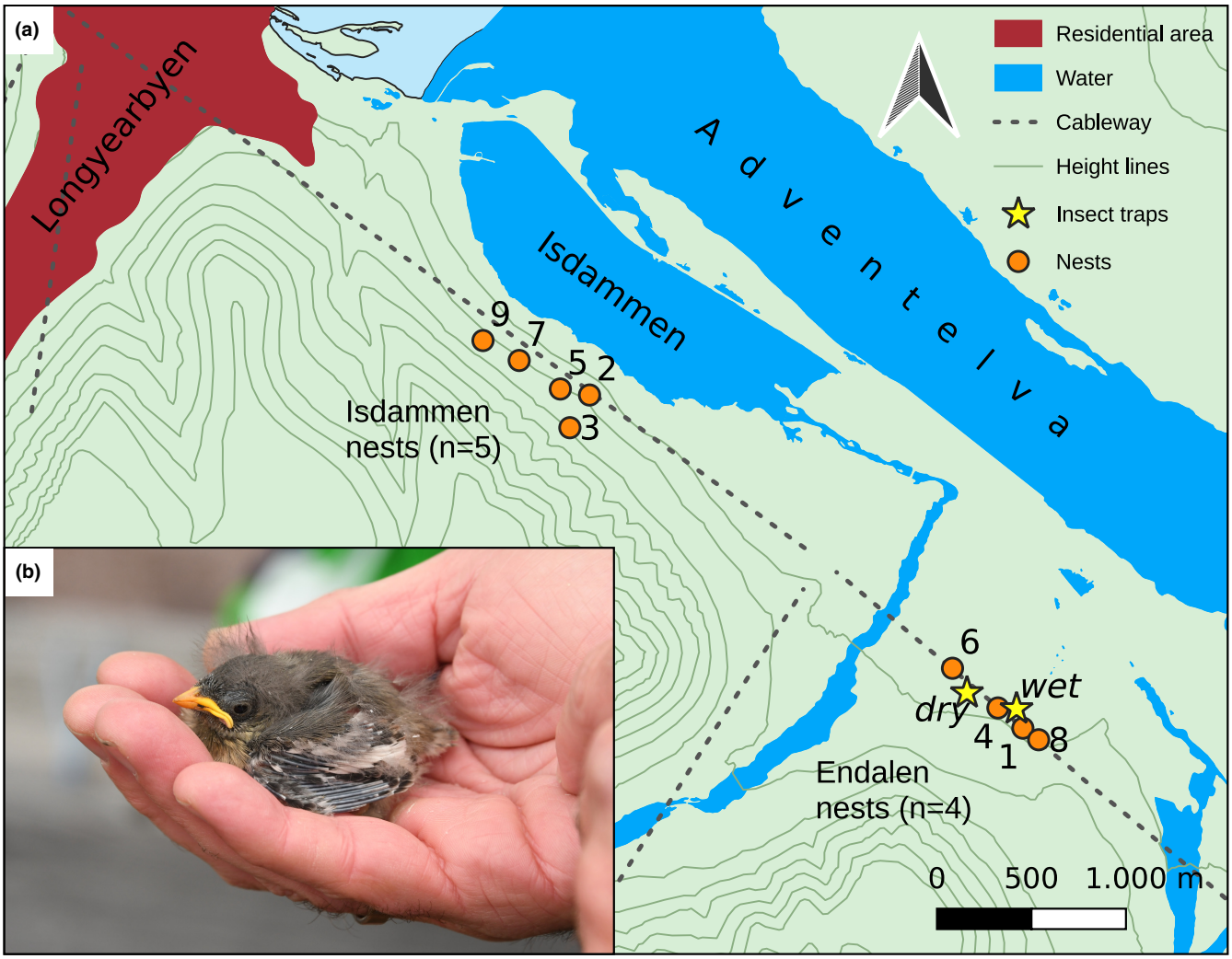

FIGURE 1 (a) Map of the study site in Adventdalen, Spitsbergen, Svalbard. Orange circles with numbers mark the location of snow bunting nests ($n = 9$) from which fecal samples were collected. Yellow stars indicate the two sites of insect trapping. The map was constructed in QGIS 3.26 (QGIS Development Team, 2022) using base data from the Norwegian Polar Institute (2014). (b) Snow bunting chick on day 8 after hatching; photograph by the authors.

temperature from June to August was 5.2°C and the average summer precipitation was 49 mm, measured at the weather station at the airport of Longyearbyen (Isaksen et al., 2017).

Snow buntings in the study area arrive in early April and breed in natural and man-made cavities such as nest boxes. Egg-laying usually commences from mid-May to late June with an average clutch size of 5.8 eggs and incubation by the female for 12–13 days (Espmark, 2016). Both parents provision the nestlings during the ca. 14-day nestling period and also postfledging (Hoset et al., 2004). The snow buntings in Adventdalen begin their migration in September heading toward their wintering grounds in the steppe region of Central Asia and western Siberia (Snell et al., 2018).

2.2 | Arthropod sampling and identification

We sampled arthropods every 4 days from June 4 to August 5, 2018 via pitfall trapping (Appendix Pitfall trap setup). The collected invertebrates were identified to family for insects and to order for other arthropods. Collembola, Acari, and dipteran larvae are difficult or impossible to identify and were, likely due to small size and a mostly subsurface or aquatic lifestyle, never (Collembola) or rarely (Acari, dipteran larvae) provisioned by snow buntings elsewhere (Asbirk & Franzmann, 1978; Hågvar et al., 2009; Hussell, 1972). We, therefore, excluded Collembola and Acari from the pitfall samples and dipteran larval stages from our analyses with higher taxonomic resolution. We considered the arthropod abundances of samples taken concurrently with the snow bunting feces samples (max. 1 day earlier/later, Table A1), as *available prey* in further analyses.

2.3 | Nestling feces collection

Fecal samples from snow bunting nestlings were collected during the breeding season from June 14 to July 29, 2018 at two locations in Adventdalen (maximum ca. 600 m between nests of each location, ca. 2 km between locations, Figure 1a). The Isdammen location featured a dry habitat dominated by *Salix polaris* Wahlenb., *Dryas*

octopetala L., and *Cassiope tetragona* D.Don and the snow bunting nests ($n = 5$; 19 nestlings) were placed in natural cavities within stone piles or stream banks. The nests ($n = 4$; 18 nestlings) at the Endalen location were surrounded by a wet graminoid-dominated habitat or by a *C. tetragona*-tundra (nest no. 6) and located in nest boxes. Fecal sample collection took place on day 8 after the hatching of the oldest chick of each brood, when the nestlings were ringed (Figure 1b). Samples were immediately preserved in 1.5 mm absolute ethanol. We collected 12 samples from four broods at Endalen and 10 samples from five broods at Isdammen (Table A1). All work was conducted under the necessary permits for scientific research, sampling of invertebrates and live capture of wild birds, from the Governor of Svalbard (ref. 16/00757-10) and the Norwegian Environment Agency (ref. 2018/272-ART-VI-ARES).

2.4 | DNA extraction, amplification, and sequencing

Before extracting DNA from each individual sample, we subsampled ca. 500 mg of feces-ethanol mixture per sample. We removed the preservative ethanol by evaporation before extracting DNA using a FastDNA Spin Kit for Soil and following the manufacturer's protocol (MP Biomedicals, 2016) with one modification: we included a second washing step with the SEWS-M Wash Solution, accounting for the high inhibitor content of fecal matter. We used the primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale et al., 2011), which target a 157 bp sequence in the cytochrome *c* oxidase subunit I (COI) gene. The primers were attached to 5′-adapter sequences complying with the 16S Metagenomic Sequencing Library Preparation protocol (Illumina, 2013) used downstream. The initial PCR was conducted in 25 μL volumes with 2.5 μL (2–12 ng μL^{-1}) sample DNA. Our Amplicon PCR consisted of an initial 3 min step at 94°C, 40 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, followed by a final phase of 10 min at 72°C. Both negative (distilled water) and positive (insect mock community) PCR controls were included in the analyses. Amplicons were purified and normalized by adding 20 μL PCR product to a SequalPrep Normalization Plate Kit (Invitrogen, 2008). A second PCR was used for adding Nextera XT indices using an initial step of 95°C for 3 min, followed by 8 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and ending with a final elongation step of 72°C for 5 min. Following a second normalization using the Invitrogen (2008) kit, we pooled all samples and performed single-end 1 × 300 bp sequencing on an Illumina NextSeq 500 System at the NTNU Genomics Core Facility, Trondheim.

2.5 | Sequence analysis and assignment of reads to arthropod taxa

We uploaded demultiplexed FASTQ files to the online Multiplex Barcoding Research and Visualization Environment (mBRAVE, Ratnasingham, 2019), which is linked to the BOLD database. From the database, we chose three different reference libraries: Insecta (including the expected main food taxa), Non-Insect Arthropoda (including Araneae), and Non-Arthropoda Invertebrates (including the closest related taxa to the two aforementioned) all last updated November 8, 2020. The libraries included reference materials of all invertebrate families caught in our pitfall traps. The mBRAVE workflow started by trimming the sequences (parameters: 30 bp front, 109 bp end, 200 bp length) to remove the primers. In the next step, the sequences were quality filtered by removing sequences that had a mean quality value (QV) of bases <10, a length of <100 bp, a maximum 4% of bases with a QV <20, or maximum 1% of bases with a QV <10. Finally, mBRAVE dereplicated and chimera-screened the sequences and compared them with clusters of equivalent sequences in the BOLD reference libraries. In BOLD, sequence clusters are represented by Barcode Index Numbers (BINs) that have Linnaean taxa or interim names assigned (Ratnasingham & Hebert, 2013). We used a 2% ID distance threshold without preclustering and proceeded with additional quality processing downstream.

Due to unequal read depth among samples, we kept only BINs that amounted to over 0.05% of total sample reads and removed one sample with low read depth from further analysis (Table A1). Filtering at 0.05% removed the most abundant species not known to occur in Svalbard, *Spilogona dispar* (Fallén, 1823), which accounted for a maximum of 0.049% of total reads in one sample. BINs associated with multiple Linnaean species names were transferred into single species records based on being local to Svalbard according to the geographic information in BOLD. We converted matches of the nonlocal species *Exechia similis* Lastovka & Matile, 1974 into the local species *Exechia frigida* (Boheman, 1865) as all matched sequences also had >98% similarity with the local species BIN as verified by single sequence comparisons with the online BOLD Identification System on January 15, 2021. We used higher-level taxonomy for BINs without association with local Svalbard species and retained BIN information for Linnaean species with multiple BIN matches.

We used two semi-quantitative metrics to report the molecular diet analysis, weighted percentage of occurrence (wPOO) and relative read abundance (RRA). The first metric wPOO is calculated based on the presence/absence of prey taxa and is considered appropriate for insectivorous diets but can lead to overrepresentation of rare taxa (Deagle et al., 2019). In contrast RRA, a measure based on the number of taxa sequence reads, is more robust toward rare taxa but prone to recovery biases (Ando et al., 2020; Deagle et al., 2019). For wPOO, we first rarefied the number of reads in the samples to the lowest total reads among samples with the R-function *rrarefy* of the *vegan* package (Oksanen et al., 2022) and then recorded the presence or absence of each taxa per sample. For summary statistics and compositional analysis, we further calculated the frequency of taxa occurrences per brood based on the presence of taxa in each individual sample. By operating on brood level rather than sample level, we controlled for a potential lack of independence among samples from nestlings of the same brood. The frequency of taxa occurrences was converted into weighted percentages based on the total number of identified

taxa in each brood and subsequentially a mean wPOO for each taxon across all broods was calculated. We computed RRA by converting the number of sequence reads for each taxon of a sample into percentages of the total sample reads. The taxon percentages were averaged for the samples of each brood and a mean RRA was calculated across all broods. The frequency of prey taxon occurrence and the average prey taxon RRA, both at brood level, were regarded as *utilized prey* for the compositional analysis.

2.6 | Statistical analysis

Statistical analyses were conducted in R version 4.2.3 (R Core Team, 2023). We created coverage-based rarefaction and extrapolation curves, and sample completeness curves for BINs and arthropod families in diet and pitfall samples with the *iNEXT* function of the *iNEXT* package to visualize our sampling process (Hsieh et al., 2022). As a visualization of the multivariate data, non-metrical multidimensional scaling (nMDS) was performed on the Jaccard distances of the presence/absence data set and the Cao distances of the rarefied RRA data set by the function *metaMDS* of the *vegan* package (Oksanen et al., 2022) with 999 tries and no autotransform. We tested whether nest location, Isdammen ($n = 8$) versus Endalen ($n = 10$), or sampling month, June ($n = 11$) versus July ($n = 7$) affected diet composition of snow buntings with nested permutational multivariate analysis of variance (NPERMANOVA, Anderson, 2017). Statistical tests were performed on the Jaccard distances of the presence/absence data set and the Cao distances of the rarefied RRA data set (both data sets with 18 samples, 33 taxa, and brood as nested factor) by the *nested.npmanova* function (parameter: permutations = 999) of the *BiodiversityR* package (Kindt & Coe, 2005). In addition, we checked the homogeneity of within-group dispersions with the help of the function *betadisper* (parameter: type = "centroid") of the *vegan* package (Oksanen et al., 2022).

To test for possible selection for certain food taxa by the snow buntings, we compared the proportions of arthropod families in *available prey* and *utilized prey* on each sampling day by performing a compositional analysis (Aebischer et al., 1993) following the methodology of Soininen et al. (2013). As zeros have to be replaced in compositional analyses, we substituted them with a number of three orders of magnitude smaller than the smallest original value before calculating the proportions of *available prey* families and *utilized prey* families for the respective sampling days. The arthropod families Aphididae, Calliphoridae, and Heleomyzidae amounted together to less than 0.4% in the *available prey* data set and were therefore omitted from the analyses as they were also not found in the *utilized prey*. The taxa proportions were centered and ln-ratio transformed via the *clr* function in the *compositions* package (van den Boogaart et al., 2022). We calculated a selectivity index by subtracting the ln-ratio-transformed *available prey* proportions from the temporally corresponding ln-ratio-transformed *utilized prey* proportions. Last we compared the selection for specific prey taxa by pairwise significance testing with the *compana* function (parameter: test = "randomization," nrep = 999) of the *adehabitatHS* package (Calenge, 2006).

3 | RESULTS

3.1 | Pitfall trap arthropod composition and phenology

The peak of arthropod abundance collected via pitfall trapping in the dry and wet habitats was reached in mid-July (Figure 2a). On the days of sample collection for feces of nestling snow buntings, dipteran flies of the families Chironomidae and Muscidae, followed by Araneae were the most trapped taxa (Table 1), but individual capture numbers varied throughout the season (Figure 2a). Araneae were captured with maximum counts early in the trapping period, whereas Chironomidae numbers peaked in the second half of June. Scathophagidae (dung flies) were trapped mainly in late June and early July. Muscid flies rarely occurred before July but dominated in the second half of the season. Hymenoptera, mainly represented by parasitoid wasps, were captured from early July until the end of the trapping period.

3.2 | Diet composition from fecal analysis

After successful DNA extraction from the fecal samples, Illumina sequencing generated 15.2 million sequences (mean 689,911, SD 538,306) for arthropods consumed by nestling buntings. Of those, the MBrave algorithm matched 8.48 million sequences (mean 385,280, SD 331,119) to 121 BINs (mean 14.0, SD 11.0) in the BOLD database (Table A1). Sequences of the negative control matched five BINs: *Scathophaga furcata* (Say, 1823) (0.003% of sample reads), *Hemineurina abbrevinervis* (Holmgren, 1869) (0.001%), *Corynoneura* sp. 15ES (0.001%), *Metriocnemus* sp. 8ES (<0.001%), and *Gattyana cirrhosa* (Pallas, 1766) (<0.001%).

After final quality filtering, 8.47 million sequences (mean 470,336, SD 304,072) representing 33 (mean 4.8, SD 4.5) unique BINs were retained in 18 samples (Table A1). The species accumulation curves of identified taxa had a sample coverage of 86% at the BIN level and 91% at the family level for the diet sampling, while the pitfall sampling had a sample coverage of 100% (Figure A2). The identified BINs comprised 11 arthropod families and 31 Linnaean species (Table 2). Dipteran flies in the families Muscidae, Scathophagidae, and Chironomidae were the most represented prey items in the feces, together accounting for 74% (wPOO) and 89% (RRA) of all detected families (Table 1). The most taxa-rich family was Chironomidae with 17 identified species, while the muscid fly *Spilogona dorsata* (Zetterstedt, 1845) and the scathophagid fly *Sc. furcata* had the highest diet percentages (Table 2). The diet composition changed over the season (Figures 2b, 3): Of the three most detected families, scathophagids were detected

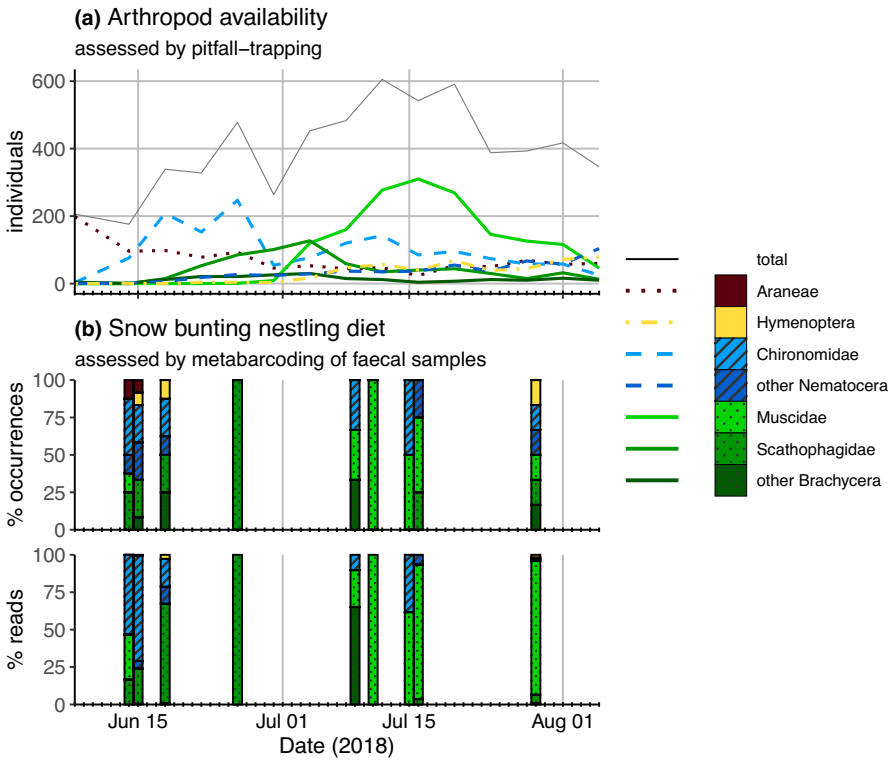

FIGURE 2 (a) Arthropod availability on the tundra in Endalen, Svalbard, in 2018 as measured by pitfall trapping in a dry *Cassiope tetragona* dominated and a wet graminoid-dominated habitat. (b) Brood-specific nestling diet of Svalbard snow buntings in Adventdalen during the breeding season 2018 as assessed by DNA metabarcoding of fecal samples. Each bar denotes the diet of one snow bunting brood by the percentage of brood-level occurrences and mean brood-level relative read abundance.

mainly in June (43.8% wPOO, 51.7% RRA) and rarely in July (8.3% wPOO, 1.9% RRA), whereas muscids occurred mainly in July (3.1% wPOO, 7.5% RRA in June, 50.0% wPOO, 73.1% RRA in July). Chironomids peaked during June (21.9% wPOO, 35.6% RRA) but were also detected later (20.0% wPOO, 9.9% RRA). The influence of sampling month was also found by statistical testing of the diet composition based on presence/absence when accounting for the influence of brood (NPERMANOVA, Pseudo-$F_{1,9}=4.17$, $R^2=0.27$, $p=0.018$) but the effect of nest location was not significant (Pseudo-$F_{1,9}=1.10$, $R^2=0.09$, $p=0.48$) (Figure A3). We found the same pattern for the diet composition based on relative sequence reads (sampling month: Pseudo-$F_{1,9}=5.97$, $R^2=0.35$, $p=0.014$, nest location: Pseudo-$F_{1,9}=0.60$, $R^2=0.06$, $p=0.74$) (Figure A3). Betadisper homogeneity tests showed no significant difference in group dispersions for either nest location (frequency: $F_{1,16}=0.22$, $p=0.65$, relative sequence reads: $F_{1,16}=0.58$, $p=0.46$) or sampling month (frequency: $F_{1,16}=2.04$, $p=0.17$, relative sequence reads: $F_{1,16}=0.26$, $p=0.62$).

3.3 | Comparison of available and utilized prey

The compositional analysis detected a positive selection for several rarely trapped taxa but also flies in the family Muscidae (Figures 4, 5). Pairwise comparisons revealed that among the most commonly trapped arthropod families, Muscidae and Scathophagidae were significantly selected over Araneae, Apocrita, and flies in the families Mycetophilidae and Sphaeroceridae, while Chironomidae was significantly less selected than Muscidae based on the frequency of occurrence data set (Tables A2, A3).

4 | DISCUSSION

By combining prey availability measurements with molecular methods for the identification of prey remains, we found that Svalbard snow buntings are generally relying on the most abundant prey taxa at the time of provisioning and opportunistically feeding their nestlings by following the seasonal succession of the arthropod community. Our results also showed that snow buntings provisioned most notably larger-sized flies in the family Muscidae, whose relatively late emergence could contribute to higher breeding success among late nesting pairs of snow buntings in Svalbard.

4.1 | Snow bunting nestling diet and selectivity

Inventories of the terrestrial arctic arthropod fauna are available, but detailed information on the arthropod food composition of higher trophic levels is scarce (Gillespie et al., 2020; Schmidt et al., 2017). Here, we present a snow bunting nestling diet at a taxonomical resolution only studies employing molecular methods can provide (Packer et al., 2009). We were successful in describing the core snow bunting nestling diet, despite a low fecal sample size typical for species that inhabit remote areas such as snow buntings (cf. Asbirk & Franzmann, 1978; Hågvar et al., 2009; Hussell, 1972), because of the relatively low species-richness in Svalbard, but also because of recent efforts to successfully develop an extensive molecular reference database for arctic and Norwegian invertebrates (Ekrem et al., 2015; Stur & Ekrem, 2020; Wirta et al., 2016). Several flies in the family Chironomidae that were identified in this study have recently been found for the first time in Svalbard (Stur & Ekrem, 2020).

TABLE 1 Arthropod prey availability and diet of snow bunting nestlings in Svalbard, 2018.

Class	Order	Family	Trapping %	Diet %	
				wPOO	RRA
Arachnidae	Araneae		15.4	2.3	<0.1
Insecta	Hemiptera	Aphididae	0.2	0	0
	Diptera	Anthomyiidae	0.2	4.2	0.2
		Calliphoridae	0.1	0	0
		Chironomidae	30.5	20.8	21.3
		Culicidae	0	2.8	0.7
		Empididae	0.1	3.7	7.2
		Heleomyzidae	0.1	0	0
		Muscidae	28.5	29.2	43.9
		Mycetophilidae	4.5	1.9	0.1
		Scathophagidae	8.1	24.1	24.0
		Sciaridae	2.3	5.6	1.8
		Sphaeroceridae	1.9	0	0
		Syrphidae	0.1	1.4	<0.1
		indet. dipteran larvae	1.6	NA	NA
	Hymenoptera	Apocritan families[a]	6.2	0	0
		Tenthredinidae	0.1	4.2	0.5

Note: The pitfall trapping percentages are based only on the trapping days corresponding to snow bunting fecal sample collection dates. The diet percentages are calculated based on the mean weighted percentage of brood-level taxon occurrence (wPOO), and mean brood-level relative read abundance (RRA). The life stage of a taxon cannot be identified by DNA metabarcoding (not applicable – NA).

[a]Diapriidae, Figitidae, Ichneumonidae, and Megaspilidae.

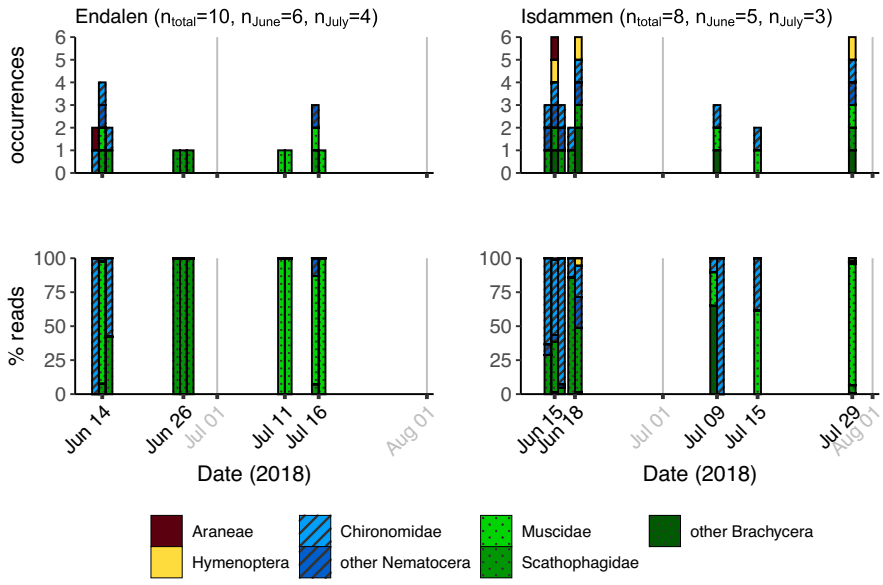

FIGURE 3 Nestling diet of Svalbard snow buntings in two nesting areas in Adventdalen, Spitsbergen, during the breeding season 2018 as assessed by DNA metabarcoding of fecal samples. The upper panels show the respective taxa occurrences per sample, the lower panels show the relative read abundance of prey taxa per sample. Each bar denotes a fecal sample from one 8-day-old chick, the clusters correspond to the chicks of a single brood with the sample collection date given on the *x*-axis.

The seasonal emergence of tundra arthropods followed our predictions based on previously observed patterns in Svalbard and other arctic sites. After peaking in the early season, Araneae trapping numbers declined, which could be due to an actual numerical decline or to less activity (Dahl et al., 2018). Among Diptera, chironomids commonly emerge earlier than muscid flies (e.g. Bolduc et al., 2013; Høye & Forchhammer, 2008) and the last group to appear during the season were parasitoid wasps in the order Hymenoptera, which emerge after the larvae of their host species have hatched. The composition of arthropods in the snow buntings nestlings' diet reflected the observed emergence patterns on the tundra: while chironomids were detected from the beginning, the muscid fly *Sp. dorsata* appeared later in the diet. The similarity between the snow bunting nestling diet and observed arthropod phenology implies that our

TABLE 2 Diet composition of nestling snow buntings as assessed by DNA metabarcoding in Svalbard, 2018.

					Diet %	
Class	**Order**	**Family**	**Species**	**BIN**	**wPOO**	**RRA**
Arachnida	Araneae	Linyphiidae	*Collinsia spetsbergensis* (Thorell, 1871)	BOLD:AAG5690	1.2	<0.1
Insecta	Diptera	Anthomyiidae	*Zaphne frontata* (Zetterstedt, 1838)	BOLD:AAG1723	2.0	0.2
		Chironomidae	*Allocladius* sp. 1ES	BOLD:ABZ1783	1.2	<0.1
			Bryophaenocladius sp.	BOLD:AAG1021	2.2	0.2
			Chironomus sp. 1TE	BOLD:AAC0592	5.1	1.9
			Cricotopus gelidus (Kieffer, 1922)	BOLD:ABX5870	1.0	0.1
			Metriocnemus euryntous (Holmgren, 1883)	BOLD:ACJ5124	0.8	0.2
			Metriocnemus euryntous (Holmgren, 1883)	BOLD:AEE4370	0.4	<0.1
			Metriocnemus fuscipes (Meigen, 1818)	BOLD:AAI1573	0.4	<0.1
			Metriocnemus sp. 1ES	BOLD:AAA9429	3.4	1.4
			Metriocnemus sp. 8ES	BOLD:ACJ4894	1.8	3.7
			Metriocnemus ursinus (Holmgren, 1869)	BOLD:AAA9434	2.2	1.6
			Orthocladiinae sp.	BOLD:ADR2796	0.7	0.8
			Paraphaenocladius brevinervis (Holmgren, 1869)	BOLD:AAE3721	2.2	1.1
			Procladius frigidus (Holmgren, 1869)	BOLD:AAB9256	7.4	3.5
			Psectrocladius limbatellus (Holmgren, 1869)	BOLD:AAD4703	1.0	<0.1
			Smittia brevipennis (Boheman, 1866)	BOLD:AAF4816	0.8	0.2
			Smittia sp. 6ES	BOLD:AAG1015	2.2	6.5
			Smittia sp. 26ES	BOLD:ACA0346	0.7	0.1
			Smittia sp. 28ES	BOLD:AAU6581	1.0	0.1
		Culicidae	*Aedes nigripes* (Zetterstedt, 1838)	BOLD:AAA3750	2.2	0.7
		Empididae	*Rhamphomyia caudata* (Zetterstedt, 1838)	BOLD:ACG1802	3.7	7.2
		Muscidae	*Spilogona dorsata* (Zetterstedt, 1845)	BOLD:AAU5038	24.8	41.7
			Spilogona megastoma (Boheman, 1866)	BOLD:AAP9046	3.2	2.1
			Spilogona megastoma (Boheman, 1866)	BOLD:ACJ5971	1.0	0.2
		Mycetophilidae	*Coelosia tenella* (Zetterstedt, 1852)	BOLD:ACZ5445	1.0	0.1
			Exechia frigida (Boheman, 1865)	BOLD:AAL9140	1.0	0.1
		Scathophagidae	*Scathophaga furcata* (Say, 1823)	BOLD:AAD0853	18.4	24.0
		Sciaridae	*Hemineurina abbrevinervis* (Holmgren, 1869)	BOLD:AAM9260	2.6	1.8
		Syrphidae	*Parasyrphus tarsatus* (Zetterstedt, 1838)	BOLD:AAC1834	0.7	<0.1
	Hymenoptera	Tenthredinidae	*Euura amentorum* (Förster, 1854)	BOLD:ACJ5900	1.0	0.2
			Euura sp.	BOLD:ACD1919	1.0	0.3
			Euura caeruleocarpus (Hartig, 1837)	BOLD:AAG3513	1.7	0.1

Note: The diet proportions are calculated based on the mean weighted percentage of brood-level taxon occurrence (wPOO) and mean brood-level relative read abundance (RRA). Species without a Linnaean name have identifier initials (ES = Elisabeth Stur, TE = Torbjørn Ekrem) as provisional names (cf. Stur & Ekrem, 2020). BIN = Barcode Index Number in the Barcode of Life Database (BOLD, Ratnasingham & Hebert, 2013).

sampling was sufficient and suggests that snow buntings provision mostly adult arthropods as captured by pitfall traps.

We found low Araneae percentages in the snow bunting nestling diet in Adventdalen, which is comparable to the diet descriptions reported from Arctic Canada, where Araneae accounted for 1.5% of all recorded diet items (Hussell, 1972) and southern Norway, where Araneae amounted up to 1.8% of diet biomass in one brood (Hågvar et al., 2009). In Eastern Greenland, Araneae constituted 57% of all diet items, however, 93% of the Araneae were collected from only one brood (Asbirk & Franzmann, 1978). Despite being rarely detected as snow bunting food, Araneae were frequently trapped in our pitfall traps and consequentially scored low in the selectivity analysis. Araneae have a high nutritional value (Arnold et al., 2007; Razeng & Watson, 2014) and the observed avoidance might be influenced by factors of study design: First, the availability of Araneae is probably overestimated by pitfall trapping (see below). Second, Araneae might be subject to a primer bias in the metabarcoding analysis. However the ZBJ-Art primers have been successfully employed to detect linyphiid spiders (Piñol et al., 2014), which comprise 98% of the Araneae found in Svalbard (Dahl et al., 2018). The low

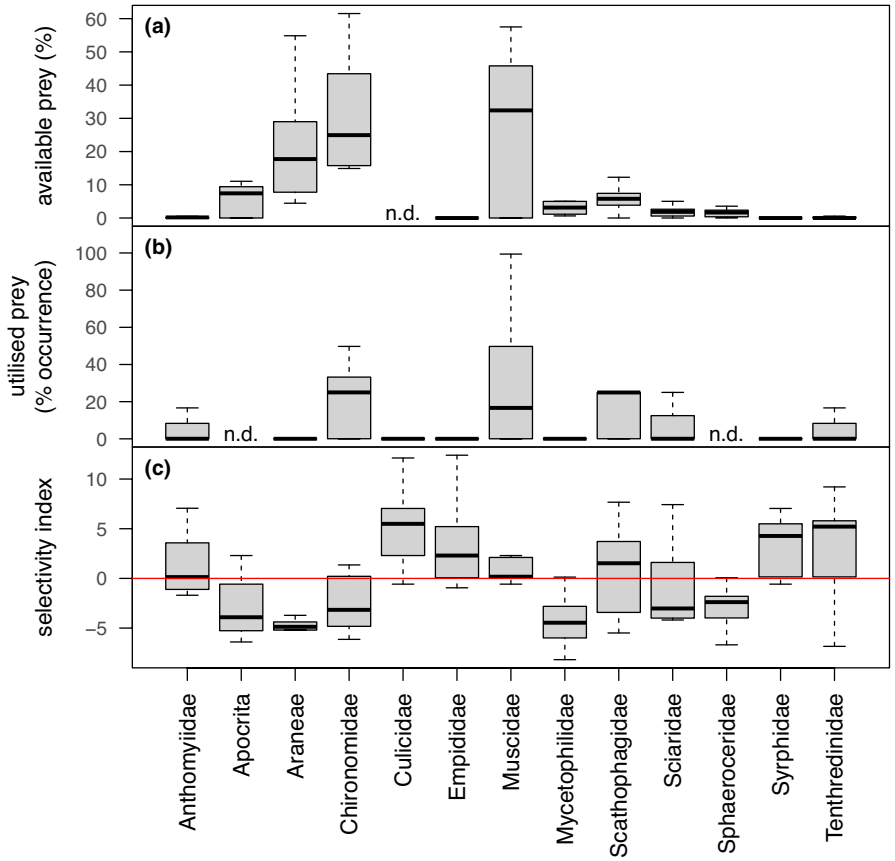

FIGURE 4 Comparison of prey availability, snow bunting diet, and calculated prey selectivity index based on the presence/absence of taxa in the diet. (a) Arthropod taxa percentages in pitfall trap samples taken during the fecal sample collection period (*available prey*). (b) Percentages of brood-level occurrences of molecular identified prey taxa (*utilized prey*). (c) Calculated prey selectivity index. Positive selectivity values indicate selection toward the specific taxon, whereas negative values indicate avoidance. The midlines represent the median, boxes upper and lower quartiles, and whiskers values that lay within 1.5 times the interquartile range. n.d. = not detected.

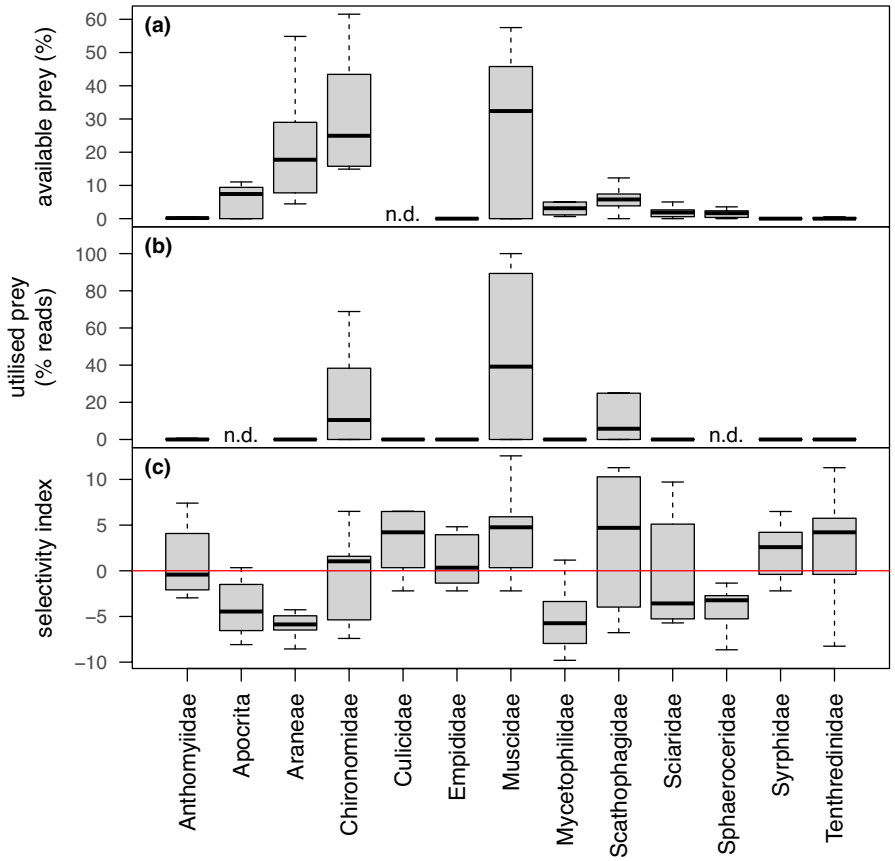

FIGURE 5 Comparison of prey availability, snow bunting diet, and calculated prey selectivity index based on relative read abundance (RRA). (a) Arthropod taxa percentages in pitfall trap samples taken during the fecal sample collection period (*available prey*). (b) Percentages of brood-level relative read abundance of molecular identified prey taxa (*utilized prey*). (c) Calculated prey selectivity index. Positive selectivity values indicate selection toward the specific taxon, whereas negative values indicate avoidance. The midlines represent the median, boxes upper and lower quartiles, and whiskers values that lay within 1.5 times the interquartile range. n.d., not detected.

proportion of Araneae in the diet might also be owing to our sampling of 8-day-old nestlings. The proportion of Araneae provisioned by other insectivorous birds decreased with nestling age (Arnold et al., 2007), a pattern also observed in snow buntings in southern Norway (no Araneae detected in the diet of 6- to 15-day-old chicks, Hågvar et al., 2009) and Araneae might be more often provisioned to younger nestlings than our results indicate.

Nutritional values of arthropods are rarely known at the family level, but larger-sized taxa are expected to have a higher energetic quality due to a lower surface area to volume ratio and therefore lower relative chitin content (Razeng & Watson, 2014). We observed that families with larger body-sized taxa such as Muscidae were selected over smaller biomass taxa such as Sphaeroceridae. The size of the provisioned prey is usually increasing as chicks grow but also the diet composition can change (Wiebe & Slagsvold, 2014). Since all nestlings were sampled at the same age, we cannot address potential age-related diet shifts. In the Scandinavian mountains, Hågvar et al. (2009) report that newly hatched snow buntings chicks were fed with smaller and more easily digestible food items than older chicks (>3 days old). Hence, a selection for smaller arthropod species during the provisioning of similarly young nestlings can also be expected in the Adventdalen population. However, we hypothesize that age-related shifts in prey size are not as noticeable in Svalbard, because the lack of tipulids and large lepidopterans implies that the upper size limit of insects is comparably small.

The scarcity of flies in the family Mycetophilidae in the nestling diet could be due to the small sample size in the later study period. Mycetophilid flies emerge in large numbers in late July (Høye & Forchhammer, 2008; Leung et al., 2018), when snow bunting nestlings have already fledged (Espmark, 2016). Mycetophilidae and other late emerging taxa such as apocritan wasps could therefore be important postfledgling food. Flies in the family Sphaeroceridae were the most frequently trapped Diptera taxa that were missing in the nestling diet; the small size of the Svalbard taxa may fall outside the preferred food size of snow buntings.

We did not capture any Lepidoptera in the pitfall traps, and we detected no lepidopteran DNA in the feces of the nestlings, despite high primer specificity (Zeale et al., 2011). In the only other study using molecular methods to identify snow bunting diet, Wirta et al. (2015) found that both adults and chicks in Greenland feed predominantly on Lepidoptera, presumably larval stages (cf. Asbirk & Franzmann, 1978). Lepidoptera imagines have been successfully caught with pitfall traps in the Arctic (Høye et al., 2014), so their absence in our study is likely due to their scarcity in Svalbard (Coulson et al., 2014; Søli et al., 2018) or insufficient sampling.

Time of season but not nest location explained variation in the diet composition of nestlings. In contrast, arthropod compositions and emergence patterns were influenced by tundra habitat (Stolz, 2019). Since individually marked birds of our study population were observed collecting food as far as ca. 700 m away from the nest (M. I. Wedege, personal observation), we assume that snow buntings have access to a variety of tundra habitats independent of nest location.

4.2 | Pitfalls of pitfall trapping

The arthropod composition was measured with pitfall traps, which only sample a subset of the true tundra community: Pitfall trapping has a bias toward surface-dwelling and active crawling species so that the resulting capture numbers represent a combination of activity and abundance rather than absolute abundance. Pitfall trapping has been widely used to measure arthropod abundance for arctic predators (Gillespie et al., 2020) and chick growth of arctic insectivorous birds was correlated to shifts in arthropod biomass recorded by pitfall traps (Reneerkens et al., 2016). While the availability of certain taxa that are strong fliers or have small body mass might be underevaluated, our diet analysis showed that the most identified snow bunting prey taxa were also numerous in the pitfall traps and followed the same phenological patterns. Thus, we believe that pitfall trapping is an adequate measure of the prey availability for the ground-feeding snow buntings. The only molecular-detected food taxon not caught in pitfall traps was the dipteran family Culicidae, which is likely underrepresented in these types of traps due to taxon-specific behavior patterns and is better caught in Malaise traps. In contrast, Araneae were exceptionally well trapped with pitfall traps (Norment, 1987) and susceptibility to capture may account for the large discrepancy between pitfall trapping numbers and the percentage identified in bunting feces.

4.3 | Considerations on molecular methods

DNA metabarcoding of fecal samples is an improvement over dietary analyses based on morphological identification of prey remains, but it is still a relatively new technique, and we identified possible sources of bias in our results.

We used a single arthropod primer pair (Zeale et al., 2011) that yields good specificity for Diptera and Lepidoptera but less so for Hymenoptera (Alberdi et al., 2018; Elbrecht et al., 2019) and Araneae. Hymenoptera seem generally difficult to detect via the COI gene region (Krehenwinkel et al., 2017; Marquina et al., 2019) and the only Hymenoptera family we detected was Tenthredinidae. Since small numbers of ichneumon wasps were identified in the diet of similar aged nestlings in Arctic Canada (Hussell, 1972), other Hymenoptera taxa might also be provisioned by Svalbard snow buntings, despite their absence in the diet as assessed by metabarcoding. The use of several primer pairs and barcoding genes or setting a different detection threshold may result in a more comprehensive diet analysis (Ando et al., 2020; Verkuil et al., 2022). Notably, plant materials as found in small amounts in the nestling diet by Hågvar et al. (2009) and Hussell (1972) could not have been detected by our method. While differentiating life stages of arthropods by metabarcoding is impossible, larvae of the taxa we recorded have rarely been provisioned by snow buntings elsewhere (Asbirk & Franzmann, 1978; Hågvar et al., 2009; Hussell, 1972) and the seasonal patterns in diet matched the observed phenology of adult emergence.

Occurrence-based molecular counts can lead to an overrepresentation of rare taxa, which for example can occur due to

contamination or tag-jumping (Deagle et al., 2019). Summaries based on relative sequence reads are more robust to inflation of rare taxa but showed similar results in our analyses. Secondary predation, where molecular detections are from amplifications of the prey DNA that resides inside the gut contents of a higher-order predator can lead to similar biases. In our study system, mesopredators such as Araneae are rather small and we therefore only consider small-sized taxa as potentially overestimated due to secondary predation. Our compositional analysis likely gives a broad overview but caution is warranted for taxa that were recorded in low amounts.

4.4 | Ecological interactions and implications

Svalbard has a depauperate fauna of arthropods because it is an island archipelago at high latitude. Accordingly, food availability for nesting snow buntings is quite different from other arctic sites. Due to the scarcity of lepidopterans and lack of tipulids, snow buntings were provisioning nestlings with mainly chironomids and the two calyptrate flies *Sc. furcata* and *Sp. dorsata* in our study. The annual importance of those food taxa could be influenced by potential interannual phenology and abundance variations. Population trends for many terrestrial arthropds in Svalbard are unknown, but *Sp. dorsata* has significantly declined in association with increased summer temperatures between 1996 and 2014 in East Greenland (Loboda et al., 2018). As for the snow bunting, while the Svalbard population is thought to be stable (Stokke et al., 2021), the Fennoscandian population has shown a significant decrease in abundance in 2002–2019, presumably mediated by increased temperatures associated with climate change (Lehikoinen et al., 2019).

Earlier egg-laying of Svalbard snow buntings was correlated with a trend toward increasing spring temperature (Fossøy et al., 2015). In many migratory bird species, early clutch initiation results in higher breeding success (Morrison et al., 2019). For example, arctic waders that are starting egg-laying as early as possible have better chick growth and survival, presumably due to a better match with the peak in food availability (Reneerkens et al., 2016; Saalfeld et al., 2019; Schekkerman et al., 2003). For snow buntings, however, late broods showed higher fledgling success with similar chick survival rates as early broods (Espmark, 2016; Hoset et al., 2009). Snow buntings usually arrive and start nesting earlier than waders and might therefore experience a higher risk of weather-related disruption (Shipley et al., 2020) or increased predation (Reneerkens et al., 2016). In addition, our results indicate that access to adequate food has to be considered because arthropod availability in the early breeding season is low, and the early emerging arthropods such as chironomids are often smaller in body size and thus have potentially less nutritional value than later emerging ones. One driver for snow buntings to nest early is to have time for a second brood during the same breeding season. While re-nesting in case of clutch failure is common, raising another brood after a successful first has only occurred in years with an early onset of egg-laying in Svalbard (Espmark, 2016; Hoset et al., 2009). The provisioning period of a second brood would typically coincide with higher availability of arthropod prey.

Further research on the breeding phenology and reproductive success of Svalbard snow buntings will rely on detailed diet information and knowledge of food availability as presented in this study. Here we have demonstrated that DNA metabarcoding is a promising technique for diet assessments and could be used for a more comprehensive study of ecological variation among years, species, and habitats.

AUTHOR CONTRIBUTIONS

The article is based on the Master's project of CS (Stolz, 2019). CS, FF, BGS, and BKS conceived the study. CS performed the fieldwork with the assistance of FF, BGS, BKS, and ØV. FF supervised the genetic analyses. CS performed taxonomic identification and analyzed and interpreted the data. CS drafted the manuscript under guidance from RAI, ØV, and FF. All authors contributed to revisions and accepted the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study is made openly available in the Dryad repository https://doi.org/10.5061/dryad.rfj6q57gg (Stolz et al., 2023).

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APPENDIX 1

PITFALL TRAP SETUP

We placed 10 pitfall traps in each of two tundra habitats 300 m apart in Adventdalen, Spitsbergen, Svalbard: a dry habitat with mainly *Cassiope tetragona* heaths and a wet marsh habitat with *Sphagnum* spp. mosses and graminoids as vegetation (Figure A1). The traps were made of two white plastic cups (68 mm diameter) stacked together and buried, without a funnel or rain guard attached, with the opening at even level with the ground. We filled the traps almost to the rim with water and added a few drops of detergent as a surfactant (Sun Light, Lilleborg AS, Oslo, Norway). By using white plastic cups with added liquid, the pitfall traps functioned also similar to white pan traps that catch flying arthropods. The traps in each habitat were placed in two parallel lines (5 m apart) consisting of five cups 2 m apart. Invertebrates were collected on the afternoon of every fourth day by sieving the trap contents over a fine cloth (mesh size

ca. 0.5 mm). The recovered invertebrates were stored immediately in vials filled with 70% ethanol. For consistency with the timing of sampling in previous years, there was a gap of 2 days without trapping after the first emptying and the second deployment. In total, we collected 15 arthropod samples.

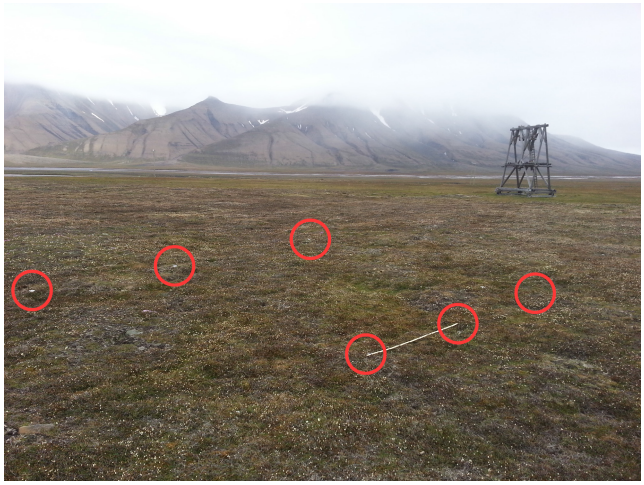

FIGURE A1 Pitfall trap setup in Endalen, Svalbard. The red circles mark buried plastic cups used as pitfall traps on two parallel lines (5 m apart). The measuring stick is 2 m long. In the background stands one of the wooden trestles of the old coal cableway, on which nesting boxes for snow buntings are attached: photograph by the authors.

SAMPLE OVERVIEW/METABARCODING OUTPUT

TABLE A1 Overview of fecal samples and DNA metabarcoding result processing.

Location	Nest number		Sampling date: Nestling feces	Sampling date: Pitfall trap	Initial reads	mBRAVE workflow: Filtered (%)	Dereplicated (%)	Chimeras	Sequences matched	BINs matched	Remaining OTUs	Reads after final filtering	BINs after final filtering
Endalen	1		14.06	14.06	99,767	44.34	98.11	0	55,531	12	13	55,407	3
					1,065,718	36.67	98.77	760	674,920	28	173	663,607	7
		*			898,242	63.64	97.42	46	326,598	12	88	223,498	4
	4		26.06	26.06	162,746	38.46	98.58	0	100,154	3	14	99,417	1
					34,868	99.97	50.00	0	12	2	3	0	0
					605,319	37.28	99.17	0	379,628	4	30	379,073	1
		*			6307	99.94	25.00	0	4	2	1	0	0
		*			342,209	39.02	99.03	0	208,669	4	22	208,298	1
	6		11.07	12.07	1,009,711	35.38	99.30	1	652,518	20	71	647,492	1
					1,723,522	35.43	99.27	0	1,112,796	16	104	1,104,539	1
	8		16.07	16.07	681,269	39.80	98.07	74	410,131	20	95	406,334	4
					1,453,649	36.37	99.27	0	925,008	19	82	919,232	1
Isdammen	2		15.06	14.06	232,068	50.13	97.32	18	115,743	7	27	114,938	6
					1,157,351	47.61	97.74	288	606,294	15	158	590,008	11
					1,237,161	58.57	97.52	714	512,498	22	177	495,370	12
	3		18.06	18.06	473,136	40.77	98.82	10	280,260	8	73	277,880	2
					729,380	48.01	97.36	104	379,169	24	91	377,516	15
	5		09.07	08.07	600,596	46.69	98.61	22	320,179	11	58	302,419	3
					14,679	99.82	73.08	0	26	3	1	0	0
					20,320	99.97	42.86	0	7	3	0	0	0
	7		15.07	16.07	1,200,842	40.02	98.72	28	720,292	32	80	715,497	3
	9		29.07	28.07	1,429,182	37.05	98.47	142	899,628	42	173	885,520	11
Neg. control (distilled water)					203,142	99.95	67.37	0	95	5	9	0	0

Note: Samples annotated with * were fresh defecations at the nest, all other samples were taken directly from the chicks. Under sampling date, the day in 2018 of fecal sample collection and the temporally closest pitfall trap sampling day are given. Initial reads: total sequence reads from Illumina NextSeq 500 sequencing, then further processed in the Multiplex Barcoding Research and Visualization Environment (mBRAVE, Ratnasingham, 2019): % filtered = percentage of filtered sequences, % dereplicated = percentage of dereplicated sequences, Chimeras = number of chimeric sequences, reads = number of sequences matched to BINs (Barcode Index Number in the Barcode Of Life Database BOLD, Ratnasingham and Hebert (2013)), BINs = number of BINs matched, OTUs = remaining OTUs (not matched in BOLD). Reads after final filtering and final BINs represent the number of sequences and the number of BINs remaining after manual filtering and were used in the analyses.

SELECTIVITY RANKING MATRICES

TABLE A2 Ranking matrix for snow bunting selectivity for different arthropod groups as nestling food, based on the proportional occurrence of arthropod taxa as food items at brood level and the proportional number of arthropod taxa trapped in pitfall traps.

	Ant	Apo	Ara	Chi	Cul	Emp	Mus	Myc	Sca	Sci	Sph	Syr	Ten
Anthomyiidae	0	+	+++	+	ns	ns	ns	+++	ns	ns	+++	ns	ns
Apocrita	−	0	ns	ns	− − −	− − −	− − −	ns	ns	ns	ns	− − −	−
Araneae	− − −	ns	0	ns	− − −	− − −	− − −	ns	−	− − −	− − −	− − −	− −
Chironomidae	−	ns	ns	0	− − −	− − −	−	ns	ns	ns	ns	− − −	− −
Culicidae	ns	+++	+++	+++	0	ns	ns	+++	ns	ns	+++	+++	ns
Empididae	ns	+++	+++	+++	ns	0	ns	+++	ns	ns	+++	ns	ns
Muscidae	ns	+++	+++	+	ns	ns	0	+	ns	ns	+++	ns	ns
Mycetophilidae	− − −	ns	ns	ns	− − −	− − −	−	0	−	ns	ns	− − −	− − −
Scathophagidae	ns	ns	+	ns	ns	ns	ns	+	0	ns	+	ns	ns
Sciaridae	ns	ns	+++	ns	ns	ns	ns	ns	ns	0	ns	ns	ns
Sphaeroceridae	− − −	ns	+++	ns	− − −	− − −	− − −	ns	−	ns	0	− − −	−
Syrphidae	ns	+++	+++	+++	− − −	ns	ns	+++	ns	ns	+++	0	ns
Tenthredinidae	ns	+	++	++	ns	ns	ns	+++	ns	ns	+	ns	0

Note: The table is read row-wise; symbols "+" and "−" indicate positive and negative selection of the arthropod taxon on the row in comparison with the taxon in the column. The number of symbols indicates significant deviation from random at $p < 0.05$ (one symbol), $p < 0.01$ (two symbols), and $p < 0.001$ (three symbols), and "ns" indicates nonsignificance. Columns are labeled with abbreviated arthropod taxon names in the same order as the rows.

TABLE A3 Ranking matrix for snow bunting selectivity for different arthropod groups as nestling food, based on the proportional relative read abundance (RRA) of arthropod taxa as food item at brood level and the proportional number of arthropod taxa trapped in pitfall traps.

	Ant	Apo	Ara	Chi	Cul	Emp	Mus	Myc	Sca	Sci	Sph	Syr	Ten
Anthomyiidae	0	+++	+++	ns	ns	ns	ns	+++	ns	ns	+++	ns	ns
Apocrita	− − −	0	ns	ns	− − −	− − −	− − −	ns	− −	−	ns	− − −	−−
Araneae	− − −	ns	0	−−	− − −	− − −	− − −	ns	−	− − −	− − −	− − −	− −
Chironomidae	ns	ns	+	0	ns	ns	ns	+	ns	ns	ns	ns	ns
Culicidae	ns	+++	+++	ns	0	ns	ns	+++	ns	ns	+++	ns	ns
Empididae	ns	+++	+++	ns	ns	0	ns	+++	ns	ns	+++	ns	ns
Muscidae	ns	+++	+++	ns	ns	ns	0	+++	ns	ns	+++	ns	ns
Mycetophilidae	− − −	ns	ns	−	− − −	− − −	− − −	0	−	ns	ns	− − −	− − −
Scathophagidae	ns	++	+	ns	ns	ns	ns	+	0	ns	+	ns	ns
Sciaridae	ns	+	+++	ns	ns	ns	ns	ns	ns	0	ns	ns	ns
Sphaeroceridae	− − −	ns	+++	ns	− − −	− − −	− − −	ns	−	ns	0	− − −	−
Syrphidae	ns	+++	+++	ns	ns	ns	ns	+++	ns	ns	+++	0	ns
Tenthredinidae	ns	+	++	ns	ns	ns	ns	+++	ns	ns	+	ns	0

Note: The table is read row-wise; symbols "+" and "−" indicate positive and negative selection of the arthropod taxon on the row in comparison with the taxon in the column. The number of symbols indicates significant deviation from random at $p < 0.05$ (one symbol), $p < 0.01$ (two symbols), and $p < 0.001$ (three symbols), and "ns" indicates nonsignificance. Columns are labeled with abbreviated arthropod taxon names in the same order as the rows.

RAREFACTION AND EXTRAPOLATION CURVES

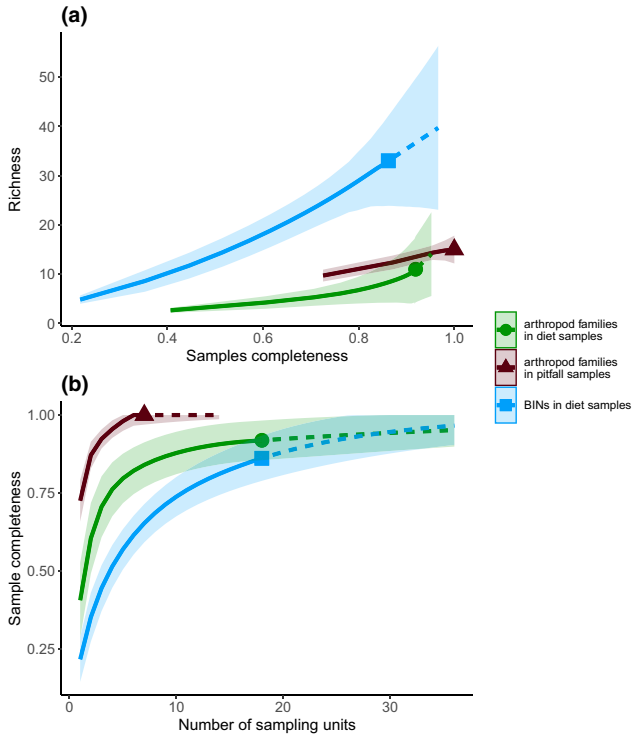

FIGURE A2 Coverage-based rarefaction and extrapolation curves (a) and sample completeness curves (b) of the identified BINs (blue) and arthropod families (green) in the snow bunting feces, and arthropod families in the pitfall traps (red), calculated with *iNEXT* package (Hsieh et al., 2022). Continuous lines show the interpolated values, dotted lines show the extrapolated values, and the shaded areas show the 95% confidence limits.

NONMETRIC MULTIDIMENSIONAL SCALING PLOTS

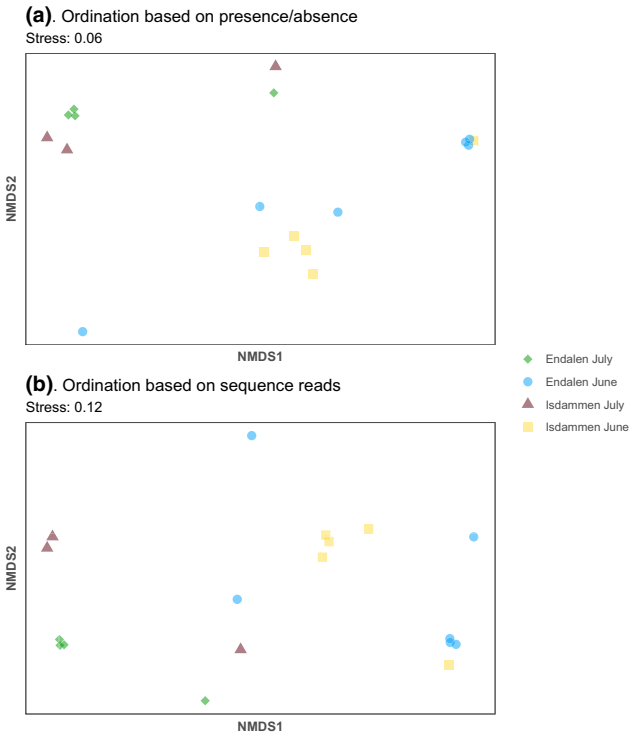

FIGURE A3 Nonmetric multidimensional scaling (nMDS) plots of snow bunting diet taxa of 18 fecal samples illustrating the differences across location (Endalen, Isdammen) and month (June, July). Dissimilarities are calculated with Jaccard distances for the presence/absence and Cao distances for sequence reads. A random jitter (width = 0.05, height = 0.05) was applied to aid visual clarity.