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NOTE

DNA metabarcoding of faecal pellets reveals high consumption of yew (*Taxus* spp.) by caribou (*Rangifer tarandus*) in a lichen-poor environment

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Abstract

Woodland caribou (*Rangifer tarandus caribou*) are threatened in Canada because of the drastic decline in population size caused primarily by human-induced landscape changes that decrease habitat and increase predation risk. Conservation efforts have largely focused on reducing predators and protecting critical habitat, whereas research on dietary niches and the role of potential food constraints in lichen-poor environments is limited. To improve our understanding of dietary niche variability, we used a next-generation sequencing approach with metabarcoding of DNA extracted from faecal pellets of woodland caribou located on Lake Superior in lichen-rich (mainland) and lichen-poor (island) environments. Amplicon sequencing of fungal ITS2 region revealed lichen-associated fungi as predominant in samples from both populations, but amplification at the chloroplast *trnL* region, which was only successful on island samples, revealed primary consumption of yew (*Taxus* spp.) based on relative read abundance (83.68%) with dogwood (*Cornus* spp.; 9.67%) and maple (*Acer* spp.; 4.10%) also prevalent. These results suggest that conservation efforts for caribou need to consider the availability of food resources beyond lichen to ensure successful outcomes. More broadly, we provide a reliable methodology for assessing ungulate diet from archived faecal pellets that could reveal important dietary shifts over time in response to climate change.

Key words: conservation, diet, DNA metabarcoding, ITS2, lichen, *trnL*, woodland caribou

Introduction

Caribou (*Rangifer tarandus*) are distributed globally throughout Arctic, sub-Arctic, and boreal forest regions (Banfield 1961). Predation, climate change, and habitat loss due to development and natural resource extraction have caused declines in population sizes across their range and continue to be major threats to their survival (Festa-Bianchet et al. 2011). In Canada, several ecotypes of caribou have been identified based on morphology, ecology, and genetic differences (COSEWIC 2002; Klütsch et al. 2016) with further classification into designatable units (DUs) for conservation (COSEWIC 2011). Within Canada, boreal caribou within central Ontario (also known as

forest-dwelling woodland caribou) are listed as Threatened under the Federal *Species at Risk Act* (COSEWIC 2014) and under *Ontario's Endangered Species Act*, 2007.

Boreal woodland caribou typically select mature conifer stands where they can forage on ground lichen (*Cladina* spp.) (COSEWIC 2002; McGreer et al. 2015; McNeill et al. 2020). Lichen colonizes soil and land plants (Favero-Longo and Piervittori 2010) and is formed through a symbiotic relationship between a fungus (mycobiont) and a population of algae (photobionts) (Bates et al. 2011; Banchi et al. 2018). Although traditionally thought to involve only a single fungal species, lichen is increasingly thought to include two or more species that make up the mycobiome of the lichen (Spribille et al. 2016; Banchi et al. 2018; Tuovinen et al. 2019). Caribou are unusual in that they are one of only a few animals for which lichen typically makes up 46%–70% of their diet (Thompson et al. 2015). Even in summer months when green plants are widely available, observational accounts suggest significant reliance of woodland caribou on lichen (Thompson et al. 2015; McNeill et al. 2020). However, Denryter et al. (2017) suggested this reliance on lichen is an oversimplification and that studies of caribou diet are often limited by seasonal sampling and biases associated with traditional postingestion techniques such as analysis of rumen contents and faecal microhistology that can lead to poor taxonomic resolution. A combination of observational video, microhistological faecal preparations, and a DNA barcoding approach with cloning and Sanger sequencing suggests that DNA-based approaches provided substantially higher taxonomic resolution (94% identified to species) over video (42% identified to species) and histological approaches (<15% identified to species) (Newmaster et al. 2013).

In Ontario, woodland caribou populations in the Lake Superior Coastal Range (**Fig. 1**) were declining dramatically in the early 1980s, so in 1982 seven individuals (1 male, 3 adult females with 3 female calves) were translocated from the Slate Islands in northern Lake Superior to join a single adult male on Michipicoten Island (Bergerud et al. 2007; Drake et al. 2018), a large, predator-free island in Lake Superior (**Fig. 1**). An additional male was added to the island in 1989 (Bergerud 2007). The caribou population on Michipicoten Island likely numbered > 500 caribou by winter 2014 (B.R. Patterson unpublished data). The vegetation on Michipicoten Island differs from that of the mainland in that lichen is very limited (Bergerud et al. 2007). Observational accounts suggest that caribou on the island have a more deer-like diet that includes leafy material, twigs, and buds from deciduous trees and shrubs (B. R. Patterson, personal communication), but this has not been confirmed with any directed diet analysis. During 2014 and 2015, wolves crossed ice bridges that temporarily connected the island to the mainland and caribou became the primary food source for the colonizing wolves. As wolf numbers increased, caribou numbers decreased, and concerns were raised about the persistence of caribou on the island so some of the remaining caribou were translocated back to the Slate Islands and Caribou Island in the winter of 2018.

Caribou recovery strategies have mainly focused on predation and habitat loss (Environment Canada 2012) with little research on diet diversity in environments with variable flora (but see also McNeill et al. 2020 and references contained therein). Currently, the diet of caribou across its various ecotypes is not well understood (Newmaster et al. 2013), but clarifying variable dietary habits is important for conservation and management policy to ensure responses such as translocation efforts are successful. This information will become increasingly important as lichen availability shifts in response to climate change and habitat alteration associated with development (Allen et al. 2019).

The approach of metabarcoding environmental DNA (eDNA) followed by next-generation sequencing has recently become a favoured approach for species identification in environmental samples, because it provides a relatively fast and efficient way to obtain species-specific data by simultaneously sequencing DNA from complex mixtures. This method has been successful in a variety of environmental DNA studies, including diet analysis (Berry et al. 2017), whereby DNA metabarcoding

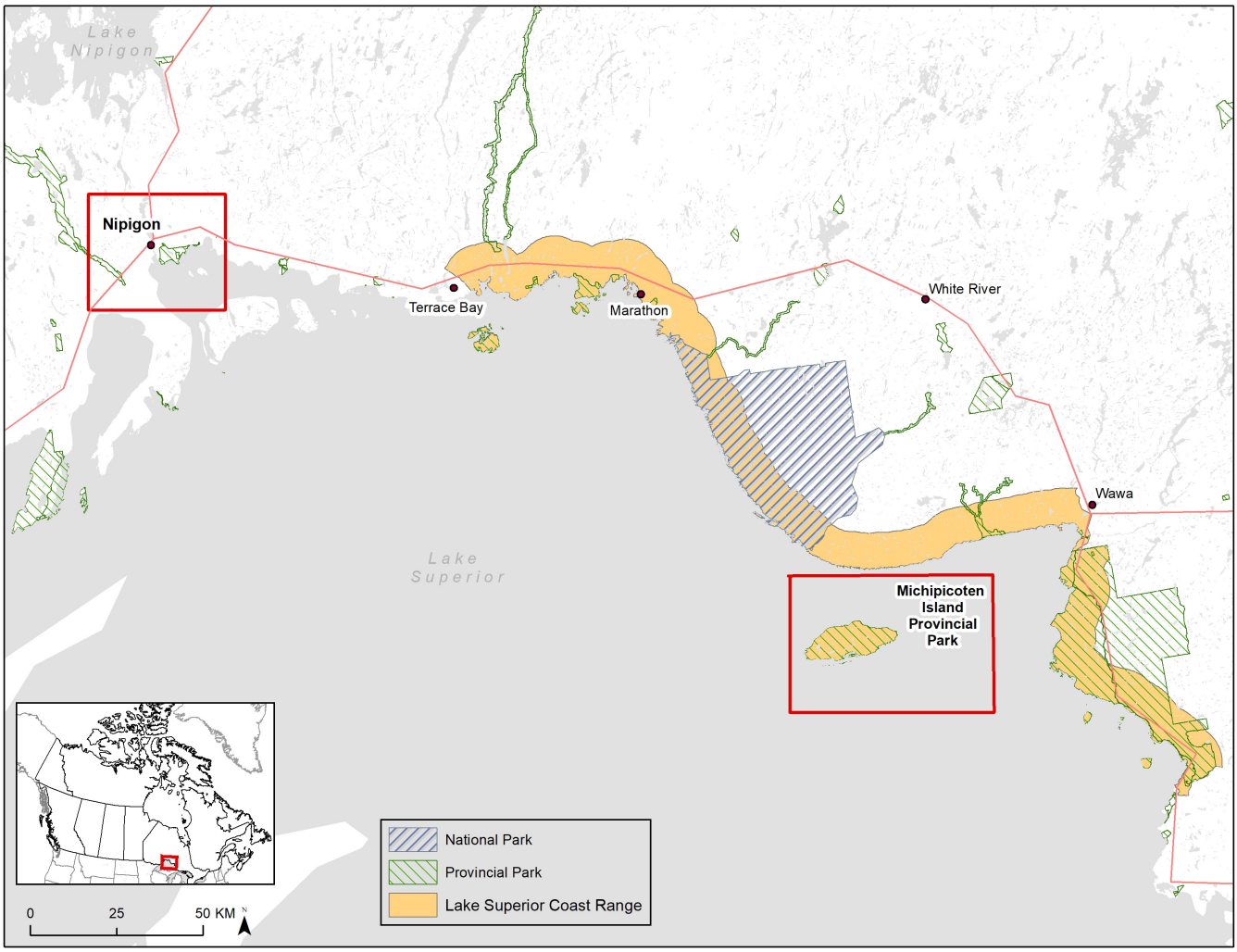

Fig. 1. Caribou faecal pellet sampling locations of Nipigon and Michipicoten Island on Lake Superior, Ontario, Canada. The base layer is from the Government of Canada, Natural Resources Canada, Earth Sciences Sector, Geomatics Canada, Surveyor General Branch. The Lake Superior Caribou Coast Range is from the Government of Canada's "Anthropogenic disturbance footprint within boreal caribou ranges across Canada (Updated 2012)" available from open.canada.ca/data/en/dataset/890a5d8d-3dbb-4608-b6ce-3b6d4c3b7dce. The projection is Canada LCC.

involves amplifying DNA from faeces with taxon-specific primers that target specific regions within the DNA (Creer et al. 2016). With careful selection of molecular markers and access to sequence databases, forage species can be reliably identified in herbivores (Kartzinel et al. 2015) and prey species can be revealed in the scat of carnivores (Monterroso et al. 2019). Overall, this process allows for a relatively inexpensive approach to identify numerous target species within a single sample (Fonseca 2018) and is quickly becoming the favoured approach to assess dietary habits of aquatic and terrestrial animals (de Sousa et al. 2019).

To develop a better understanding of the potential for woodland caribou to adapt their diet to different environments, we used DNA metabarcoding and next-generation sequencing to analyze caribou scat samples collected in a lichen-rich environment on the mainland (Nipigon region) and in a lichen-poor environment on Michipicoten Island (**Fig. 1**). We used two different markers, one to

capture the diversity of green plants (*trnL* region of the chloroplast genome) and another to identify lichen-associated fungal species (ITS2 region of the nuclear genome). Taxonomic identification of the lichen through amplification of the ITS2 region of the associated fungal species has been successful with previous Sanger sequencing methods (Newmaster et al. 2013). Although caribou diet shifts seasonally (Bergerud 1972; Thompson et al. 2015; Denryter et al. 2017), this research focuses on the winter diet due to the ease of scat collection in winter months and the availability of previously collected samples.

Materials and methods

Sampling, DNA extraction, and amplification

Archived caribou scat samples were used for this study. Samples were collected between February and March of 2007 from the Nipigon mainland region (n = 10) and from Michipicoten Island on 27 February 2006 (n = 10) (**Fig. 1**) and stored at −20 °C. Although the Michipicoten samples were collected on a single day, they were collected by at least two different field biologists and each sample represents a different pellet cluster. Additional metadata (e.g., GPS coordinates) are unavailable for these archived samples. Additionally, scat samples from captive reindeer *(Rangifer tarandus)* were collected in September 2018 from the enclosure at the Peterborough Riverview Zoo as a positive control to validate our methods (n = 3). Although visibly fresher samples were chosen from the reindeer enclosure, the actual age of each sample is unknown. Zoo reindeer were fed a diet of Zukudla Herbivore Pellets along with alfalfa, various browse (willow, dogwood, poplar, and maple) when available, and a small daily amount of apples and carrots (4 apples and 2 carrots) were made available. These animals are also free to eat available browse within the outdoor enclosure. The commercial pellets include (in order of abundance) wheat shorts, soybean meal, oat screenings, soy hulls, ground wheat, alfalfa, barley, beet pulp, ground flax, and wheat middlings. Although it is expected that the captive animals consumed primarily the pellets and woody browse, the specific daily relative consumption patterns of each item on any given day is not known.

For the samples collected from Nipigon and Michipicoten regions, two extraction replicates of 10 samples from each location were used with an additional two (*trnL*) or three (ITS2) positive control samples from the zoo. Faecal samples were prepared by thawing individual pellets, mashing within the collection bag, and then collecting approximately 50 mg per extraction into lysis buffer (Zymo Research Corporation, Irvine, CA). DNA was extracted with the Zymo Quick-DNA Faecal/Soil Microbe Kit (Zymo Research Corporation, Irvine, CA) according to manufacturer's instructions. Negative controls were used throughout the extraction process to track any possible contamination. DNA from all samples was recovered by elution in a 100 µL volume of low TE (Tris-ethylenediaminetetraacetic acid (EDTA); 10 mM Tris pH 8, 0.1 mM EDTA).

Test amplification for both markers (*trnL* and ITS2; **Table 1**) was done on DNA from three zoo samples and three previously extracted water hyacinth (*Eichhornia crassipes*) samples. Each polymerase chain reaction (PCR) used 1.0 mM PCR buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 mM bovine serum albumin (BSA), 0.3 µM of each primer, 0.025 U of *Taq* DNA polymerase, and 2 µL of DNA template in a final volume of 10 µL. Amplicons were visualized with gel electrophoresis on a 1.5% agarose gel containing Ethidium Bromide to ensure amplification of targeted size fragment.

Library preparation

To determine the optimal annealing temperatures, a temperature gradient PCR was conducted for both primer pairs using the following annealing temperatures; 63.8 °C, 59.1 °C, 55.7 °C, 63.8 °C and 51 °C. The 25 µL reaction consisted of 12.5 µL 2 × NEBNext Q5 Ultra II Q5 Master Mix

Table 1. Sequences of primer pairs used to amplify the *trnL* region of the chloroplast (GP6F & HP6R) and the ITS region of the fungal nuclear genome (ITS86F & ITS4R).

Primer Name	Primer Sequence (5′ – 3″)	Reference
GP6F	GGGCAATCCTGAGCCAA	Taberlet et al. (2007)
HP6R	CCATTGAGTCTCTGCACCTATC	Taberlet et al. (2007)
Adapt_GP6F	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGGCAATCCTGAGCCAA	
ADAPT_HP6R	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CCATTGAGTCTCTGCACCTATC	
ITS86F	GTGAATCATCGAATCTTTGAA	Op De Beeck et al. (2014)
ITS4R	TCCTCCGCTTATTGATATGC	Op De Beeck et al. (2014)
Adapt_ITS86F	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GTGAATCATCGAATCTTTGAA	
Adapt_ITS4R	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCCTCCGCTTATTGATATGC	

Note: Sequences that are bold and underlined are the Illumina overhang sequences added to allow dual indexing.

(New England BioLabs, Whitby, ON, Cat No: M0543S), 0.3 µM of each primer (with Illumina overhang adaptor sequences (Table 1)), 4 µL of DNA template and 7 µL molecular grade DNAase/RNAase free water. The amplification included four replicates of DNA from a zoo faecal sample (unquantified), and four replicates of DNA previously extracted from water hyacinth (standardized to 2 ng/µL) and a PCR negative. The reactions were carried out under the following conditions: initial denaturation at 98 °C for 30 seconds, denaturation at 98 °C for 10 seconds, annealing (63.8–51 °C) for 30 seconds and extension at 65 °C for 45 seconds, followed by 35 cycles and a final extension at 65 °C for 5 minutes. Based on these results, 62 °C was selected for future annealing temperature for the first stage PCR amplification in the library preparation protocol. All samples were independently amplified twice, and duplicate PCR products were pooled prior to indexing. Samples from both sampling locations and the zoo amplified at the ITS2 region, but samples from Nipigon did not amplify at the *trnL* region and were therefore excluded from further analysis at this DNA marker.

Following the first-stage PCR, the pooled *trnL* amplicon PCR products were cleaned with 1.5X AMPure magnetic beads (Beckman Coulter, Mississauga) and the ITS2 amplicon PCR products were cleaned with 0.8X AMPure magnetic beads according to methods described in the 16S Illumina Metagenomic Sequencing Library Preparation protocol. The cleaned amplicon PCR product was visualized with an Agilent Tape Station 4200 (Agilent Technologies, Mississauga) with the D1000 reagent kit according to manufacturer's directions to ensure amplification of target fragments. A second PCR was used to attach unique Nextera XT Dual Indices to our amplicon product (Nextera XT 24 Index kit; Illumina, Cat No. FC-131-1001) according to manufacturer's instructions. Briefly, we used 25 µL of the 2x NEBNext Q5 Ultra II Q5 Master Mix, 5 µL of each Nextera XT index primers, 10 µL of DNase/RNase free molecular grade water and 5 µL of the amplicon DNA template. The index PCR included 10 cycles with the following conditions: initial denaturation at 98 °C for 30 seconds, denaturation at 98 °C for 10 seconds, annealing/extension at 65 °C for 75 seconds followed by final extension at 65 °C for 5 minutes. A second bead clean-up was done on the indexed PCR product with the same reagents and protocol as the first PCR bead clean-up with the exception that we used 1.12X (56 µL) of AMPure magnetic beads to capture our target fragment. Cleaned product was quantified with the High Sensitivity assay on a Qubit Fluorometer (Thermo-Fisher Scientific, Markham). Sample concentration was converted to nM and samples were standardized to 4 nM with elution buffer (EB; 10 mM Tris-Cl, pH 8.5) (Qiagen, Toronto). Samples at 4 nM were pooled (5 µL each) and the final library was visualized on the Agilent 4200 Tape Station and quantified with Qubit Fluorometer. In summary, 5 µL of all the Michipicoten (n = 10), Nipigon (n = 10), and zoo

(n = 3) ITS2 samples were combined to form a single library (n = 23) and 5 uL of the Michipicoten (n = 10) and zoo (n = 2) *trnL* samples were combined into a second library (n = 12) for independent sequencing.

Sequencing, classification, and analysis

Pooled libraries were diluted to 6 pM and denatured with sodium hydroxide (NaOH) diluted with hybridizing buffer followed by heat denaturation according to Illumina sequencing instructions. Sequencing of the ITS2 library was completed with the MiSeq Reagent Nano Kit v2 (500 cycles; MS-103-1003), and for the *trnL* library we used the MiSeq Reagent Nano Kit v2 (300 cycles; MS-103-1002) with 2 million paired-end reads expected for each library. We included 30% PhiX (PhiX control kit v3, FC-110-3001) in each sequencing run to serve as an internal control and to ensure sufficient clustering on the flow cell when low diversity libraries are sequenced.

Sequences were automatically de-multiplexed on the MiSeq BaseSpace platform, and sequences were identified to species or genus following an adapted Metagenomics protocol in Geneious v 11.1.4 (Biomatters Ltd.). Briefly, reads were paired and trimmed to remove remaining Illumina adaptors, bases with a quality score below 30 and reads less than 100 bp (ITS) or 80 bp (*trnL*) were removed. Paired reads were merged to create a single consensus sequence. We then used the *de novo* assembly to cluster similar reads into operational taxonomic units (OTUs). We used a maximum mismatch per read of 2%, maximum per read gap of 1%, and minimum overlap identify of 98%. To create an ITS2 fungal taxonomy database locally within Geneious we: (*i*) downloaded the NCBI ITS project database (Fungi RefSeq ITS), (*ii*) uploaded it into Geneious, (*iii*) batch blasted the OTU consensus sequences against the Fungi RefSeq ITS database within Geneious with a maximum hit of 1 and scoring mismatch of 1–2, (*iv*) removed duplicates, (*v*) downloaded the hits list, and (*vi*) extracted the BLAST hit regions to create a database for sequence classification. We then classified the ITS2 amplicons with Geneious Sequence Classifier plug-in with high sensitivity and minimum overlap of 95%. We summarized the hits based on sequences that could be classified to the genus level, and then combined hits for each of Michipicoten, Nipigon, and zoo samples. Taxa that had fewer than 20 total reads were excluded. The process for classifying the *trnL* sequences was similar, except that initial BLAST of OTU consensus sequences was done within the online NCBI nucleotide database, and samples only include Michipicoten and two zoo samples because amplification at this marker was unsuccessful on the Nipigon samples. The third zoo sample (Sample Zoo1) was not included in the *trnL* library due to limited availability of DNA for the that sample. Only sequences that were at least 80 bp in length and had 100% identity based on BLAST results were included in the *trnL* summaries.

Relative read abundance was calculated for each detected item (RRA_i) based on sequence counts for both markers according to the following formula (Deagle et al. 2019):

$$\mathrm{RRA}_i = \frac{1}{S}\sum_{k=1}^{S}\frac{n_{i,k}}{\sum_{i=1}^{T} n_{i,k}} \times 100$$

where S is the number of samples, T is the number of taxa, and $n_{i,k}$ is the number of sequences for item i in sample k. To explore consistency of plant taxa detected across samples, we used *ggplot2* (Wickham 2016) within the R (R Core Team 2020) to create stacked bar charts of RRA for individual samples from Michipicoten and the zoo samples.

Results

ITS2

The ITS2 library produced 1,019,901 total reads with an average Q30 of 83.02%. Overall, the variety of fungal species detected was highest in the zoo samples (S = 17), but none seem to be affiliated with lichen (Supplementary Material, Table S1). Within the wild samples, the number of fungal species detected was higher in Michipicoten (S = 13) compared to Nipigon (S = 10) (Table 2). The obligately

Table 2. Proportional representation of ITS2 fungal sequences identified in caribou scat samples from Michipicoten Island and Nipigon and for reindeer samples from the Peterborough Riverview Park and Zoo.

Classification (no. of samples)	Total reads	Relative read abundance, (%)	SD
Michipicoten Island			
Lichenoconium aeruginosum (10)	4116	70.54	19.44
Antennariella placitae (5)	675	7.62	9.83
Cladosporium antarcticum (5)	471	5.56	6.83
Stagonosporopsis lupini (3)	310	7.17	11.77
Sphaeropsis citrigena (1)	92	1.71	n/a
Nectriopsis lindauiana (1)	80	1.36	n/a
Talaromyces thailandensis (1)	71	1.21	n/a
Nothophoma macrospora (1)	68	0.69	n/a
Aureobasidium melanogenum (1)	61	0.54	n/a
Nigrograna norvegica (1)	58	0.99	n/a
Loratospora luzulae (1)	41	1.14	n/a
Diplodia sapinea (1)	39	0.66	n/a
Capnobotryella renispora (1)	37	0.80	n/a
Nipigon			
Lichenoconium aeruginosum (10)	2658	62.19	25.26
Antennariella placitae (3)	1408	8.77	19.61
Tremella aurantia (1)	410	1.89	n/a
Stagonosporopsis lupini (3)	403	12.71	22.69
Cladosporium antarcticum (4)	187	4.44	6.61
Sphaeropsis citrigena (2)	99	3.69	8.19
Nectriopsis lindauiana (1)	98	2.61	n/a
Penicillium spp. (2)	61	1.37	2.89
Gelasinospora saitoi (1)	54	1.44	n/a
Talaromyces coalescens (1)	33	0.88	n/a

Note: For Michipicoten samples, percentages are based on 6119 total classified reads; for Nipigon samples percentages are based on 5411 total classified reads. Numbers in parentheses beside each taxonomic group indicate the number of samples within which the taxon was identified. Average proportion (%) and standard deviation (SD) are based on proportions found within each of the 10 samples for each population; n/a indicates the taxon was found in only one sample so average proportion and SD are not reported. Species names in bold represent those found in both Michipicoten and Nipigon sampling locations.

lichen-associated (i.e., lichenicolous) fungus (*Lichenoconium aeruginosum*) was the most predominant species in Michipicoten and Nipigon samples with a relative read abundance (RRA) of 70.54% (±19.44 SD, n = 10) for Michipicoten samples and an overall RRA of 62.19% (±25.26 SD, n = 10) for the Nipigon samples, followed by *Antennariella placitae* (Michicpicoten = 7.62% ± 9.83 SD, n = 10; Nipigon = 8.77% ± 19.61% SD, n = 10), a fungus not known to be affiliated with lichen (**Table 2**). Both wild populations also had *Cladosporium antarcticum* (Michicpicoten = 5.56% ± 6.83 SD, n = 10; Nipigon = 4.44% ± 6.61 SD, n = 10) which is a species identified as "probably" lichenocolous (Lawrey and Diederich 2018). Overall, *L. aeruginosum* and *C. antarcticum* were the only two lichenocolous fungal species identified in our dataset. The percentage of unclassified reads due to low identity or no match was high in both Michipicoten (77.0% ± SD 3.6, n = 10) and Nipigon (77.5% ± SD 7.1, n = 10).

trnL

The *trnL* library produced a total of 1,150,042 reads with an average Q30 of 89.89%. In the zoo samples, the top five food items overlapped in both samples (**Fig. 2**) with cereal grasses (RRA = 39.59% ± 15.31 SD; n = 2), maple (RRA = 31.36% ± 35.9 SD; n = 2), and oats (RRA = 24.27% ± 26.76 SD; n = 2) making up over 95% of the RRA (**Table 3**). In most cases, the sequences had multiple species matches and lacked sufficient resolution to distinguish among species within a genus or family. However, given the diet of the captive animals, we can infer that the cereal grass is common wheat (*Triticum aestivum*), the oat is common oat (*Avena sativa*), the soybean/guar is cultivated soybean (*Glycine max*), and the burclover is alfalfa (*Medicago sativa*). These results are generally consistent with the known diet of these animals (see Materials and methods), although soybean was expected to have a higher RRA given that it is the second ingredient in the commercial pellets. Not all food items made available to the animals (e.g., apples/carrots) or that are known to be in the commercial pellets (e.g., beet pulp) were detected, most likely due to lack of consumption

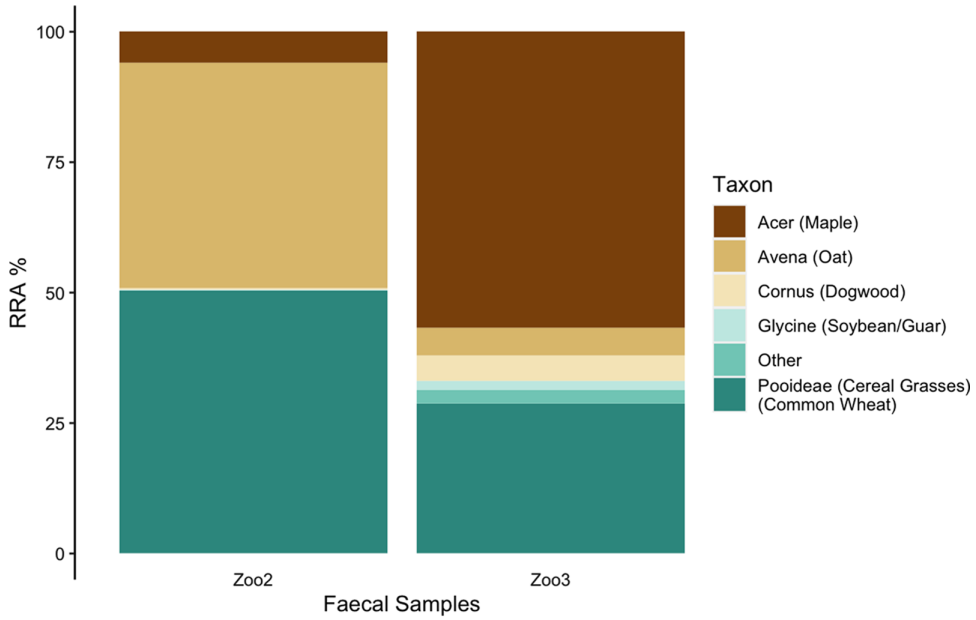

Fig. 2. Relative read abundance (RRA) as a percentage of *trnL* sequence read counts for each taxon in each zoo sample. Note that *Cornus* and *Glycine* were detected in sample Zoo2, but at comparatively low levels (0.291 and 0.130 %, respectively) and are not readily evident in the bar chart.

Table 3. Summary of *trnL* sequence matches found with sequencing of the *trnL* region in faecal samples from Peterborough Riverview Park and Zoo reindeer ($n = 2$).

Common name (no. of samples)	Scientific name	No. of sequence reads	Relative read abundance (%)	SD
Cereal grasses (2) (includes Common Wheat)	Poaceae: Pooideae (includes *Triticum aestivum*)	24,443	39.59	15.31
Maple (2)	*Acer* spp.	16,695	31.36	35.90
Oat (2)	*Avena* spp.*	15,948	24.27	26.76
Dogwood (2)	*Cornus* spp.	1,346	2.56	3.21
Soybean/guar (2)	*Glycine spp./Cyamopsis tetragonoloba*	487	0.92	1.12
Burclover (1) (includes alfalfa)	*Medicago spp.* (includes *M. sativa)*	148	0.29	n/a
Rose gamily (1)	Rosaceae	124	0.24	n/a
Grass family (1) (includes corn)	Poaceae (includes *Zea mays*)	140	0.27	n/a
Hackberry (1)	*Celtis* spp.	114	0.22	n/a
Aster/daisy family (1)	Asteraceae*	65	0.13	n/a
Brome grasses (1)	*Bromus* spp.	40	0.08	n/a
Toad rush (1)	*Juncus bufonius*	22	0.04	n/a
Barley (1)	*Hordeum vulgare*	21	0.04	n/a

Note: Reads reported are between 80 - 100 bp in length and had a 100% sequence match based on at least 20 sequence reads.
*Sequences where a single mismatch was attributed to an R designated nucleotide (which codes for G/A) in the contig compared to a G in the reference database. Total assigned reads per sample: Zoo 2 = 33,725; Zoo3 = 25868. Note that Sample Zoo1 was not sequenced at this marker.

on that particular day, low proportional representation in the commercial pellets, or lack of sequence resolution in the reference database.

The *trnL* amplification was unsuccessful on the Nipigon samples, but the most predominant species identified in all the Michipicoten caribou samples was yew (*Taxus* spp.), with an RRA of 83.68% (±9.62 SD; $n = 10$). This species is presumably Canada yew (*Taxus canadensis*) since that is a primary caribou forage species on Michipicoten Island (Kuchta 2012). Other species that were detected consistently include dogwood (RRA = 9.67% ± 7.92 SD; $n = 10$)), maple (RRA = 4.10% ± 2.62 SD; $n = 10$), and apple (*Malus* sp.; 2.14% ± 1.86 SD; $n = 10$) (Table 4). Overall, every sample had the highest RRA for yew (range: 72.13%–97.0%), with dogwood, maple, and apple making up most of the remaining reads (Fig. 3).

Discussion

Here, we demonstrate an optimized eDNA metabarcoding approach for taxonomic classification of plants and lichen in >10-year-old caribou faecal pellets. We found that metabarcoding and sequencing amplicons at the ITS2 region was useful for identifying lichen-associated fungi, although the methodology is limited by the reference samples in the fungal NCBI database. Similarly, DNA metabarcoding and sequencing of amplicons from the *trnL* chloroplast region provided reliable identification of plants at the genus (and sometimes family) level.

Lichen-associated fungus (*Lichenoconium aeruginosum*) was evident in both wild populations, but the majority of fungal species identified are not known to be associated with lichen. These are presumably from alternate environmental sources. The identification of lichen-associated fungi in the Michipicoten samples suggests that caribou do have access to at least some terrestrial lichen on the island. Fungal species within the *Lichenoconium* genus, including *L. aeruginosum,* are known to

Table 4. Proportional representation of plant sequences identified in caribou scat samples from Michipicoten Island ($n = 10$).

Classification (common came) (no. of samples)	Total reads per genus	Releative read abundance (%)	SD
Taxus sp. (Yew) (10)	266494	83.68	9.62
Cornus sp. (Dogwood) (9)	30717	9.67	7.92
Acer sp. (Maple) (10)	12798	4.10	2.62
Malus sp. (Apple) (10)	6752	2.14	1.86
Abies sp. (Fir) (7)	521	0.19	0.20
Viburnum sp. (Flowering Shrubs) (6)	256	0.08	0.09
Carpinus sp. (Hornbeam) (1)	131	0.049	n/a
Rubus sp. (Raspberry/Blackberry) (2)	109	0.04	0.08
Solidago sp. (Goldenrod) (2)	89	0.03	0.06
Prunus sp. (Pitted Fruit Tree) (1)	54	0.014	n/a
Achillea sp. (Yarrow) (1)	46	0.017	n/a
Glycine sp. (Soybean) (1)	44	0.009	n/a
Sambucus sp. (Elder) (1)	25	0.005	n/a

Note: Sequences were included that had at least 20 reads that were identified to at least the genus level. Percentage of total reads is based on a total of 318 036 total assigned reads. Numbers in parentheses beside each taxonomic group indicate the number of samples within which the taxon was identified. Relative read abundance (RRA; %) and standard deviation (SD) are based on average proportion of reads across the 10 samples; n/a indicates the taxon was found in only one sample so SD is not reported.

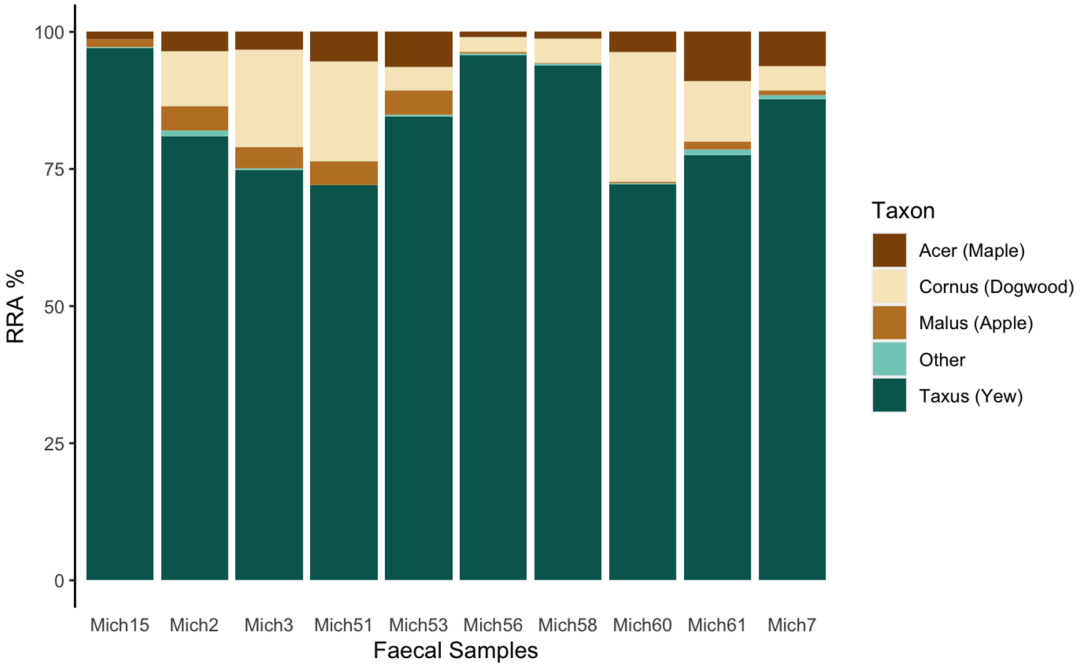

Fig. 3. Relative read abundance (RRA) as a percentage of *trnL* read counts for each taxon in each Michipicoten Island sample.

associate with *Cladonia* spp. of lichen (Jando et al. 2000; Zhurbenko and Pino-Bodas 2017), the predominant terrestrial lichen available to woodland caribou in Ontario (Newmaster et al. 2013). Whether or not caribou will access lichen sources in their most recent translocation environment back on the Slate Islands, where arboreal lichen sources are more readily available than terrestrial sources (Bergerud et al. 2007), will require sampling and analysis of caribou faecal pellets from that location. The high percentage of unclassified reads suggests that identification may be limited by the NCBI ITS2 fungal reference database and so a targeted effort to sequence the ITS2 region of various arboreal and terrestrial lichen could improve the database and potentially increase species assignments.

Although the *trnL* region only amplified in zoo and Michipicoten samples, the results provide support for our methodology and insight into the reliance of island caribou on alternative plant sources. In the zoo samples, the predominance of sequence reads from the three main ingredients in the commercial pellets (wheat, oats, soybean) and the supplemental woody browse (maple and dogwood) provide support that sequence reads reflect relative abundance, at least in broad terms. Although not every item listed in the commercial pellets nor every supplemental item made available was detected in the zoo samples, it is possible that items not detected were in small quantities in the pellets and (or) that high consumption of woody browse (e.g., maple) on any given day may have impacted the relative abundance in the faecal pellets collected. Alternatively, the parameterization and thresholds set for analysis may also have contributed to lack of detection of food items consumed in small quantities.

Woodland caribou are often considered to be obligate lichen foragers. It may seem surprising then that all individual Michipicoten samples had yew as the predominant plant consumed, and that on average yew accounted for >80% of the sequences, since *Taxus* spp. contain relatively high concentrations of taxine alkaloids and are generally toxic to domestic grazers (reviewed by Wilson et al. 2001 and Windels and Flaspohler 2011). Wild cervids, however, readily browse on yew, and toxic effects have rarely been documented (e.g., Handeland 2008). Also, a decline in yew is often associated with overgrazing by ungulates. For example, the disappearance of Canada yew from Isle Royale within 2 decades of the arrival of moose in 1909 is well documented (Murie 1934; Snyder and Janke 1976). Similarly, Canada yew was considered functionally extirpated from the Slate Islands by 1985 (Bergerud et al. 2007), putatively due to overbrowsing by caribou. In contrast, the high consumption of yew found in our study is consistent with a forage survey conducted on Michipicoten in 2011 that determined yew to be the dominant available forage for caribou despite almost three decades of increasing caribou abundance on the island (Kuchta 2012).

The heavy consumption of yew by Michipicoten Island caribou has been noted in historical observational accounts (Eason pers. Comm. Cited in Cumming 1992), but unconfirmed until now. Although yew is abundant on Michipicoten, it is rare in more northern regions of Ontario (Cumming 1992), making it an unlikely food source in more northern landscapes. The near-extirpation of yew from the Slate Islands, and the fact that caribou were known to be threatened by food limitations there in the past (Bergerud et al. 2007), may impact the fitness of caribou that have been recently translocated back there. Green forage plants provide higher protein (>30%) compared to terrestrial lichens (<4%) and should be preferred when available, but lichens are known to be important year-round even when alternatives are available (Thompson et al. 2015). Continued monitoring of the diet and fitness of caribou in different environments with variable browse will provide important information on dietary shifts of caribou in response to environmental changes and food accessibility.

The ability of DNA metabarcoding to correlate sequence reads with proportional consumption has been controversial. Issues surrounding variable DNA copies across tissue sources, DNA degradation, and potential preferential binding of primers to specific species template have been presented as challenges to proportional consumption based on sequence read frequency (Pompanon et al. 2012).

However, Kartzinel et al. (2015) found that mean sequence RRA of *trnL* sequences was a reliable predictor of proportional consumption based on correlation with stable isotope analysis, although they focus on coarse taxonomic differentiation of plant families. The main food items identified in our *trnL* analysis were yew (Taxacea: *Taxus*), dogwood (Sapindaceae: *Cornus*), maple (Cornales: *Acer*), and apple (Rosaceae: *Malus*), which all belong to different plant families, and therefore most likely reflect some relative level of biomass. Furthermore, Deagle et al. (2019) reviewed the topic of interpreting sequence counts and conducted simulations to conclude that, in general, relative read abundance provides a more accurate view of diet analysis than does frequency of occurrence, particularly when the same food taxa occur across many samples. They also noted that sequence read counts are most reliable for population-level diet analysis where common items are represented by the highest number of reads. Our *trnL* data reveal yew as the highest RRA in every sample, suggesting this species made up the bulk of caribou plant diet at that particular time. Furthermore, the results from the zoo samples, while imperfect and not specifically designed as a controlled feeding trial, do reflect the major food items made available. Therefore, it seems a reasonable conclusion that the high proportion of yew sequences found in our samples is representative of relative population-level consumption during winter.

We faced two main challenges with this work. First, the ITS2 analysis was limited by the reference sequences of the fungal NCBI database. Database coverage is often a limiting factor in DNA metabarcoding studies (Pompanon et al. 2012), especially for uncommon sequencing regions like ITS2 and *trnL*. The most common databases utilize mitochondrial DNA markers, especially cytochrome oxidase I (e.g., Barcode of Life Data (BOLD; boldsystems.org) developed at the Centre for Biodiversity Genomics in Canada, and the International Barcode of Life (iBOL; ibol.org), which are often not useful for dietary analysis where alternate markers are often used. In these cases, making a customized database may be required to fully disentangle dietary niches (Deagle et al. 2009; Rayé et al. 2011). To more thoroughly assess lichen diversity and alternative plant sources in caribou diet, we recommend collection of lichen and flora samples from faecal collection sites to create independent and comprehensive databases for regional lichen (ITS2) and plant (*trnL*) sequences. In doing so, future work will be able to more accurately assess dietary needs of caribou by identifying lichen-associated fungi and plant sequences more reliably to the species level.

Second, amplification at the *trnL* region failed in the mainland Nipigon samples, even though the samples were of similar age to the Michipicoten samples where amplification was successful. Degradation of DNA can be problematic in diet analysis studies and is often the cause of failed amplification (Deagle et al. 2006). However, the *trnL* primers amplify a chloroplast fragment that is about half the size of the nuclear ITS2 fragment we successfully amplified in all samples, which suggests DNA was not degraded in the Nipigon samples. Also, the *trnL* primers amplify a wide variety of plant species (Taberlet et al. 2007), so it is unlikely that mismatched primer binding sites were the reason for failed amplification. Therefore, the failed amplification may truly be reflective of the late winter diet of mainland caribou in which they rely heavily, and possibly exclusively, on lichen in regions where it is abundant and accessible (Thompson et al. 2015). Further exploration with larger sample sizes and broader temporal sampling would help corroborate our results.

Maintaining and stabilizing woodland caribou populations is a key objective for caribou conservation and recovery in Canada (Environment Canada 2012). Predation, industrial development, and climate change have been identified as the major threats to caribou persistence (Festa-Bianchet et al. 2011), but the variability in food resources and bottom-up regulation has received limited recent attention for woodland caribou populations (but see Bergerud 1972, 1996; Newmaster et al. 2013). Although lichen is recognized as a critical winter food source for caribou, the dependence on alternative dietary sources for woodland caribou in lichen-poor environments has not been fully recognized. Our results suggest that yew (*Taxus* spp.) provides a substantial proportion of dietary needs to caribou

on Michipicoten Island and may be the preferred browse when lichen is less available. We recognize, however, that interpretation is limited by small sample sizes and that our results do not exclude the possibility that other species (e.g., dogwood, maple) could be heavily targeted in the absence of yew (Bergerud 1996). We also recognize that it is currently unknown if yew contributes significantly to the overall caloric and nutritional requirements of caribou or if it serves primarily the needs of the gut microbiome. Regardless, our results are important for conservation efforts that include translocation of animals to environments with variable flora, or where yew or other woody browse is inaccessible or overgrazed, such as the Slate Islands (Bergerud 1996). Although the current lack of wolves on the Slate Islands will limit top-down regulation, the lack of yew and maple as an alternative food source could impact caribou viability through bottom-up limitations (Bergerud et al. 2007; Thompson et al. 2015). Although caribou diet may be region specific, with different food resources supporting different caribou populations, the nutrient availability in the variable forage will also need to be considered.

Overall, our results provide preliminary eDNA data that corroborates historical accounts of the diet versatility of caribou (Bergerud 1972) and flags the importance of variable food resources to caribou persistence. Furthermore, we document a reliable methodology that can be applied to historical/archived samples to support caribou conservation on the Slate Islands, but more broadly to ungulate species across more extensive spatial and temporal scales.

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Author contributions

GM and LYR conceived and designed the study. PJW, MM, BR, BRP, and LYR were responsible for project administration. GM and LYR performed the experiments, collected the data, and analyzed and interpreted the data. PJW, MM, BR, BRP, and LYR contributed resources. GM and LYR drafted the initial manuscript. LYR revised the manuscript. MM, BRP, PJW, LYR edited the manuscript.

Competing interest statement

Authors have no competing interests.

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Data availability statement

Sequence data is available from the Dryad Digital Repository: doi.org/10.5061/dryad.hqbzkh1j3.

Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2021-0071.

Supplementary Material 1

References

Allen JL, Mcmullin RT, Tripp EA, and Lendemer JC. 2019. Lichen conservation in North America: A review of current practices and research in Canada and the United States. Biodiversity and Conservation, 28: 3103–3138. DOI: 10.1007/s10531-019-01827-3

Banchi E, Stankovic D, Fernández-Mendoza F, Gionechetti F, Pallavicini A, and Muggia L. 2018. ITS2 metabarcoding analysis complements lichen mycobiome diversity data. Mycological Progress, 17: 1049–1066. PMID: 30956650 DOI: 10.1007/s11557-018-1415-4

Banfield AWF. 1961. A revision of the reindeer and caribou, genus Rangifer. National Museum of Canada, Bulletin No. 177. Queen's Printer, Ottawa. 137 p.

Bates ST, Cropsey GWG, Caporaso G, Knight R, and Fierer N. 2011. Bacterial communities associated with lichen symbiosis. Applied and Environmental Microbiology, 77: 1309–1314. PMID: 21169444 DOI: 10.1128/AEM.02257-10

Bergerud AT. 1972. Food habits of Newfoundland Caribou. The Journal of Wildlife Management, 36: 913–923. DOI: 10.2307/3799448

Bergerud AT. 1996. Evolving perspectives on caribou population dynamics, have we got it right yet? Rangifer, Special Issue No. 9: 95–116.

Bergerud AT. 2007. The need for the management of wolves – an open letter. Rangifer, 27: 39–50. DOI: 10.7557/2.27.4.319

Bergerud AT, Dalton WJ, Butler H, Camps L, and Ferguson R. 2007. Woodland caribou persistence and extirpation in relic population son Lake Superior. Rangifer, 27: 57–78. DOI: 10.7557/2.27.4.321

Berry TE, Osterrieder SK, Murray DC, Coghlan ML, Richardson AJ, Grealy AK, et al. 2017. DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Noephoca cinerea*). Ecology and Evolution, 7: 5435–5453. PMID: 28770080 DOI: 10.1002/ece3.3123

COSEWIC. 2002. COSEWIC assessment and update status report on the woodland caribou Rangifer tarandus caribou in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa. xi + 98 pp.

COSEWIC. 2011. Designatable units for Caribou (*Rangifer tarandus*) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 88 pp.

COSEWIC. 2014. COSEWIC assessment and status report on the Caribou *Rangifer tarandus*, Newfoundland population, Atlantic-Gaspésie population and Boreal population, in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa. xxiii + 128 pp. [online]: Available from registrelep-sararegistry.gc.ca/default_e.cfm.

Creer S, Deiner K, Frey S, Porazinska D, Taberlet P, Thomas WK, et al. 2016. The ecologist's field guide to sequence-based identification of biodiversity. Methods in Ecology and Evolution, 7: 1008–1018. DOI: 10.1111/2041-210X.12574

Cumming HG. 1992. Woodland caribou: Facts for forest managers. The Forestry Chronical, 68: 481–491. DOI: 10.5558/tfc68481-4

Deagle BE, Eveson JP, and Jarman SN. 2006. Quantification of damage in DNA recovered from highly degraded samples – a case study on DNA in faeces. Frontiers in Zoology, 3: 11. PMID: 16911807 DOI: 10.1186/1742-9994-3-11

Deagle BE, Kirkwood R, and Jarman SN. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. Molecular Ecology, 18: 2022–2038. PMID: 19317847 DOI: 10.1111/j.1365-294X.2009.04158.x

Deagle BE, Thomas AC, McInnes JC, Clarke LJ, Vesterinen EJ, Clare EL, et al. 2019. Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? Molecular Ecology, 28: 391–406. PMID: 29858539 DOI: 10.1111/mec.14734

De Sousa LL, Silva SM, and Xavier R. 2019. DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. Environmental DNA, 1: 199–214.

Denryter KA, Cook RC, Cook JG, and Parker KL. 2017. Straight from the caribou's (*Rangifer tarandus*) mouth: Detailed observations of tame caribou reveal new insights into summer–autumn diets. Canadian Journal of Zoology, 95: 81–94. DOI: 10.1139/cjz-2016-0114

Drake CC, Manseau M, Klütsch CFC, Priadka P, Wilson PJ, Kingston S, et al. 2018. Does connectivity exist for remnant boreal caribou (Rangifer tarandus caribou) along the Lake Superior Coastal range? Options for landscape restoration.

Environment Canada. 2012. Recovery Strategy for the Woodland Caribou (*Rangifer tarandus caribou*), Boreal population, in Canada. Species at Risk Act Recovery Strategy Series. Environment Canada, Ottawa. xi + 138pp.

Favero-Longo SE, and Piervittori R. 2010. Lichen-plant interactions. Journal of Plant Interactions, 5: 163–177. DOI: 10.1080/17429145.2010.492917

Festa-Bianchet M, Ray JC, Boutin S, Côté SD, and Gunn A. 2011. Conservation of caribou (*Rangifer tarandus*) in Canada: An uncertain future. Canadian Journal of Zoology, 89: 419–434. DOI: 10.1139/z11-025

Fonseca VG. 2018. Pitfalls in relative abundance estimation using eDNA. Molecular Ecology Resources, 18: 923–926. DOI: 10.1111/1755-0998.12902

Handeland K. 2008. Acute yew (*Taxus*) poisoning in moose (*Alces alces*). Toxicon, 52: 829–832. PMID: 18793664 DOI: 10.1016/j.toxicon.2008.08.008

Jando K, Kowalewska A, and Kukwa M. 2000. The first records of two lichenicolous fungi, Lichenoconium pyxidatae and Taeniolella beschiana, in Poland. Fragmenta Floristica et Geobotanica Ann, 45: 529–532.

Kartzinel TR, Chen PA, Coverdale TC, Erickson DL, Kress WJ, Kuzmina ML, et al. 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. Proceedings of the National Academy of Sciences, 112: 8019–8024. DOI: 10.1073/pnas.1503283112

Klütsch CFC, Manseau M, Trim V, Polfus J, and Wilson PJ. 2016. The eastern migratory caribou: The role of genetic introgression in ecotype evolution. Royal Society Open Science, 3: 150469. PMID: 26998320 DOI: 10.1098/rsos.150469

Kuchta B. 2012. Bottom-up and top-down forces shaping Caribou Forage availability on the lake superior coast. Masters of Forestry Thesis, Lakehead University, Thunder Bay ON Canada. [online]: Available from knowledgecommons.lakeheadu.ca/handle/2453/402.

Lawrey JD, and Diederich P. 2018. Lichenicolous fungi: Worldwide checklist, including isolated clutures and sequences available. [online]: Available from lichenicolous.net.

McGreer MT, Mallon EE, Vander Vennen LM, Wiebe PA, Baker JA, Brown GS, et al. 2015. Selection for forage and avoidance of risk by woodland caribou (*Rangifer tarandus caribou*) at coarse and local scales. Ecosphere, 6: art288–11. DOI: 10.1890/ES15-00174.1

McNeill EP, Thompson I, Wiebe PA, Street GM, Shuter J, Rodgers AR, et al. 2020. Multi-scale foraging decisions made by woodland caribou (*Rangifer tarandus caribou*) in summer. Canadian Journal of Zoology, 98: 331–341. DOI: 10.1139/cjz-2019-0197

Monterroso P, Godinho R, Oliveira T, Ferreras P, Kelly MJ, Morin DJ, et al. 2019. Feeding ecological knowledge: The underutilized power of faecal DNA approaches for carnivore diet analysis. Mammalian Review, 49: 97–112. DOI: 10.1111/mam.12144

Murie A. 1934. The moose of Isle Royale. University of Michigan Museum of Zoology, Miscellaneous Publications, 25.

Newmaster SG, Thompson ID, Steeves RAD, Rodgers AR, Fazekas AJ, Maloles JR, et al. 2013. Examination of two new technologies to assess the diet of woodland caribou: Video recorders attached to collars and DNA barcoding. Canadian Journal of Forest Research, 43: 897–900. DOI: 10.1139/cjfr-2013-0108

Op De Beeck M, Lievens B, Busschaert P, Declerck S, Vangronsveld J, and Colpaert JV. 2014. Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. PLoS ONE, 9: e97629. PMID: 24933453 DOI: 10.1371/journal.pone.0097629

Patterson BR. unpublished data. Research scientist. Ontario Ministry of Natural Resources and Forestry.

Pompanon F, Deagle B, Symondson WOC, Brown DS, Jarman SN, and Taberlet P. 2012. Who is eating what: Diet assessment using next generation sequencing. Molecular Ecology, 21: 1931–1950. PMID: 22171763 DOI: 10.1111/j.1365-294X.2011.05403.x

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [online]: Available from R-project.org/.

Rayé G, Miquel C, Coissac E, Redjadj C, Loison A, and Taberlet P. 2011. New insights on diet variability revealed by DNA barcoding and high-throughput pyrosequencing: Chamois diet in autumn as a case study. Ecological Research, 26: 265–276. DOI: 10.1007/s11284-010-0780-5

Snyder JD, and Janke RA. 1976. Impact of moose browsing on boreal-type forests of Isle Royale National Park. American Midland Naturalis, 95: 79–92. DOI: 10.2307/2424235

Spribille T, Tuovinen V, Resl P, Vanderpool D, Wolinski H, Aime MC, et al. 2016. Basidiomycete yeasts in the cortex of ascomycete macrolichens. Science, 353: 488–492. DOI: 10.1126/science.aaf8287

Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, Valentini A, et al. 2007. Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding. Nucleic Acids Research, 35: e14. PMID: 17169982 DOI: 10.1093/nar/gkl938

Thompson ID, Wiebe PA, Mallon E, Rodgers AR, Fryxell JM, Baker JA, et al. 2015. Factors influencing the seasonal diet selection by woodland caribou (*Rangifer tarandus tarandus*) in boreal forests in Ontario. Canadian Journal of Zoology, 93: 87–98. DOI: 10.1139/cjz-2014-0140

Tuovinen V, Ekman S, Thor G, Vanderpool D, Spribille T, and Johannesson H. 2019. Two Basidiomycete fungi in the cortex of wolf lichens. Current Biology, 29: 476–483.e5. PMID: 30661799 DOI: 10.1016/j.cub.2018.12.022

Wickham H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York. ISBN: 978-3-319-24277-4. [online]: Available form ggplot2.tidyverse.org.

Wilson CR, Sauer J-M, and Hooser SB. 2001. Taxines: A review of the mechanism and toxicity of yew (*Taxus* spp.) alkaloids. Toxicon, 39: 175–185. PMID: 10978734 DOI: 10.1016/S0041-0101(00)00146-X

Windels SK, and Flaspohler DJ. 2011. The ecology of Canada Yew (*Taxus canadensis Marsh*): A review. Botany, 89: 1–17. DOI: 10.1139/B10-084

Zhurbenko M, and Pino-Bodas R. 2017. A revision of lichenicolous fungi growing on Cladonia, mainly from the Northern Hemisphere, with a worldwide key to known species. Opuscula Philolichenum, 16: 188–266.