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Faculty of Biosciences, Fisheries and Economics,
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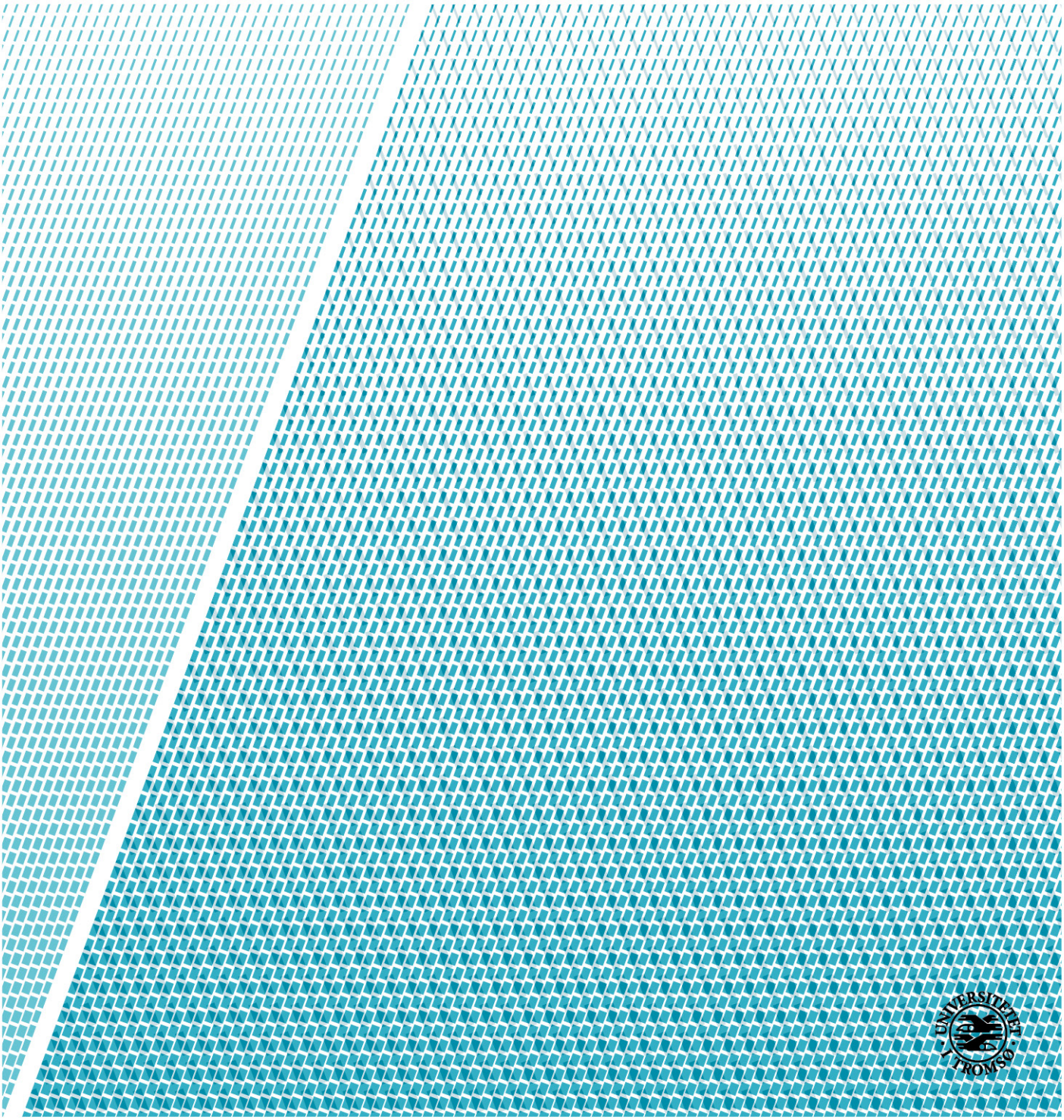
Christian Stolz

BIO-3950 Master thesis in Biology, Northern Populations and Ecosystems, May 2019



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Abstract

Tundra arthropods have considerable ecological importance as a food source for several bird species that are reproducing in the Arctic. The actual arthropod taxa comprising the chick diet are however rarely known, complicating assessments of ecological interactions. In this study, I identified the nestling diet of Svalbard snow bunting (*Plectrophenax nivalis*) for the first time. Faecal samples of snow bunting chicks were collected in Adventdalen, Svalbard in the breeding season 2018 and analysed via DNA metabarcoding. Simultaneously, the availability of prey arthropods was measured via pitfall trapping. The occurrence of 32 identified prey taxa in the nestling diet changed according to varying abundances and emergence patterns within the tundra arthropod community: Snow buntings provisioned their offspring mainly with the most abundant prey items which were in the early season different Chironomidae (Diptera) taxa and *Scathophaga furcata* (Diptera: Scathophagidae), followed by *Spilogona dorsata* (Diptera: Muscidae). An influence of breeding location on the diet could not be established, although tundra habitat explained significant differences in the trapped arthropod compositions. A selectivity analysis revealed a selection towards larger sized prey taxa, which could have implications for observed variations in snow bunting nestling success. This one year study shows the promising results of DNA metabarcoding as a non-invasive technique to assess diet variations and trophic interactions.

Keywords: arctic food web; diet analysis; fecal/faecal samples; insectivore; pitfall trap; scatology; Spitsbergen; tundra arthropod community

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Introduction

It is fundamental for species in seasonal environments to coincide the times of growth, reproduction, energy storage and migration with the availability of resources during the productive season (Varpe 2017). Changes in seasonal patterns caused by climate warming are well documented (Parmesan 2006) and how they can effect the phenology of species is an ongoing topic of research. When phenological changes happen unevenly among trophic levels, given populations can undergo mismatches between resource availability and demand (Durant et al. 2007; Stenseth and Mysterud 2002; Visser and Both 2005). Migratory birds are one species group likely to experience mismatches. In these species, timing of migration and breeding can be so constrained that adjustments of annual routines to match phenological shifts of resources availability are not possible. (Dunn and Winkler 2010; Renner and Zohner 2018; Visser et al. 2012).

The rapid warming of the Arctic has led to phenology changes in terrestrial, freshwater and marine ecosystems (Descamps et al. 2017; Post 2017; Wrona et al. 2016). In tundra ecosystems, increasing spring temperatures can facilitate an earlier emergence of arthropods, which are important food for terrestrial birds (Gilg et al. 2012; Høye and Forchhammer 2008; Tulp and Schekkerman 2008). Mismatch studies on insectivorous birds in the Arctic are scarce and show varying results (Corkery et al. 2019; Leung et al. 2018; McKinnon et al. 2012). None of these studies recorded the actual diet composition of the species in question, but based their conclusions on diet reports from previous studies. There is in fact a considerable knowledge gap regarding the detailed diet of many arctic insectivorous birds (Gillespie et al. 2019; Schmidt et al. 2017).

The snow bunting (*Plectrophenax nivalis*) is a migratory songbird of alpine and arctic regions in the northern hemisphere. Adult snow buntings are ground feeding birds with a main diet consisting of seeds and other plant material, but also ingest a substantial arthropod fraction during the breeding season (Cramp and Perrins 1994; De Graaf et al. 1985; Gabrielson 1924; Longstaff 1932; Montgomerie and Lyon 2011). It has therefore a position as both a herbivore (Jónsdóttir 2005) and insectivore (Ims et al. 2013) in the arctic food-web. Regarding chick diet, the provisioned food consists almost solely of arthropods (Asbirk and Franzmann 1978; Hågvar et al. 2009; Hussell 1972) and breeding success should therefore be closely linked to arthropod availability as shown in other insectivorous arctic birds (Meltofte et al. 2007).

The Fennoscandian snow bunting population shows a significant decrease (Lehikoinen et al. 2014; Lehikoinen et al. 2019). Declining trends, although non-significant, have also been observed in North America (Butcher and Niven 2007) and Iceland (Icelandic Institute of Natural History cited in Hayhow et al. 2018). A warming Arctic and subsequent mismatches might un-

derlie these observations, making it all the more important to fill remaining knowledge gaps in the food ecology of this species. In the high arctic archipelago of Svalbard for instance, one snow bunting population has shown an average decline of nestling weight and earlier onset of breeding from 1998 to 2012 (Fossøy et al. 2015), leading to speculations about a phenological mismatch between start of breeding and peak abundance of prey items (Espmark 2016; Fossøy et al. 2015). Evaluation of this possibility remains unclear, as variations in seasonal availability of arthropod prey are poorly understood and the actual nestling diet on Svalbard is unknown.

The nestling diet of snow buntings has been described in detail on Devon Island in Arctic Canada (Hussell 1972), in Scoresby Land in Eastern Greenland (Asbirk and Franzmann 1978) and the Hardangarvidda mountain plateau in central South Norway (Hågvar et al. 2009). These studies have revealed considerable regional differences in prey composition. However, Tipulidae (Diptera; crane flies) have been an important food item at all those study sites. In addition, accounts of provisioning snow buntings in the Koryak Mountains in Siberia (Kishchinsky 1980), West Greenland (Longstaff 1932), Melville Peninsula in Arctic Canada (Lyon et al. 1987) and the Scottish mountains (Nethersole-Thompson 1966; Watson 1997) mention Tipulidae as well. Tipulidae are not a part of the arthropod fauna of Svalbard (Coulson et al. 2014), which is holding considerably fewer resident insect and spider species than most other arctic sites (Coulson 2007; Gillespie et al. 2019; Hodkinson 2018). The nestling diet of Svalbard snow buntings might thus differ from other places and make them more dependent on certain prey taxa, which could in turn increase the vulnerability towards phenological mismatches (Miller-Rushing et al. 2010).

Diet studies have traditionally used invasive or lethal methods to attain crop or stomach samples (Rosenberg and Cooper 1990). The use of faecal samples reduces the stress of the animals greatly and next-generation DNA sequencing allows the analysis of even 5-day-old scat samples as well as detection of otherwise unidentifiable soft-bodied prey (Oehm et al. 2011). The method is increasingly used to study the diet of diverse bird species (McInnes et al. 2017; Sullins et al. 2018; Trevelline et al. 2018) and is important for food-web construction (Roslin and Majaneva 2016). In this context, Wirta et al. (2015) assessed the arctic food-web of Eastern Greenland, which included the first analysis of snow bunting diet via metabarcoding of faeces. However, detailed nestling diet information of other populations and how it changes during the breeding season is still missing.

For a future assessment of a possible mismatch between the timing of brood-rearing in buntings versus food availability on Svalbard, a detailed nestling diet analysis is needed. Therefore my objective was to reveal for the first time the nestling diet of Svalbard snow buntings with the help of DNA metabarcoding of faecal samples. Furthermore, I used simultaneous insect trappings to compare the actual bird diet to the prey availability and was thus able to evaluate the selection

and avoidance of certain diet components. As for arthropod availability, I anticipated that a peak of Araneae abundance would be followed by peaks of Diptera and lastly Hymenoptera, similar to what has been observed at other arctic sites (Bolduc et al. 2013; Høye and Forchhammer 2008; MacLean and Pitelka 1971). Accordingly, I expected that snow bunting nestling diet would change similarly over time, but with variation due to breeding location. For individual prey items, I hypothesised that the main nestling food would consist of Araneae and Chironomidae as those taxa were frequently found in other diet analyses (Asbirk and Franzmann 1978; Hussell 1972) and are common on Svalbard (Coulson et al. 2003; Dahl et al. 2018; Hodkinson et al. 1996). Finally, I also expected that snow buntings would positively select larger sized prey items based on food choice analyses of other insectivorous birds (f.e. Kaspari and Joern 1993; Schwagmeyer and Mock 2008; Turner 1982).

Materials and methods

Study site and species

Snow buntings are the only wide-spread passerine bird species on the high arctic archipelago of Svalbard and are important bioindicators for anthropogenic pollution (Kristoffersen et al. 2012; Warner et al. 2019). The valley Adventdalen adjacent to the town of Longyearbyen (15.38°E, 78.13°N; Fig. 1) was chosen as study location as it holds a well studied snow bunting population (Espmark 2016). Adventdalen is characterised by moss-rich mire and marsh plant communities on the valley bottom and a snowbed vegetation dominated by heaths along the slopes (Brattbakk 1984; Elvebakk 1994). The mean summer air temperature from June to August was 5.9 °C in the years 2007-2016 and the average summer precipitation was 49 mL from 1986-2015, both measured at the closest weather station at the airport of Longyearbyen (Isaksen et al. 2017). After arrival in early April, the snow buntings breed in artificial nest boxes as well as natural rock crevices before migrating back to their wintering grounds in Western Siberia in September (Snell et al. 2018). Egg-laying commences usually from mid-May to late June with an average clutch size of 5.8, after which the birds are incubating for 12-13 days (Espmark 2016). Snow buntings are socially monogamous with moderate rates of extra-pair fertilisations (Hoset et al. 2014) and while males feed females during incubation, both parents share the provisioning of nestlings, which stay ca. 12 days in the nest after hatching (Espmark 2016).

Arthropod sampling and identification

I sampled arthropods to assess the availability of snow bunting food items from 4. June to 5. August 2018 via pitfall trapping in Endalen, one of the site valleys of Adventdalen (Fig. 1). Ten pitfall traps were each set up in two tundra habitats: a *dry* habitat with mainly *Cassiope tetragona* heaths and in a 300 m apart *wet* marsh habitat with *Sphagnum* spp. mosses and graminoids as vegetation (Fig. 2). The traps were made out of two white plastic cups (68 mm diameter) stacked onto each other and buried into the soil with the rim at even level with the ground surface. No funnel or rain guard was used. The traps were filled with ca. 200 mm water plus a few drops of a detergent (Zalo Ultra, Lilleborg AS, Oslo, Norway). In each habitat, the cups were arranged to form 2 parallel lines (3 m apart) consisting of five traps with 2 m spacing between cups of one line. Emptying happened on the afternoon of every fourth day by sieving the captured arthropods over a fine cloth (mesh size ca. 0.5 mm). To be consistent with sampling in previous years, there was a gap of 2 days after the first emptying and setting the traps for the next trapping period. The recovered invertebrates of each day were pooled by habitat and stored immediately in vials filled with 70% ethanol. In total, 15 arthropod samples of each habitat were collected. I identified insects minimum to family level, other invertebrates minimum to order level using the keys of Søli (2018) and Oosterbroek (2006). Collembola, Acari and dipteran larval stages

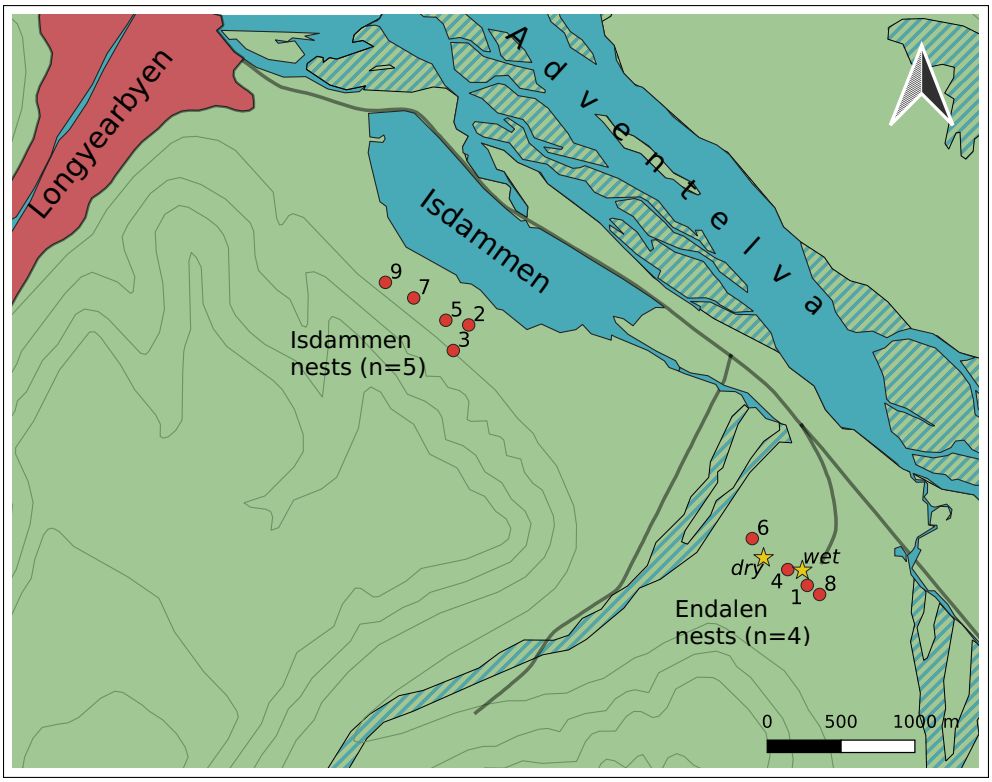

Figure 1: Study site in Adventdalen, Svalbard. Red dots with numbers mark the location of nests (n=9) from which faecal samples were collected. Yellow stars show the two sites of insect trapping. The map was constructed in QGIS 3.6 (QGIS Development Team 2019) using base data from © Norwegian Polar Institute www.npolar.no

were removed prior to analysis as they were not expected to be part of the main snow buntings diet (due to small size or lifestyle). All remaining taxa were regarded as potential snow bunting food items and grouped at family level in a species matrix.

Faeces collection

Faecal samples from snow bunting broods were collected in the 2018 breeding season from two two distinct locations (Fig. 1) within the study area. The Isdammen nests (n=5) were in a dry habitat dominated by *Salix polaris*, *Dryas octopetala* and *C. tetragona* and placed in natural cavities within stone piles or stream banks. The Endalen nests (n=4) were close to the pitfall trapping location with the three southern nests surrounded by a wet graminoid dominated habitat and the nest farther north (nr 6) by a *Cassiope tetragona* tundra. All Endalen nests were located in nesting boxes. Sample collection happened on day 8 after hatching of the oldest chick during annual weighing and banding of the nestlings. As a protection against wind, cold and rain, the nestlings were put into a cloth bag during the procedure and defecation happened often therein, so not all samples could be assigned to an individual bird. In addition, three fresh faecal samples were picked directly from the nest (see Table A1). All samples were immediately preserved in 1.5 mL absolute alcohol.

All fieldwork was performed in line with the necessary permits for handling and banding birds

Figure 2: Part of the pitfall trap setup in Endalen. The red circles mark individual pitfall traps on two parallel (3 m apart) lines. The picture was taken in the dry habitat on 03.06.2018. © C. Stolz

from the Governor of Svalbard (16/00757-10) and the land owner, Store Norske Spitsbergen Kulkompani AS.

DNA extraction, amplification and sequencing

DNA was extracted from the scat samples by drying ca. 500 mg of the faecal matter to remove the storage ethanol before applying a slightly modified FastDNA Spin Kit for Soil protocol (MP Biomedicals 2016), which was successful in extracting target DNA from house sparrows (*Passer domesticus*) in preceding workflows. The modification included a second washing step with the SEWS-M Wash Solution to account for high amount of inhibitor proteins in faecal matter. Amplification of arthropod DNA within the sample was carried out using the general arthropod primer pair ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale et al. 2011), which target a 157 bp sequence in the ”Folmer” region of the cytochrome c oxidase *c* subunit I (COI) gene (Folmer et al. 1994). Although degenerate primers like the one presented in Elbrecht and Leese (2017) have a better performance in amplifying a large assemblage of arthropod taxa (Piñol et al. 2019; Marquina et al. 2018), earlier metabarcoding of house sparrow scat samples included over 95% reads of house sparrows DNA using their primer (*F. Fossøy, pers. obs.*). In contrast, the Zeale-primers I used have a low chance to identify non-invertebrate DNA in environmental and faecal samples (Alberdi et al. 2018; Esnaola et al. 2018). A 5’ adapter sequence was added to the primers to make them applicable for the downstream high-throughput DNA sequencing protocol. The polymerase chain reaction was conducted in 25 µL volumes following the instructions from the 16S Metagenomic Sequencing Library Preparation protocol (Illumina 2013) with 2 to

12 ng µL^{-1} sample DNA. The procedure consisted of an initial 3 min step of 94 °C, 40 cycles of 30 sec at 94 °C, 30 sec at 55 °C and 30 sec at 72 °C, followed by a final phase of 10 min at 72 °C. The amplified DNA was purified according to the Illumina (2013) protocol and 20 µL template were normalised using the SequalPrep Normalization Plate Kit (Invitrogen 2008). Unique indices and sequencing adapters were added and the resulting DNA library purified following the Illumina (2013) protocol. After a second normalization using the same kit, single-end 1x300 bp sequencing with an Illumina NextSeq 500 System at the NTNU Genomics Core Facility (GCF), Trondheim (`www.ntnu.edu/mh/gcf`), was performed.

Sequence analysis and assignment of reads to arthropod taxa

Demultiplexed .fastq files were uploaded to and analysed on mBRAVE (Multiplex Barcoding Research and Visualization Environment; `www.mbrave.net`), which has direct access to the Barcode of Life Database (BOLD) reference libraries (Ratnasingham and Hebert 2007). The libraries used (Insecta, Non-Arthropoda Invertebrates and Non-Insect Arthropod, all last updated 7. April 2019) have an especially good record of COI gene region references (Andújar et al. 2018; Hebert et al. 2003). The workflow started by trimming the sequences (parameters: 30bp front, 109bp end, 200bp length) to remove the primers. Then they were quality filtered by removing sequences not matching a mean Quality Value (QV) of <10, a length of <200bp or those with more than 25% of their bases with a QV <20). mBRAVE then clustered the dereplicated and chimaera-screened sequences into Operational Taxonomic Units (OTUs) with slightly adjusted default parameters (exclude from OTU threshold: none, minimum OTU size: 1, OTU threshold: 2%). Finally, the generated OTUs were matched to OTUs of BOLD, represented by the Barcode Index Numbers (BINs), the unique registry unit of BOLD (Ratnasingham and Hebert 2013). I used the standard 3% ID distance threshold and followed with additional quality processing further downstream, including keeping only BINs with sequences reads that amounted to over 0.05% of total sample reads. The threshold of 0.05% is frequently used in samples with high number of reads (Deagle et al. 2019), which resulted in removing the most abundant contaminant *Tipula paludosa* (this taxa is not local on Svalbard and was identified due to likely tag-jumping (Schnell et al. 2015) from simultaneous sequenced water samples). In addition, all sequences with less than 10 reads were discarded. Remaining BINs matching multiple species were transferred into single records based on being local to Svalbard. Non-local BINs were removed when their records were associated with much higher reads of closely related local BINs or converted into local BINs when single sequences comparison via the BOLD Identification System (Ratnasingham and Hebert 2007) gave the same similarity for the local BIN. The resulting dataset was list of identified arthropod prey species with their respective sequence reads per sample.

While the number of sequences generated by metabarcoding is in general proportional to the entered biomass, non-quantitative data can be randomly generated with any given sequence run

(Lamb et al. 2019). Furthermore, molecular diet studies face the issue of potential non-uniform digestion across food items within the subject (Pompanon et al. 2012). Therefore, my analysis follows the recommendation from Deagle et al. (2019) and uses both relative read abundance (RRA) and weighted percentage of occurrence (wPOO) to present the metabarcoding results. For RRA, the number of sequence reads per taxa and sample was divided by the total number of reads of that sample. Occurrence data was created by transforming sequence reads into a presence/absence dataset and calculating the individual species percentages based on the total number of identified food items of the respective sample (see Deagle et al. 2019). These values were summed among all samples and scaled to sum to 100% for presentation.

Statistical analysis

All data analysis was performed in R version 3.5.3 (R Core Team 2019). For investigating the effects of both time and habitat on the prey community, non-metrical multidimensional scaling (nMDS) of the *vegan* package (Oksanen et al. 2019) was performed on the Bray-Curtis distances of the ln(x+1) transformed species matrix generated by pitfall trapping (function *metaMDS*, 999 tries). As an unconstrained ordination method, the nMDS collapses the multivariate community data into a two-dimensional plot suitable for visualisation. To test for significant differences over time (day of the season) and the two habitats, I used a permutational multivariate analysis of variance (PERMANOVA, Anderson 2017) on the same Bray-Curtis dissimilarity matrix. To account for the repeated measurement design, permutations were blocked to habitat-type and 5 time segments of the entire sampling period. The test was performed by the *adonis* function in the *vegan* package (Oksanen et al. 2019).

To avoid pseudoreplication within the diet analysis, the RRA and occurrences of each sample were summarised per clutch for hypothesis testing. To test whether nest location and/or time in the season is affecting the diet choice, a PERMANOVA was performed on the Bray-Curtis distances of the RRA dataset and on the Jaccard distances of the occurrence dataset. As an exploratory analysis, the diet similarity within each clutch was compared with a non-metric hierarchical cluster analysis. This method groups faecal samples with similar diet composition together without a priori knowledge about which brood they belong to. Clustering with the *hclust* function and group-average sorting was performed on the RRA dataset by calculating the Bray-Curtis dissimilarity and on the occurrence matrix by using the Jaccard dissimilarity.

To identify possible preferences in snow bunting food choice and/or biases in pitfall trap sampling, a compositional analysis (Aebischer et al. 1993) following the methodology of Soininen et al. (2013) on molecular data was performed. Two centred ln-ratio transformed (clrt) datasets, one with identified diet proportion in the faecal samples and the other with available diet proportions measured by the pitfall trapping, allow the calculation of a selectivity index (sensu Soininen et al. 2013). I again used the brood-level summarised and scaled to sum to 100% RRA and oc-

currences datasets to account for non-independence between samples from the same brood. For the comparison with available prey, the closest pitfall trapping date for each faeces collection date was chosen. Of those trapping dates, the total (i.e. wet plus dry habitat) proportion of each arthropod group was calculated. Those proportions were centred ln-ratio transformed via the *clr* function in the *compositions* package (van den Boogaart et al. 2018). Then, the selectivity index for each clutch-level sample was calculated by subtracting the clrt proportions of available arthropod groups from the clrt proportions of the identified diet composition. As zeros have to be replaced in compositional analyses (Aebischer et al. 1993), zeros prior to transforming were substituted with a value three orders of magnitude lower than the lowest observed in the original dataset. Furthermore, Aphidae, Coleoptera, Calliphoridae, Coelopidae, Heleomyzidae, Sphaeroceridae and Apocrita were omitted as they were not detected in the nestlings diet. Culicidae were not captured in the pitfall traps and therefore removed prior to this analysis as well. Whether the selectivity was significant different among taxonomic group was tested with the randomisation version of the *compana* function of the *adehabitat* package (Calenge 2006). This function calculates pairwise significance among the arthropod groups using a Wilks lambda.

Results

Arthropod phenology

A total of 8119 arthropods were collected via pitfall trapping of which 6009 were regarded as potential snow bunting food items and further analysed (Table 1). Of those, insects amounted to 82.2% and spiders to 17.8%. Diptera was the most abundant insect order with 12 families and 73.9% of all individuals, whereas Hymenoptera followed with 7.9%. At insect family level, Muscidae and Chironomidae were the most abundant (26.3% and 24.5% of the total individuals respectively) followed by Scathophagidae (10.8%) which consisted of the one species *Scathophaga furcata*.

Table 1: Arthropod abundance collected via pitfall trapping from 04. June to 05. August 2018 in two tundra habitats in Endalen, Svalbard. Due to lifestyle and small size only Araneae and Insecta (without Diptera larvae) were regarded as potential snow bunting food items for the analysis.

				Habitat		
Class	Order	Family	Species	Dry	Wet	total
Arachnida	Acari	indet.		175	112	287
	Araneae	indet.		859	208	1067
Entognatha	Collembola	indet.		570	1165	1735
Insecta	Aphidae	indet.		9	16	25
	Coleoptera	Curculionidae	*Isochnus flagellum*	1	-	1
	Diptera	Anthomyiidae	*Zaphne frontata*	22	2	24
		Calliphoridae	*Protophormia* sp.	6	-	6
		Chironomidae	indet.	540	935	1475
		Coelopidae	*Coelopa frigida*	1	-	1
		Empididae	*Rhamphomyia caudata*	7	1	8
		Heleomyzidae	indet.	1	2	3
		Muscidae	*Spilogona* spp.	1129	451	1580
		Mycetophilidae	*Mycoma islandica*	5	-	5
			indet.	107	274	381
		Scathophagidae	*Scathophaga furcata*	251	398	649
		Sciaridae	indet.	137	13	150
		Sphaeroceridae	indet	7	149	156
		Syrphidae	*Parasyrphus tarsatus*	3	-	3
		larvae indet.		14	74	88
	Hymenoptera	Braconidae	indet.	3	-	3
		Diapriidae	indet.	24	6	30
		Figitidae	indet.	3	1	4
		Ichneumonidae	*Gelis* sp.	-	1	1
			indet.	240	157	397
		Megaspilidae		24	6	30
		Tenthredinidae	indet.	3	3	6
			larvae indet.	-	4	4
Total				4141	3978	8119

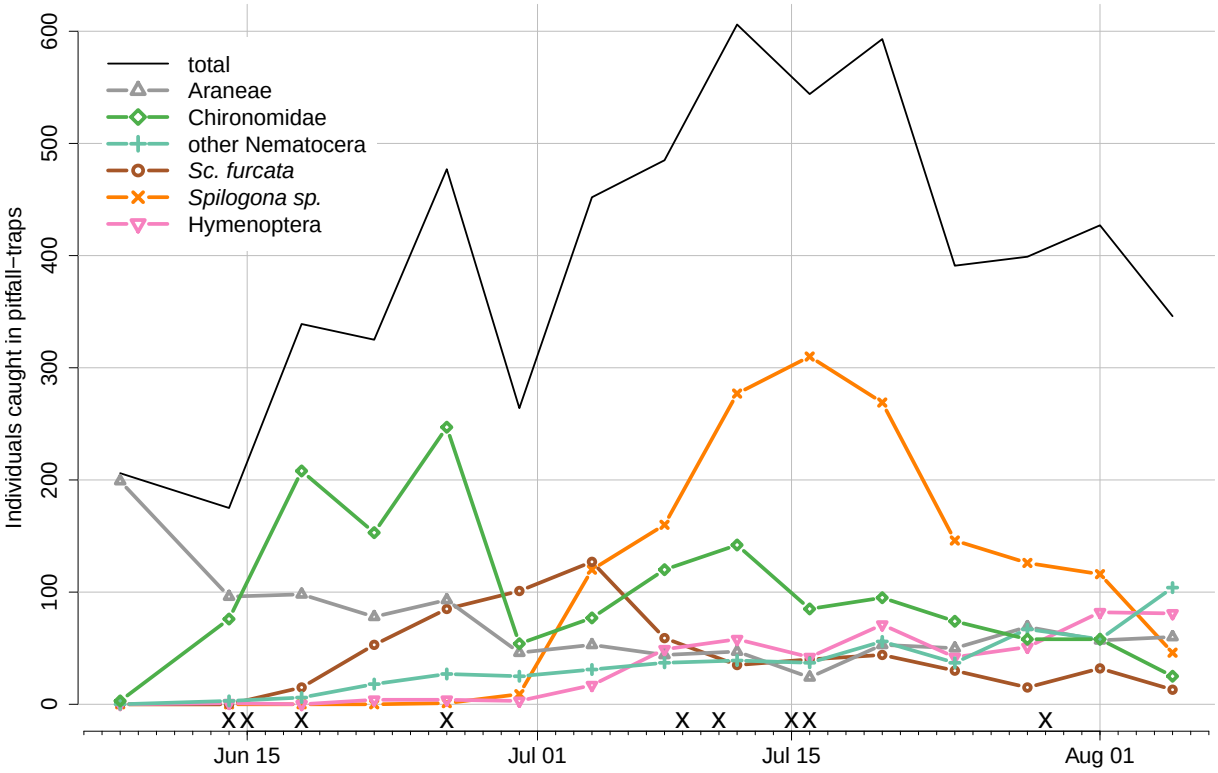

Figure 3: Phenology of insect and spider emergence on the tundra in Endalen, Svalbard, in 2018 as measured by 20 pitfall traps in a dry *Cassiope tetragona* dominated and a wet graminoid dominated habitat. X marks the dates on which snow bunting faecal samples where taken.

The community composition showed patterns of both sampling habitat and trapping period in the best nMDS-solution (Fig. A1): The ordination separated samples according to the two habitats and sorted the trapping periods along the first nMDS-axis, thereby displaying the largest variation among samples. The grouping was confirmed by the PERMANOVA, as arthropod composition changed significantly with day of the season (Pseudo-$F_{1,27}$=25.21, R^2=0.41, P=0.001) and was significantly different between the two habitats (Pseudo-$F_{1,27}$=9.35, R^2=0.15, P=0.001).

As for insect composition, a succession of different species was detected and the peak abundance across all taxa was reached in mid-July (Fig. 3). Regarding individual taxa, Araneae were mainly trapped in the dry habitat with maximum numbers in the beginning of the trapping period, while Chironomidae showed a sharp increase to reach a maximum in late June although capture numbers in the wet habitat were fairly stable (Fig. 4). The numbers of other Nematocera increased constantly over the trapping period (Fig. 3) with Sciaridae as the main captured family in June in the dry habitat followed by Mycetophilidae in later July and August in the wet habitat (Fig. 4). Scathophagidae were trapped in peak numbers in early July (Fig. 3) while Muscidae appeared rarely before July, but increased to a maximum in mid-July with larger numbers in the dry habitat (Fig. 4). Hymenoptera appeared in early July and increased until the end of trapping in early August (Fig. 3).

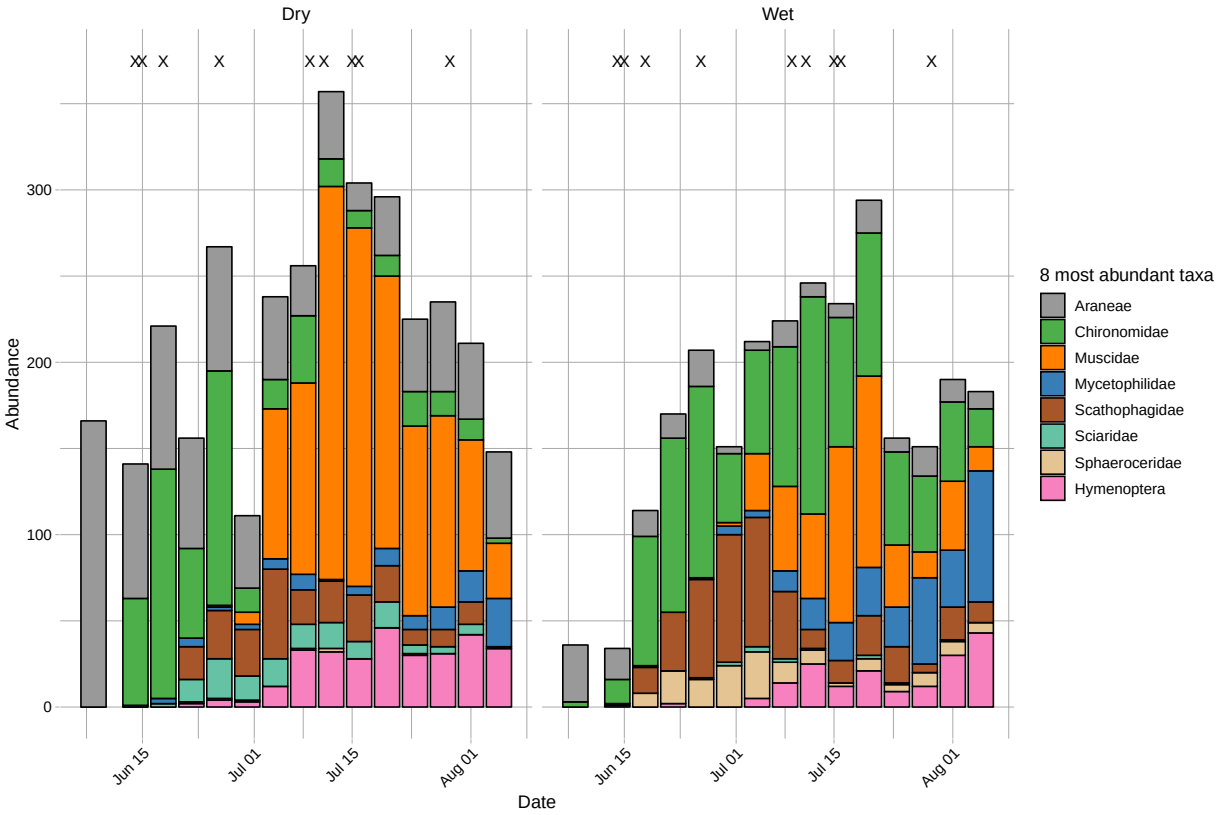

Figure 4: Tundra habitat differences in phenology and abundance of arthropod groups in Endalen, Svalbard 2018. The specimens were captured by 20 pitfall traps in a dry *Cassiope tetragona* dominated and a wet graminoid dominated habitat. X marks the dates on which snow bunting faecal samples where taken.

Faeces analysis

A total of 22 scats were collected consisting of 12 samples from 4 broods in Endalen and 10 samples from 5 broods situated in Isdammen (Table A1). After successful DNA extraction, Illumina sequencing generated 15.2 million sequences. Low-quality sequences were removed and the remaining de-replicated into 2.1 million unique sequences. Those were clustered into 687,150 OTUs which matched 724 BINs in the BOLD databases. After quality filtering, 11.2 million sequences representing 94 BINs were retained across all samples and used for further diet analysis.

A total of 11 arthropod families and 32 species were identified, among them 9 formally yet undescribed species, that have identifier initials as provisional names (Table 3). Both in wPOO and RRA, Scathophagidae followed by Muscidae and Chironomidae were the most represented prey items in the faeces, amounting together to 80.6% (wPOO) and 93.7 % (RRA) of all identified families (Table 2). On species level, the taxa-richest family was Chironomidae with 16 matched taxa, while the Scathophagidae *Sc. furcata* (33.2% wPOO, 36.9% RRA) and the Muscidae *Sp. dorsata* (19.2% wPOO, 28.8% RRA) were the most identified single species (Table 3). As my analysis of the RRA and occurrence datasets resulted in similar findings, the occurrence based results are presented in the appendix.

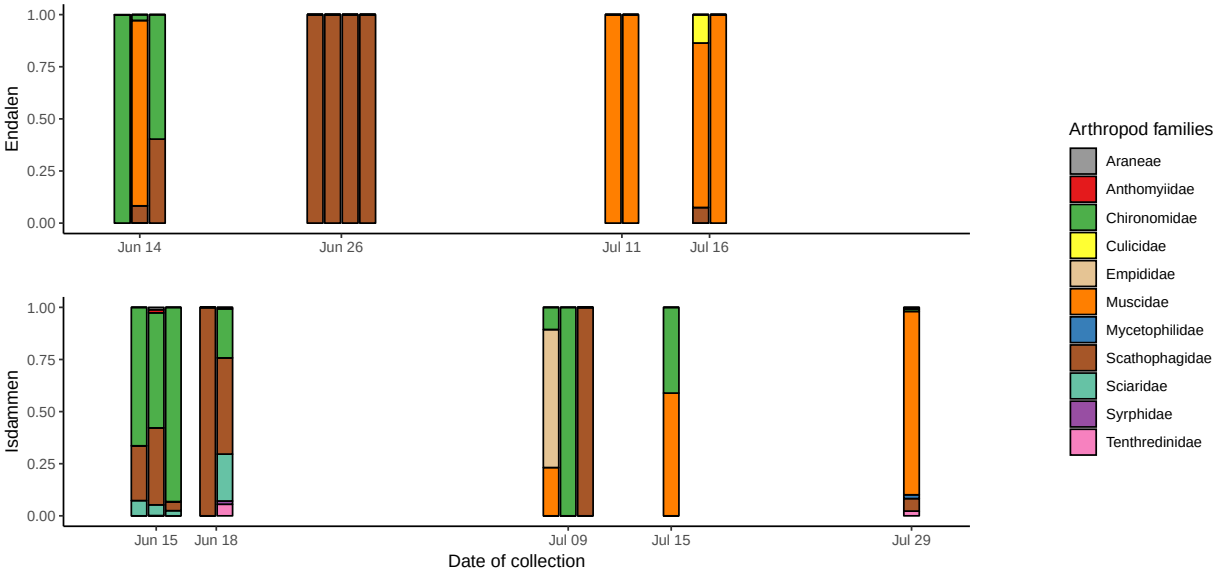

Figure 5: Clutch specific nestling diet of Svalbard snow buntings in two breeding locations during the breeding season. The height of each bar (=sample) represents the relative read abundance of each taxon within the faecal sample.

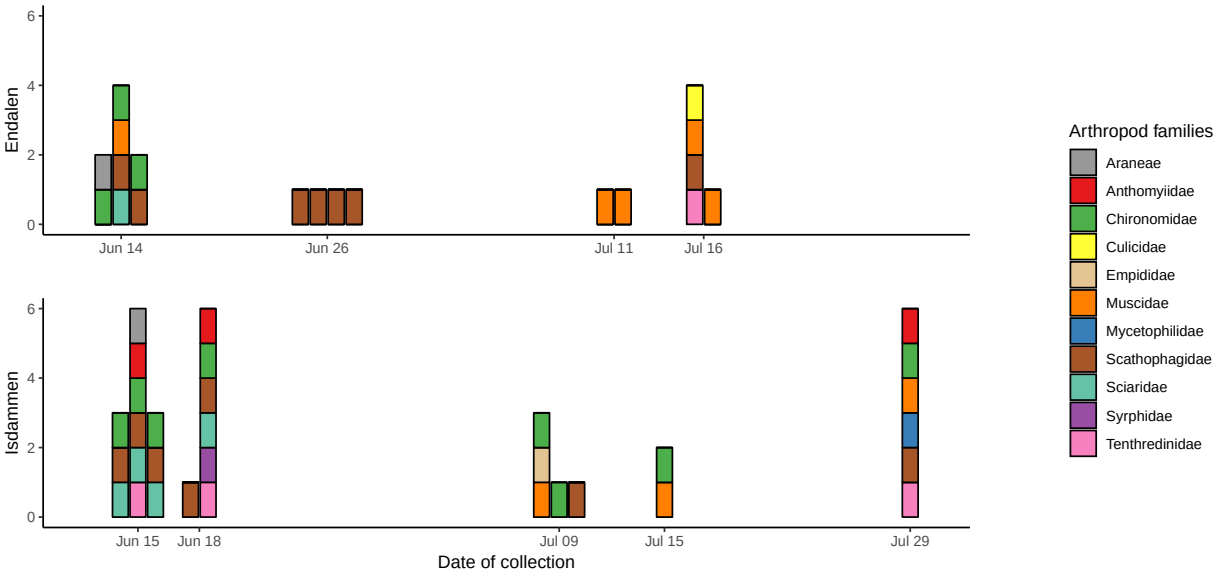

Figure 6: Clutch specific nestling diet of Svalbard snow buntings in two breeding locations during the breeding season. The height of each bar (=sample) represents the number of occurrences of each taxon within the faecal sample.

Table 2: Comparison of arthropod group proportions in snow bunting nestling diet identified by metabarcoding and arthropod groups collected on the tundra via pitfall traps. RRA = relative read abundance, wPOO = weighted percentage of observation.

Class	Order	Suborder	Family	RRA	wPOO	total % in pitfall traps
Arachnidae	Araneae			<0.1	3.1	17.8
Insecta	Coleoptera			-	-	<0.1
	Aphidae			-	-	0.4
	Diptera		Anthomyiidae	0.1	2.4	0.4
			Calliphoridae	-	-	0.1
			Chironomidae	26.4	20.2	24.6
			Coelopidae	-	-	<0.1
			Culicidae	0.6	1.2	-
			Empididae	3.2	1.6	0.1
			Heleomyzidae	-	-	<0.1
			Muscidae	30.4	21.4	26.3
			Mycetophilidae	<0.1	0.8	6.4
			Scathophagidae	36.9	38.9	10.8
			Sciaridae	1.8	6.0	2.5
			Sphaeroceridae	-	-	2.6
			Syrphidae	<0.1	0.8	<0.1
	Hymenoptera	Apocrita		-	-	7.8
		Symphyta		0.4	3.6	0.1

Though no grouping occurred according to the two nest locations in the nMDS, some clustering according to collection date was observable especially in the occurrence based ordination Fig. A2). This was supported by statistical testing of the nest-level sums: the RRA dataset showed a significant influence of collection day (PERMANOVA, Pseudo-$F_{1,6}$=4.58, R^2=0.39, P=0.017) but not nest location (Pseudo-$F_{1,6}$=1.12, R^2=0.10, P=0.347), which was confirmed by the same test on the occurrence dataset (PERMANOVA, Pseudo-$F_{1,6}$=2.42, R^2=0.26, P=0.009 for collection day; Pseudo-$F_{1,6}$=0.10, R^2=0.11, P=0.417 for nest location).

The faecal samples of chicks from the same clutch were not very similar in terms of identified taxa (Figs. 5 and 6). which resulted in mixed nest groupings in the cluster analysis (Figs. A3 and A4). However, the two main identified clusters based on RRA represented approximately the date of sample collection, with nests 1-4 before 1st of July and nests 5-9 after (Fig. A3). Based on occurrence of foot items, this pattern emerged again, however the latest collection date of all (nest 9) grouped here with the earlier samples (Fig. A4).

The compositional analysis revealed a significant avoidance of Araneae and Mycetophilidae based on both RRA and occurrence data (Tables A2 and A3 and Figs. 7 and A5). Chironomidae were also significantly avoided in favour of Muscidae and Empididae in both analyses, and significantly in favour of Scathophagidae, Syrphidae and Tenthredinidae in the occurrence-based analysis.

Table 3: Overview over species-level components of snow bunting nestling diet as assessed via metabarcoding of faecal samples (n = 21). wPOO = weighted percentage of occurrence, RRA = relative read abundance. Formally yet undescribed species have identifier initials as provisional names.

Class	Order	Family	Species	wPOO	RRA
Arachnida	Araneae	Linyphiidae	*Collinsia spetsbergensis*	2.0	0.1
Insecta	Diptera	Anthomyiidae	*Zaphne frontata*	1.2	0.1
		Chironomidae	*Allocladius* sp. 1ES	1.0	<0.1
			Bryophaenocladius sp. 8ES	2.4	0.3
			Chironomus sp. 1TE	3.1	0.9
			Cricotopus gelidus	0.4	<0.1
			Metriocnemus euryntous	1.2	0.3
			Metriocnemus fuscipes	0.4	<0.1
			Metriocnemus sp. 1ES	3.4	1.6
			Metriocnemus sp. 8ES	2.0	5.0
			Metriocnemus ursinus	2.1	2.0
			Paraphaenocladius brevinervis	1.8	1.5
			Procladius crassinervis	7.9	6.4
			Psectrocladius limbatellus	0.4	0.0
			Smittia brevipennis	1.4	0.3
			Smittia sp. 6ES	4.2	7.9
			Smittia sp. 26ES	0.3	0.1
			Smittia sp. 28ES	0.7	0.1
		Culicidae	*Ochlerotatus (Aedes) nigripes*	0.8	0.6
		Empididae	*Rhamphomyia caudata*	1.6	3.2
		Muscidae	*Spilogona dorsata*	19.2	28.8
			Spilogona megastoma	1.2	0.1
			Spilogona sp. GS01	1.2	1.5
		Mycetophilidae	*Exechia frigida*	0.4	<0.1
			Coelosia tenella	0.4	0.1
		Scathophagidae	*Scathophaga furcata*	33.2	36.9
		Sciaridae	*Lycoriella abbrevinervis*	2.5	1.8
			Lycoriella modesta	0.3	<0.1
		Syrphidae	*Parasyrphus tarsatus*	0.3	0.1
	Hymenoptera	Tenthredinidae	*Amauronematus amentorum*	0.4	0.1
			Amauronematus sp. BOLD:ACD1919	1,5	0.3
			Nematus caeruleocarpus	0.7	0.1

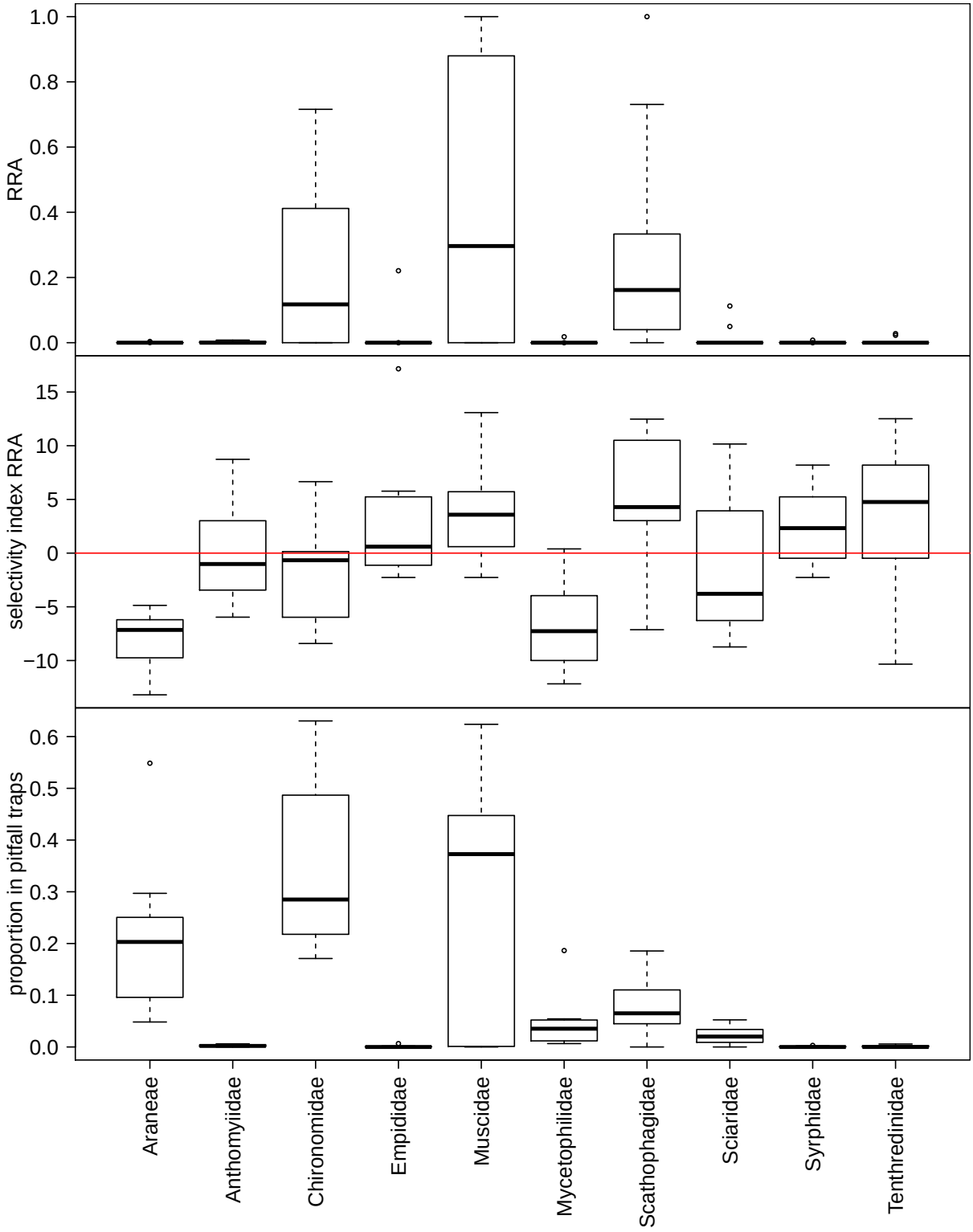

Figure 7: Snow bunting diet based on molecular scatology, selectivity and availability of arthropod groups based on pitfall trapping. The upper panel shows the relative read abundance (RRA) of identified prey taxa on nest-level summaries, the middle panel the selectivity index (see methods) and the lower panel the proportion in pitfall trap samples taken at the corresponding time. Positive selectivity values indicate selection towards the specific taxa, whereas negative values indicate avoidance. The midline represents the median, boxes upper and lower quartiles, whiskers values that lay within 1.5 times the interquartile range and points outliers.

Discussion

This study is to my best knowledge the first to assess simultaneous arthropod availability on the tundra and the actual taxa consumed by their predator in the Arctic. Thereby, I found that the snow buntings are opportunely provisioning their nestlings by following the phenology of arthropod emergence on the tundra and relying generally on the most abundant prey taxa available. The results are also indicating a preference towards larger-sized Diptera, whose comparable late emergence could have implications for previously observed patterns in snow bunting fledgling success.

Snow bunting diet and selectivity

Many knowledge gaps about the ecological importance of arctic arthropods remain (Høye and Culler 2018). For instance, the lack of data on predation pressure (Ávila-Jiménez et al. 2010) and exactly which arthropods species compose the diet of higher trophic levels is notable (Gillespie et al. 2019; Schmidt et al. 2017). Here, I present snow bunting nestling diet with a taxonomic resolution only metabarcoding studies can provide. In fact, the number of taxa identified to species level is larger than similar molecular diet assessments of other insectivorous birds (f.e. Jedlicka et al. 2017; McClenaghan et al. 2019; Moran et al. 2019), which is likely owing to comparable low species-richness of Svalbard, but also highlights the recent efforts to create a extensive molecular reference database for arctic and Norwegian invertebrates (Ekrem et al. 2015; Hodkinson 2018; Wirta et al. 2016).

The emergence pattern of tundra arthropods in 2018 as assessed by pitfall trapping followed my initial expectations. As observed on Svalbard before, the highest numbers of Araneae were trapped in the early season, although it remains unclear if the decrease in the later season is due to lower activity or an actual numerical decline (Dahl et al. 2018). The early peak of Chironomidae contrasting with later emerging Muscidae was also observed in different places in the Canadian Arctic (Bolduc et al. 2013). The latest group to appear were Hymenoptera, mainly driven by ichneumon wasps which as parasitoids of Araneae and Diptera commonly appear after their host species (Hodkinson 2018). The arthropods which formed the main diet of Svalbard snow buntings followed the observed emergence pattern on the tundra. In the early season different Chironomidae species and the Scathophagidae (*Sc. furcata*) were frequently found in the faecal samples, while later the muscid fly (*Sp. dorsata*) dominated.

Counter to my predictions, Araneae were only a small part of the identified prey taxa despite high availability and scored therefore low in the selectivity analysis. Several explanations are possible: In other insectivorous birds, the proportion of provisioned Araneae decreased as chicks grew (Arnold et al. 2007; García-Navas et al. 2012; Naef-Daenzer et al. 2000; but see confound-

ing of prey size in chestnut-crowned babblers (*Pomatostomus ruficeps*) in Browning et al. 2012), so the amount of Araneae provisioned to 8 day old nestlings might be fundamentally lower than to freshly hatched chicks. Secondly, different passage times of food items through the gastrointestinal tract of songbirds can lead to unequal results in metabarcoding studies (Pompanon et al. 2012). Custer and Pitelka (1975) found that snow buntings digest adult muscid flies up to 12 min slower than Araneae and thus the latter might be under-represented in my study. Lastly, Araneae might be subject to a primer bias in the metabarcoding analysis. While several studies have used the Zeale-primer to amplify Araneae DNA (Alberdi et al. 2018; Clarke et al. 2014; Trevelline et al. 2016), others were not successful (Esnaola et al. 2018) and the COI barcode region seems generally not optimal for Araneae barcoding (Krehenwinkel et al. 2017). That Araneae are readily taken by snow buntings is shown by analysis of snow bunting nestlings diets in Kamchatka (Kishchinsky 1980), South Norway (Hågvar et al. 2009), Arctic Canada (Hussell 1972), West Greenland (Longstaff 1932) and in Eastern Greenland, where they were the most abundant group of arthropods (Asbirk and Franzmann 1978).

The selectivity analysis showed in addition a positive selection towards the dipteran families Empididae, Muscidae, Scathophagidae, Syrphidae and the hymenopteran family Tenthredinidae. As predicted, those families have a larger size, and therefore biomass (Sample et al. 1993), in comparison to the less selected Chironomidae and Sciaridae. A selection for larger prey items in the nestling diet is known from other insectivorous birds (Davies 1977; Gibb and Betts 1963; Schwagmeyer and Mock 2008), where the prey size and composition changes also related to the age of the chick (Biermann and Sealy 1982; Royama 1966; Wiebe and Slagsvold 2014). Potential age-related diet shifts could not be addressed in this study, as all chicks had the same age. The almost complete lack of Mycetophilidae in the nestling diet could be a shortcoming of the low sample size in the later study period (only one faecal sample in late July). On average the snow bunting laying season ends in late June (Espmark 2016), whereas Mycetophilidae emerge usually in larger numbers later in July (Høye and Forchhammer 2008; Leung et al. 2018).

In the only other study using molecular methods to identify snow-bunting diet, Wirta et al. (2015) found that Greenlandic birds prey predominantly on Lepidoptera, presumably larval stages (cf. Asbirk and Franzmann 1978). Here, this order was not identified in the snow bunting faeces, despite high primer-specificity to lepidopteran DNA (Piñol et al. 2019; Zeale et al. 2011), and neither was captured in the pitfall traps. Pitfall traps have successfully been used to collect Lepidoptera imagines in the Arctic (Høye et al. 2014), so the absence might be connected to the scarcity of this order on Svalbard with only 3 resident species (Coulson et al. 2014); one of them only recently rediscovered after more than 140 years (Søli et al. 2018). They can however be an incidental part of the buntings diet, as a male bird provisioning with an unidentified Lepidoptera imago was found (*C. Stolz, pers. obs.*).

Based on my analysis, snow-bunting nest location cannot explain variations in diet composition, although the type of tundra habitat had significant influence on the arthropod composition and emergence patterns. Snow buntings generally collect food in an area of up to 250m around the nest (Falconer et al. 2008; Lyon and Montgomerie 1987; Kareila 1958), although similar data is lacking from the Svalbard population, where some nesting places are high up on steep slopes without any vegetation close by. Casual observations of individually marked birds have been as far as ca. 700m from the nest during active breeding (*M. I. Wedege, pers. comm.*). Because of the patchy distribution of vegetation types in Adventdalen (Brattbakk 1984), I can therefore assume that snow buntings have access to different tundra habitats independent of nest location.

Since I had several faecal samples from the same brood available, I was able to do an exploratory analysis of intra-clutch diet variations, but found no clear pattern. While some parents seemed to provision only one type of arthropod prey, other broods lacked intra-clutch similarity. As a multiple prey loader, snow buntings should be able to feed several chicks during a single nest visit, but the extent of such behaviour is unknown. Parental sex-differences in specialisation towards certain prey types is known from other insectivorous birds (f.e. Rasmann et al. 2013) and might also be relevant for snow buntings, as Falconer et al. (2008) showed that male and female snow buntings are searching for food at different distances from the nest.

Pitfalls of pitfall trapping

The arthropod composition as determined in this study cannot be regarded as a direct representation of the true tundra composition, as pitfall traps only sample a subset of the community. The method targets surface arthropods and has a bias towards more active species, so that the resulting capture numbers should be regarded as a activity-abundance measure and not taken by pure quantitative value (Brown and Matthews 2016; Woodcock 2005; Yi et al. 2012). Here, I use pitfall traps to assess the prey availability for a ground-feeding bird, which is coming practice (f.e. Corkery et al. 2019; Høye and Forchhammer 2008; Pérez et al. 2016) and recommended (Gillespie et al. 2019) in arctic environments where high flying insects are rare (Coulson et al. 2003). In the Arctic, pitfall traps are functioning more like pan traps (Loboda et al. 2018) and are able to catch substantial amounts of Lepidoptera and Diptera (Bowden et al. 2015; Høye et al. 2014; Loboda et al. 2018). Furthermore, shifts in arctic insect abundance and biomass captured this way were correlated to chick growth in insectivorous birds (Machín et al. 2018; Reneerkens et al. 2016; Schekkerman et al. 2003), suggesting an adequate measure of prey availability. My simultaneous diet analysis showed that all identified prey taxa except one were captured in the pitfall traps. Moreover, the most common prey taxa were also the most numerous captured by the trapping method. The one exception was the dipteran family Culicidae, which are likely under-represented in ground traps (Høye and Forchhammer 2008; Silver 2008). Abundance biases could also be expected for Calliphoridae, Empididae, Syrphidae and Tenthredinidae as they are good fliers. In contrast, Araneae are exceptionally well trapped with pitfall traps (Nor-

ment 1987) and this could account for the large discrepancy between pitfall trapping numbers and amount of identified food items in bunting faeces (but see also discussion about Araneae above).

While the general emergence pattern among tundra arthropod groups is recurrent (Muscidae after Chironomidae), there are considerable variations in timing and abundance among years (Bolduc et al. 2013; Høye and Forchhammer 2008; Leung et al. 2018). These variations are thought to be related to the number of individuals in previous years as well as temperature and snow-melt of the current season (Danks 1999; Hodkinson et al. 1996). The phenology and abundances of individual arthropod taxa as observed in 2018 should therefore not be generalised and further sampling to assess inter-annual variation is recommended. This is also reinforced by the fact, that four of the recorded chironomid species of this study (*Allocladius sp. 1ES, Metriocnemus sp. 1ES, Smittia sp. 6ES and Smittia sp. 28ES)* have not previously been registered in Western Svalbard despite considerable sampling effort (Ávila-Jiménez et al. 2019).

Considerations on molecular methods

The molecular methods applied in this study are not without limitations in assessing the diet via analysis of faecal samples. PCR amplification errors, primer biases and different digestibility and therefore varying gut-passage time will lead also to differing metabarcoding results (King et al. 2008; Oehm et al. 2011; Pompanon et al. 2012). Therefore it is still uncertain how sequence read numbers should be interpreted. Piñol et al. (2019) are expecting that molecular diet analysis of insectivorous birds yields quantifiable results, while others disagree (Deagle et al. 2019). Therefore I used both RRA and weighted occurrence to present the metabarcoding records and show that my findings are consistent among the two methods.

There might be a bias towards larger biomass specimens in samples with low sequencing depth as dilution effects can render smaller taxa undetectable (Braukmann et al. 2019; Elbrecht et al. 2017). Therefore some rare, small sized taxa could have been missed, but likely this would not change the overall proportions of the abundant taxa (Elbrecht et al. 2017). Another problem can be secondary predation, i.e. detecting the prey DNA that was inside the gut contents of a consumed predator species is possible via metabarcoding (Sheppard et al. 2005). Here, Araneae and Empididae could have potentially a diverse composition of consumed arthropod DNA in their guts (Eitzinger et al. 2019), but since most identified prey taxa are non-predatory, the influence of this bias should be low.

I used a single general arthropod primer (Zeale et al. 2011) that yields good specificity for Coleoptera, Diptera, Lepidoptera and Trichoptera, but less so for Hymenoptera (Alberdi et al. 2018; Clarke et al. 2014; Esnaola et al. 2018), which are generally difficult to detect via the COI gene region (Brandon-Mong et al. 2015; Krehenwinkel et al. 2017; Marquina et al. 2018). The

Hymenoptera identified in the diet were solely Tenthredinidae, whereas Apocrita were absent. Apocrita were identified in low numbers in snow bunting nestlings diet in Arctic Canada (Hussell 1972), and Hymenoptera (unclear which families) were also found in nestling stomachs in the Scandinavian mountains (Hågvar et al. 2009). Since 7.8% of all pitfall trap individuals were apocrite Hymenoptera (Table 2), they might be a taken occasionally by Svalbard snow buntings, despite their absence in the diet as assessed by this study. The use of several different primer pairs and barcoding genes may result in a more comprehensive diet analysis (Alberdi et al. 2018; Esnaola et al. 2018; Piñol et al. 2019). Notably, plant material in the nestling diet as found in low amounts by Hågvar et al. (2009) and Hussell (1972) could not have been detected by my method. Primer biases like the ones mentioned here, can be assessed by using mock communities of which the composition is known (Lamb et al. 2019), which should be applied to this type of study going further.

Implications

Though knowledge about the ecological interactions in an arctic arthropod-vertebrate food web is increasing (Wirta et al. 2015), my study shows that there are considerable regional peculiarities that have to be taken into account. For Svalbard the lack of Tipulidae has to be considered, which for example can account for up to 86% of the daily arthropod biomass in Taimyr, Siberia (Tulp and Schekkerman 2008). In eastern Greenland, were Tipulidae are also rather scarce (Høye and Forchhammer 2008), snow buntings are utilising increasingly Lepidoptera (Asbirk and Franzmann 1978; Wirta et al. 2015). By contrast, the snow buntings of Svalbard were provisioning mainly the two abundant Brachycera *Sc. furcata* and *Sp. dorsata*, the latter especially as the season progressed. *Sp. dorsata* has significantly declined between 1996 and 2014 in East Greenland, correlating with increasing summer temperature (Loboda et al. 2018); the status on Svalbard is unknown.

Increasing spring temperature on Svalbard were correlated with a shift towards earlier egg-laying in snow buntings (Fossøy et al. 2015). In many bird species, early clutch initiation results in higher breeding success (Perrins 1970; Pérez et al. 2016; Verhulst and Nilsson 2008), but in the Svalbard snow bunting population late broods had higher fledgling success (Espmark 2016; Hoset et al. 2009). Furthermore, the mean nestling weight was lower when egg-laying commenced early (Fossøy et al. 2015). Based on my results, one explanation is that in the early laying season the general prey availability is lower but also that the early arthropods are generally smaller Chironomidae than larger-sized Diptera. A future phenological mismatch analysis must therefore also consider that snow buntings are simply nesting ”too early” (Leung et al. 2018). Temperature and snow-melt are important factors in explaining earlier clutch initiation in arctic birds (Grabowski et al. 2013; Liebezeit et al. 2014) as well as emergence and abundance of arctic arthropods (Bolduc et al. 2013; Høye and Forchhammer 2008; Schekkerman et al. 2003) which itself could explain variations in breeding phenology of arctic waders (Meltofte et al.

2007). For cavity-nesting birds like the snow bunting, the ecological mechanisms might be different: In temperate regions, only temperature and not food availability could explain the clutch initiation of several hole-nesting bird species (Drake and Martin 2018). In this framework, untangling the underlying causal relationships of variations in snow bunting nestling success will rely on detailed diet information like presented in this study. Hereby I 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.

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Appendix

Table A1: Overview over all faecal samples used for DNA metabarcoding and their respective bioinformatical yields. Samples annotated with * were taken from the nest.

Location	Nest	Sampling date faeces	Sampling date pitfall	Initial reads	Unique sequences	OTUs	BINs	BINs kept	Final reads	Reads kept (%)
Endalen	1	14.06	14.06	99,767	15,652	6,700	26	3	74,000	74.2
				1,065,720	129,639	40,079	63	9	853,621	80.1
	*			898,242	184,351	73,121	28	5	315,902	35.2
	4	26.06	26.06	162,746	20,789	6,810	5	1	134,059	82.4
				34,868	7,987	2,243	3	1	17	<0.1
				605,319	63,651	19,468	10	1	505,402	83.5
	*			6,307	1,851	727	4	0	0	0
	*			342,209	39,449	12,407	7	1	277,272	81.0
	6	11.07	12.07	1,009,710	105,686	30,133	49	1	828,387	82.0
				1,723,520	184,430	47,741	55	1	1,410,977	81.9
	8	16.07	16.07	681,269	100,255	31,373	43	6	541,794	79.5
				1,453,650	166,337	46,718	59	1	1,173,159	80.7
Isdammen	2	15.06	14.06	232,068	43,822	17,643	20	6	172,459	74.3
				1,157,350	185,970	63,918	43	11	814,956	70.4
				1,237,160	206,429	73,314	35	11	709,969	57.4
	3	18.06	18.06	473,136	61,126	22,191	31	1	320,222	67.7
				729,380	139,982	54,601	52	16	531,215	72.8
	5	09.07	08.07	600,596	83,312	29,007	24	3	406,577	67.7
				14,679	4,885	1,651	4	1	22	0.1
				20,320	5,362	1,673	6	1	18	0.1
	7	15.07	16.07	1,200,840	167,202	52,197	70	3	956,950	79.7
	9	29.07	28.07	1,429,180	185,224	53,435	87	11	1,149,854	80.5
				15,178,036	2,103,391	687,150	724	94	11,176,832	60,5
All										

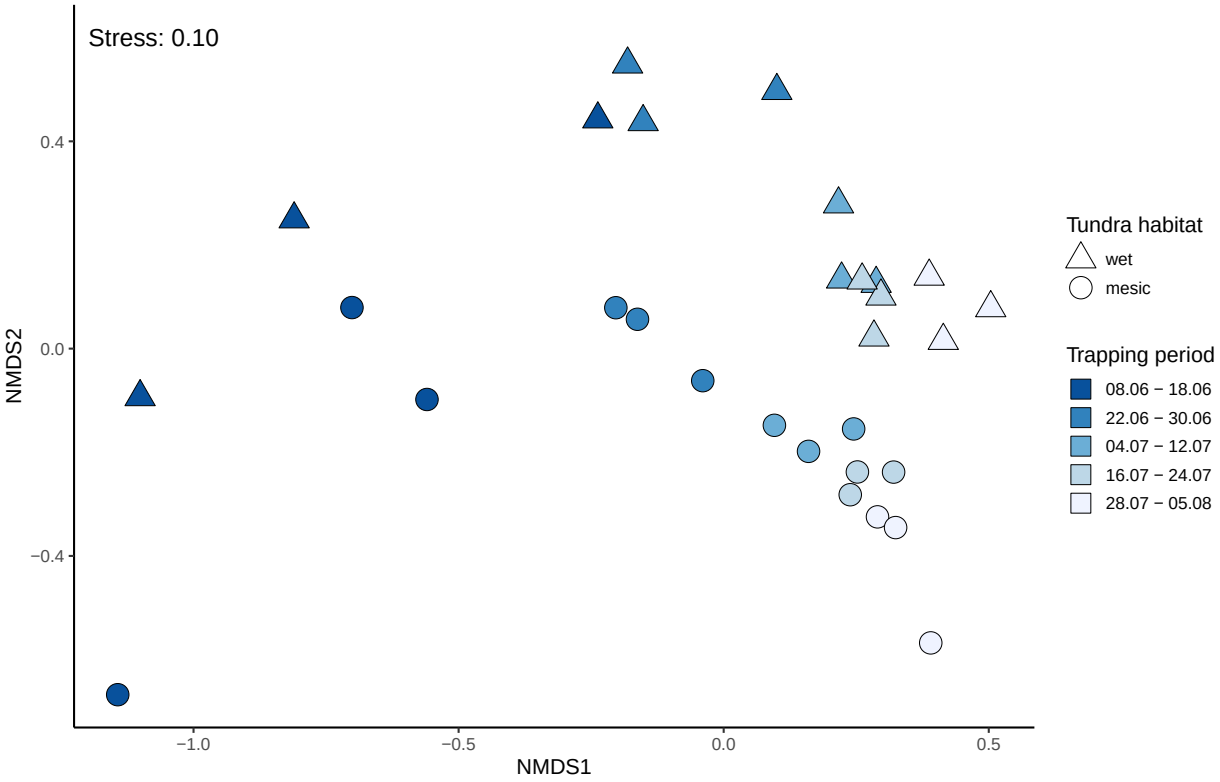

Figure A1: Non-metric multidimensional scaling (nMDS) of Bray-Curtis dissimilarities of log(x+1) transformed arthropod community as measured by pitfall trapping in Endalen, Svalbard in 2018. The differences in time (colour) and habitat (shape) of each pitfall trap sample are shown. The stress value is a goodness of fit measure that describes how well the two-dimensional representation fits the actual community dissimilarities with 0 being a perfect fit.

Table A2: Simplified ranking matrix for snow bunting selectivity for arthropod groups, based on relative read abundance (RRA) of arthropod DNA in individual faecal samples summed at nest-level and proportion of trapped prey taxa in the same period as the faecal sample was taken. The table is read row-wise; "+" indicates that the food item on the row was selected more and "-" that it was selected less than the one in the column. Significant differences are indicated by a triple sign as calculated by compositional analysis. Columns are labelled with abbreviated group names in the same order as the rows.

	Ara	Ant	Chi	Emp	Mus	Myc	Sca	Sci	Syr	Ten
Araneae	0	- - -	- - -	- - -	- - -	-	- - -	- - -	- - -	- - -
Anthomyiidae	+++	0	+	-	-	+++	- - -	+	-	-
Chironomidae	+++	-	0	- - -	- - -	+++	-	+	-	-
Empididae	+++	+	+++	0	-	+++	-	+	+	-
Muscidae	+++	+	+++	+	0	+++	-	+++	+	+
Mycetophilidae	+	- - -	- - -	- - -	- - -	0	- - -	-	- - -	- - -
Scathophagidae	+++	+++	+	+	+	+++	0	+++	+	+
Sciaridae	+++	-	-	-	- - -	+	- - -	0	-	-
Syrphidae	+++	+	+	-	-	+++	-	+	0	-
Tenthredinidae	+++	+	+	+	-	+++	-	+	+	0

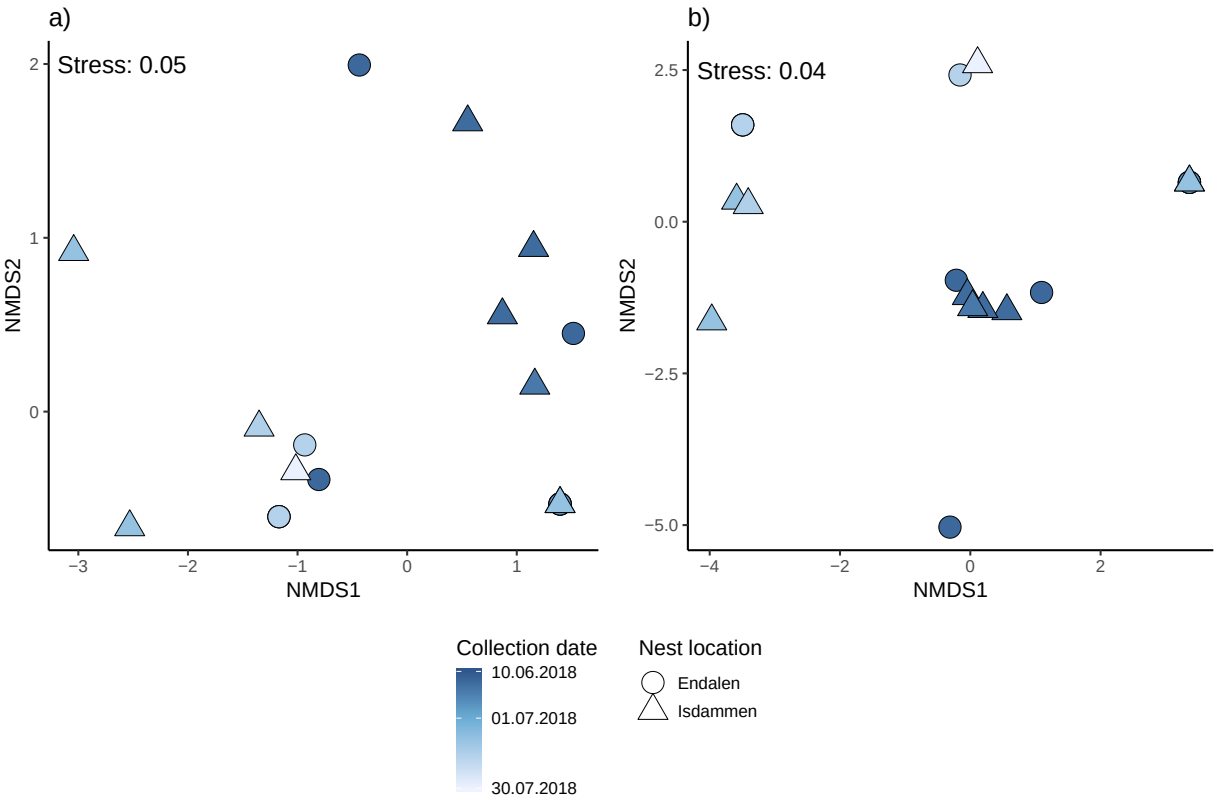

Figure A2: Non-metric multidimensional scaling (nMDS) of snow bunting diet dissimilarities showing the differences in time (colour) and habitat (shape) of each faecal sample. The stress value is a goodness of fit measure that describes how well the two-dimensional representation fits the actual community dissimilarities with 0 being a perfect fit. Data bases: a) Bray-Curtis dissimilarity of relative read abundance (RRA), b) Jaccard dissimilarity of occurrences (presence/absence)

Table A3: Simplified ranking matrix for snow bunting selectivity for arthropod groups, based on proportional occurrence of of arthropod DNA at nest-level and proportion of trapped prey taxa in the same period as the faecal sample was taken. The table is read row-wise; "+" indicates that the food item on the row was selected more and "-" that it was selected less than the one in the column. Significant differences are indicated by a triple sign as calculated by compositional analysis. Columns are labelled with abbreviated group names in the same order as the rows.

	Ara	Ant	Chi	Emp	Mus	Myc	Sca	Sci	Syr	Ten
Araneae	0	- - -	- - -	- - -	- - -	-	- - -	- - -	- - -	- - -
Anthomyiidae	+++	0	+	-	-	+++	-	+	-	-
Chironomidae	+++	-	0	- - -	- - -	+++	- - -	-	- - -	- - -
Empididae	+++	+	+++	0	+	+++	+	+	-	-
Muscidae	+++	+	+++	-	0	+++	-	+	-	-
Mycetophilidae	+	- - -	- - -	- - -	- - -	0	- - -	-	- - -	- - -
Scathophagidae	+++	+	+++	-	+	+++	0	+++	-	-
Sciaridae	+++	-	+	-	-	+	- - -	0	-	-
Syrphidae	+++	+	+++	+	+	+++	+	+	0	-
Tenthredinidae	+++	+	+++	+	+	+++	+	+	+	0

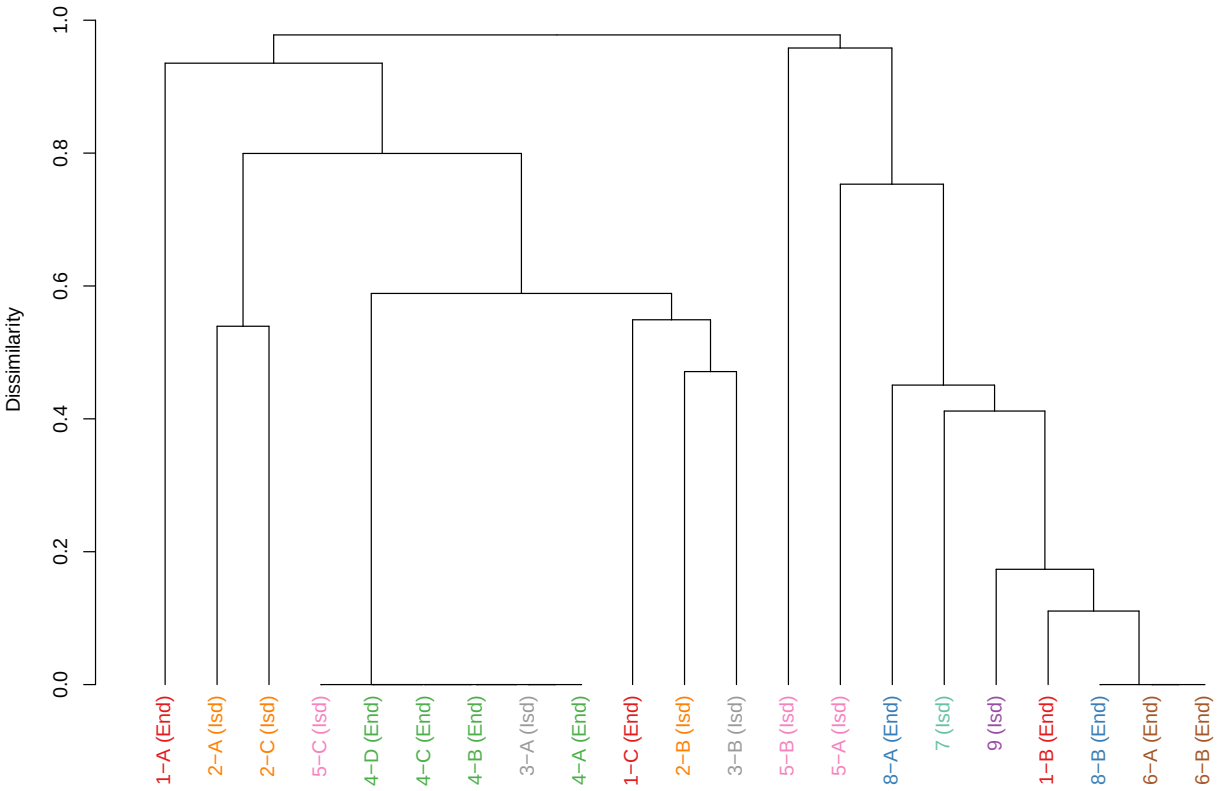

Figure A3: Hierarchical cluster analysis of individual faecal samples based on relative read abundance (RRA) of prey arthropod taxa. Clustering was performed with group-average sorting on the Bray-Curtis dissimilarities of the faecal sample arthropod communities.

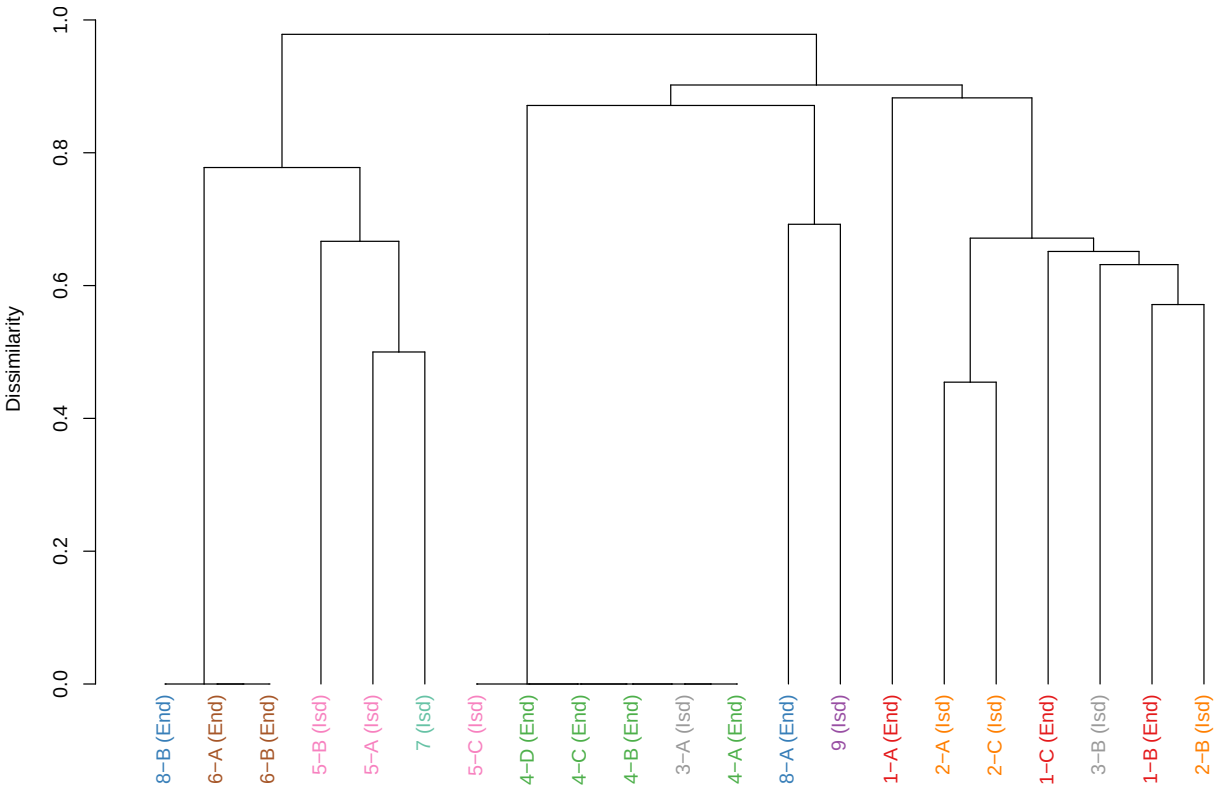

Figure A4: Hierarchical cluster analysis of individual faecal samples based on occurrence of prey arthropod taxa. Clustering was performed with group-average sorting on the Jaccard dissimilarities of the faecal sample arthropod communities.

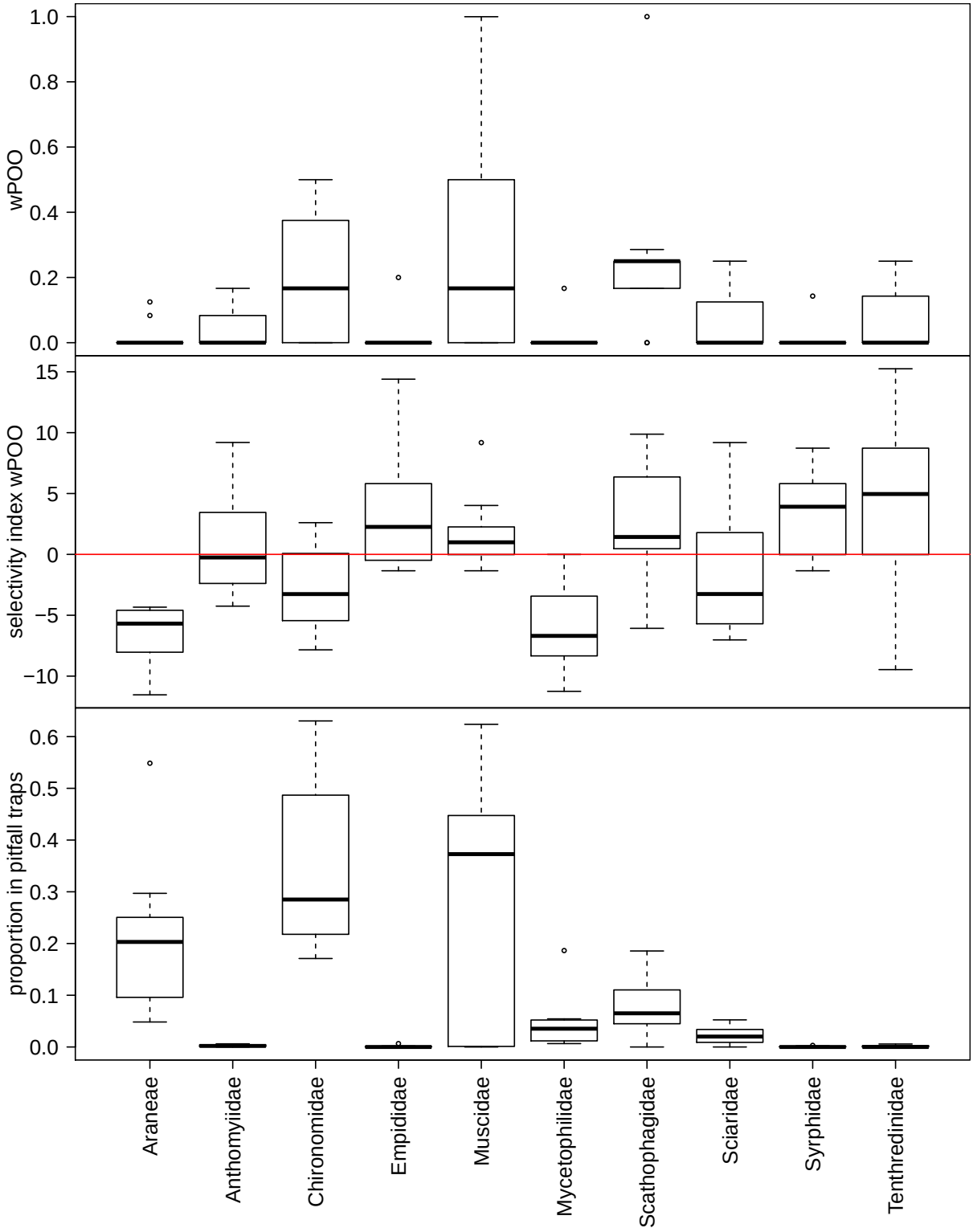

Figure A5: Snow bunting diet based on molecular scatology, selectivity and availability of arthropod groups based on pitfall trapping. The upper panel shows the weighted percentage of occurrence (wPOO) of identified prey taxa on nest-level summaries, the middle panel the selectivity index (see methods) and the lower panel the proportion in pitfall trap samples taken at the corresponding time. Positive selectivity values indicate selection towards the specific taxa, whereas negative values indicate avoidance. The midline represents the median, boxes upper and lower quartiles, whiskers values that lay within 1.5 times the interquartile range and points outliers