



## A comparison of morphological and molecular diet analyses of predator scats

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An understanding of a species' diet is required to make sound conservation and management decisions. Traditionally, morphological analyses of undigested hard parts from food items remaining in scats have been used to assess diets. More recently, molecular analyses of scats have been used to identify plant and prey species' DNA, but no studies have compared morphological and molecular diet analyses for large, terrestrial carnivores. We used molecular tools to determine the percentage of black bear and coyote scats that contained 3 common prey species (caribou, moose, and snowshoe hares) in Newfoundland and compared the results to a traditional morphological analysis. We found that a ranking of relative prey frequencies was consistent between the 2 methods, but molecular methods tended to detect prey species in a greater percentage of scats for all prey species. However, there were individual scats in which a prey species was detected by morphological methods only, and we provide evidence that molecular methods could result in false negatives if prey DNA is not uniformly distributed throughout a scat or as a result of PCR inconsistency. We also found that the per sample cost comparison between morphological and molecular analyses was dependent upon whether or not a molecular test was needed to identify scats to the predator species, the cost of developing molecular methods, and the number of samples being processed. We recommend that controlled feeding studies be performed to validate molecular methods and investigate the utility of molecular techniques to estimate the proportions of food items consumed.

Key words: black bear, *Canis latrans*, caribou, coyote, diet analysis, Newfoundland, noninvasive genetic sampling, *Rangifer tarandus*, scat detection dog, *Ursus americanus*

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Knowledge of a species' resource requirements is particularly important for understanding habitat needs (Litvaitis 2000). In particular, the diet of a species can provide information regarding how it consumes and/or competes with other members of its community (Klare et al. 2011). Examination of stomach contents and scats is the most common method used to evaluate the diet of terrestrial animals (Litvaitis 2000). Scat analysis is particularly attractive because of the ease of collection and implementation and its non-destructive and noninvasive nature, particularly when studying rare or elusive species (Valentini et al. 2009) or species of conservation concern (Mills 1996).

Traditionally, hard part or morphological scat analysis entails the identification of undigested animal or plant matter (Casper et al. 2007a), such as bones, teeth, hair, feathers, scales, exoskeletons (Litvaitis 2000), otoliths (Casper et al. 2007b), and macro- or microscopic plant material (Valentini

et al. 2009). Determining the proportion of scats containing a food item (frequency of occurrence) is commonly performed; however, identifying the volume or mass of food parts from a specific food item in proportion to all food items, in conjunction with species-specific correction factors, is preferred since it provides a better estimate of the contribution of food items to a species' diet (Klare et al. 2011). Regardless of the approach, the accuracy of morphological methods can be limited by a lack of identifiable hard parts (Casper et al. 2007a), variable delays in the excretion of hard parts between food items (Casper et al. 2007a), and difficulty distinguishing between hard parts of closely related species (Spaulding 2000; Zeale et al. 2011). Recently, employed molecular methods have the potential to limit or eliminate some of these challenges by permitting the objective identification of food items from both soft and hard matter present in scats (Tollit et al. 2009), but also bring their

own set of limitations, such as the inability to amplify DNA from highly degraded scats (Tollit et al. 2009) and expensive, specialized equipment.

Researchers have used molecular methods to study diet across a range of species. The scats of pinnipeds (Deagle et al. 2005; Casper et al. 2007a, 2007b; Matejusová et al. 2008; Tollit et al. 2009; Bowles et al. 2011), penguins (Deagle et al. 2007, 2010), bottlenose dolphins (*Tursiops truncatus*—Dunshea 2009), chamois (*Rupicapra rupicapra*—Rayé et al. 2011), domestic sheep (Pegard et al. 2009), snow leopards (*Panthera uncia*—Shehzad et al. 2012a), leopard cats (*Prionailurus bengalensis*—Shehzad et al. 2012b), and bats (Zeale et al. 2011) have been analyzed with molecular methods. Valentini et al. (2009) demonstrated the utility of a DNA barcoding approach by identifying the diets of primarily herbivorous vertebrate and invertebrate species via a DNA fragment of the chloroplast genome (P6 loop of *trnL* [UAA] intron). Some researchers have followed a similar approach by utilizing restriction sites that are found across a broad range of species (Dunshea 2009; Pegard et al. 2009; Deagle et al. 2010; Rayé et al. 2011; Shehzad et al. 2012a, 2012b), but others have relied on species- or taxon-specific markers to identify known diet items (Matejusová et al. 2008; Bowles et al. 2011; Zeale et al. 2011).

To our knowledge, only 3 studies have compared the findings of morphological and molecular analyses. A feeding trial study of captive fur seals (*Arctocephalus* spp.) determined that molecular methods detected prey species sooner after feeding, more frequently, and over a more predictable time period in comparison to morphological methods (Casper et al. 2007a). However, in a study of wild Stellar sea lions (*Eumetopias jubatus*), molecular analyses failed to identify a prey species in 22% of scats but were able to identify prey to species when morphological methods were limited to genus (Tollit et al. 2009). Tollit et al. (2009) also reported significant differences in detection rates between the 2 methods for certain prey species. Molecular methods also provided greater species resolution in an insectivorous bat study and found several additional orders (Zeale et al. 2011). However, despite the importance of understanding the diets of large, terrestrial carnivores (Mills et al. 1992), no studies have compared the results of morphological and molecular analyses in a terrestrial system containing a large, carnivorous species. Interpretation of predator–prey relationships in such a system, and thus management decisions, might vary as a result of differences in detection rates of different prey species.

The island of Newfoundland, Canada, presents an excellent study system for comparing morphological and molecular diet analyses because of the relative simplicity of the predator–prey system. Black bears (*Ursus americanus*) depend heavily on vegetation but also consume large amounts of meat, particularly in spring, when an abundance of winter-killed moose (*Alces alces*) and caribou (*Rangifer tarandus*), and especially neonate calves, are present. Coyotes (*Canis latrans*) only recently immigrated to Newfoundland (1980s) but have been confirmed as one of the major predators of caribou calves (Mahoney and Weir 2009; Lewis and Mahoney 2014; Mumma et al. 2014) and also consume moose, snowshoe hares (*Lepus americanus*), and

other small mammals. Given the recent decline (> 66% since 1998) in Newfoundland's caribou population, partially caused by an increase in calf predation (Mahoney and Weir 2009), an assessment of the techniques used to evaluate diet of the 2 major predators of caribou calves is warranted.

Our goals were to evaluate the feasibility of using molecular tools to detect 3 potentially common prey items in scats of 2 large, terrestrial carnivores and determine how these results compare to morphological analyses. We focused on a limited number of prey items to enable a straightforward comparison between methods. We hypothesized that molecular methods would identify a prey species in a greater percentage of scats, but that morphological methods would identify a prey species in some scats where molecular methods did not as a result of PCR failure or a lack of prey DNA uniformity throughout a scat. We evaluated both of these possible explanations for molecular method failures and also compared costs between the 2 techniques.

## MATERIALS AND METHODS

**Study site.**—Newfoundland (111,390 km<sup>2</sup>) is an island off Canada's eastern coast with a cool, maritime climate characterized by interspersed coniferous forest, windswept barrens, and peatland (McManus and Wood 1991). We selected 3 study sites (Fig. 1) inhabited by 4 of Newfoundland's caribou herds (La Poile, Northern Peninsula, St. Anthony, and Middle Ridge),

### 1 - Island of Newfoundland Study Sites

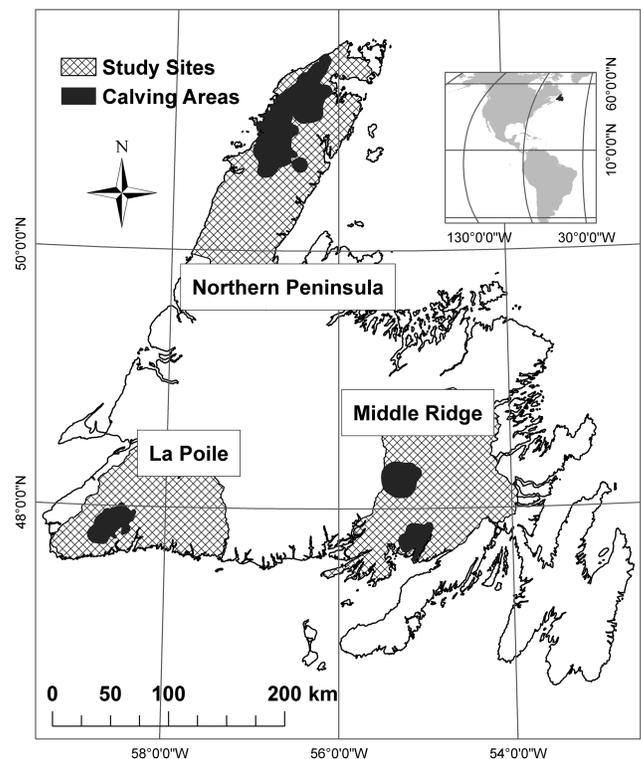


Fig. 1.—The location of our 3 study sites and associated calving grounds on the island of Newfoundland, Canada.

along with moose, snowshoe hares, and predatory black bears and coyotes (Rayl et al. 2014).

**Scat sampling.**—From June through August of 2009, black bear and coyote scats were collected along roads and in areas adjacent to roads across La Poile (~1,700 km<sup>2</sup>) and the Northern Peninsula (~2,000 km<sup>2</sup>) using a trained scat detection dog (MacKay et al. 2008). Sampling locations were spread across La Poile (17 search locations) and the Northern Peninsula (18 search locations) to increase the number of habitat patches and individual predators sampled. Scat detection dog searches varied in distance (5–10 km) and search time (2–6 h) depending on the number of scats found and the weather. Scats were placed in plastic bags by dog handlers wearing latex gloves and frozen at the end of each field day when possible. Prior to lab processing, scats were thawed and a total of ~0.5 ml of fecal material was removed from multiple locations on the outside of each scat (Stenglein et al. 2010) and placed in 2-ml collection tubes containing DETS buffer to prevent DNA degradation (Frantzen et al. 1998). Plastic bags and tubes were labeled with a sample number and date of collection, which linked samples to an electronic record of their GPS coordinates.

**Predator species identification.**—Samples were extracted using the Qiagen QIAamp DNA stool mini kit (Qiagen Inc., Valencia, California) in a laboratory dedicated to low quantity and quality DNA samples, and DNA extractions included a negative control. We tested each sample along with negative controls, using a mitochondrial DNA (mtDNA) control region fragment analysis test to identify each sample to species (Murphy et al. 2000; Dalen et al. 2004; Onorato et al. 2006; Mumma et al. 2014; De Barba et al. 2014b), because there was the potential to misidentify nontarget Canada lynx (*Lynx canadensis*) and red fox (*Vulpes vulpes*) scats as coyote. Since the identification of each scat to species relies on sloughed epithelial cells from the gastrointestinal tract of the predator, we assumed that prey DNA would exceed predator DNA; therefore, we considered a positive black bear or coyote result an indication of good sample quality and that prey DNA, if present, would have a high probability of amplification. We determined allele sizes using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, California) and associated GeneMapper 3.7 software (Applied Biosystems 2004).

**Molecular prey species identification development.**—Three common prey species were selected for our molecular prey species identification test: caribou, moose, and snowshoe hare. Reference samples of each prey species were collected from Newfoundland (caribou = 4 hair, moose = 3 tissue, and snowshoe hare = 3 tissue). DNA was extracted from the hair samples in a laboratory dedicated to low-quality DNA and from tissue samples in a separate lab using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, California). Each extraction included a negative control to monitor for contamination of PCR reagents.

All sequences available for the target species at the cytochrome *b* (*Cytb*) region of mtDNA were downloaded from Genbank ([www.ncbi.nlm.nih.gov/genbank/](http://www.ncbi.nlm.nih.gov/genbank/)). These sequences,

8 caribou (accession numbers AY726672–8), 4 moose (accession numbers AY090099, AY245520, EF077657, and M98484), and 3 snowshoe hares (accession numbers AF010152, AY292733, and LAU58932), were used to design primers that would target ~200 base pairs (bps) in caribou and moose and ~375 bps in snowshoe hare of the *Cytb* region. We then used these primers to generate sequence data from the reference prey individuals from our study area (Table 1). The PCR for sequencing caribou and moose contained 0.06 μM of primers NFCytb F and NFCytb R, 1.5 mM MgCl<sub>2</sub>, 0.4 mM dNTPs, 1X Amplitaq gold PCR buffer, and 0.5 U of Amplitaq Gold DNA Polymerase (Applied Biosystems Inc., Foster City, California) in a final reaction volume of 10 μL. The thermal profile for this reaction was an initial denaturation step of 95°C for 10 min, followed by 35 cycles of 95°C for 30s, 50°C for 30s, and 72°C for 1 min. The PCR and thermal profile for sequencing snowshoe hare was the same as above except using primers LepusA F and LepusSeq R. Prior to sequencing, PCR product was cleaned using ExoSAP-IT (Affymetrix, Santa Clara, California) according to the manufacturer's protocol. Sequencing was carried out in 10 μL reactions using BigDye Terminator v3.1 (Applied Biosystems Inc., Foster City, California). Sequencing products were cleaned using a BigDye XTerminator Purification Kit and then run on a 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, California). We used the program Sequencher 4.7 (Gene Codes 2000) to edit and align the sequences with those from Genbank. The program MacClade 4.0 was used to determine the number of unique haplotypes (Maddison and Maddison 2003).

DNA isolated from scat samples can be degraded, which often hinders PCR amplification of longer DNA fragments (Kohn et al. 1995; Murphy et al. 2000). Thus, the goal was to design species-specific primers to amplify relatively short DNA fragments. The primer NFCytb F was designed to anneal to both caribou and moose DNA. A species-specific reverse primer was designed for each species from the aligned sequence data above to produce bands of different sizes when combined with the primer NFCytb F (Tarandus3 R and Alces3 R; Table 1). The Tarandus3 R primer amplified a 105-base pair (bp) fragment, while the Alces3 R primer amplified a 129-bp fragment (Table 1). A species-specific reverse primer was designed for snowshoe hare that produced a 179-bp fragment when combined with the primer LepusA F (Table 1).

**Table 1.**—DNA primers used during the development of the molecular prey identification test.

Primer name	Dye	Primer sequence 5' to 3'	Frag size (base pairs)
NFCytb F	6-FAM	ACCCACCCATTAATAAAAATTGT	
Tarandus3 R		GAATTAAGCAGATTCTAG	105
Alces3 R		GAATAGTCCTGTAAGGATTGTAAG	129
LepusA F	6-FAM	TTAACCACTCCCTAATTGAC	
LepusA R		TTACGTCTCGGCAGATATG	173
NFCytb2 R		TCGTCCTACATGTATAATA	210
LepusSeq R		TAGGGTTGTCCCAATGTAG	370

All primers were multiplexed into a single PCR reaction to minimize the amount of time and cost accrued when analyzing samples. This PCR contained 0.57  $\mu\text{M}$  of primer NFCytb F; 0.14  $\mu\text{M}$  of primers Caribou3 R, Moose3 R, LepusA F, and LepusA R; 0.5X Q solution; and 1X Multiplex Mastermix (Qiagen Inc., Valencia, California) in a final reaction volume of 7  $\mu\text{L}$ . The thermal profile for this reaction was an initial denaturation step of 95°C for 10 min, followed by 15 cycles of 94°C for 30s, touchdown starting at 65°C and decreasing by 0.7°C each cycle for 30s, 72°C for 1 min, followed by 35 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 1 min. The NFCytb F and LepusA F primers were labeled with 6-FAM dye. PCR products were run on a 3130xl Genetic Analyzer using Genescan 500 LIZ Size Standard (Applied Biosystems Inc., Foster City, California). The results were visualized using Genemapper 3.7 (Applied Biosystems 2004).

*Validation of molecular prey species identification test.*—To validate the prey species identification test, samples of known species origin were collected for caribou ( $n = 20$  hair), moose ( $n = 17$  fecal pellets), and snowshoe hares ( $n = 19$  fecal pellets). The 3 tissue samples used for sequencing from moose and snowshoe hares were included in the validation. DNA was extracted from the hair samples using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, California). DNA from the pellet samples was extracted using QIAamp Stool Kit (Qiagen Inc., Valencia, California) with 1 protocol modification. Given the large size of moose pellets, up to 3 mL of buffer ASL was used to submerge the pellet in order to wash cells from the largest surface area possible. We then removed 1.4 mL of buffer ASL from around the pellet and moved to the next step in the protocol. DNA extractions were performed in a laboratory dedicated to low-quality DNA samples, and each extraction contained a negative control to test for reagent contamination. The DNA extracts were then tested using the molecular prey species identification test, and the results were cross-referenced with the known prey species of origin.

*Molecular prey species identification.*—We used the molecular prey species identification test to determine the presence of caribou, moose, and snowshoe hare in all scats that tested positive as black bear or coyote collected from La Poile and the Northern Peninsula study sites in 2009. All PCR runs included a negative control to monitor contamination. All samples were tested in duplicate and samples with unclear results were retested up to 2 additional times to clarify the presence or absence of prey species. A positive prey species identification required a clear peak exceeding 200 fluorescent units, which resembled the shape of positive controls and distinguished itself from nonspecific noise, in at least one of the duplicate PCRs for each sample. Samples were retested if uncertainty remained following the examination of the duplicate PCRs. We compared the final result to the first 2 PCRs to determine the consistency of PCR results. Both molecular and morphological analyses were conducted blindly to the results of the other prey identification method to avoid the potential for bias.

*Morphological prey species identification.*—All scats analyzed by our molecular prey species identification test were

also analyzed using standard morphological analyses of undigested hard parts from food items remaining in scats (Reynolds and Aebischer 1991). Thawed scats were individually cleaned by washing in a 2.0-mm mesh sieve and then dried to a constant mass in a drying oven at 40–50°C for 24–48 h. We placed the dried contents of a scat onto a 30×20-cm tray with a 2×5-line grid (10 intersections) at the bottom and evenly spread scat contents across the grid. We selected the food item part crossing or nearest to each grid intersection to get a representative sample of scat content. We secured hair samples on a plastic cover slip between 2 glass slides clipped with 4 paper clamps. The hairs were placed within a conventional toaster oven at high power for 10 min to melt the cuticular mosaic pattern onto the plastic cover slip and then removed from the plastic cover slip. Both the hair and cover slip were taped to a datasheet and examined under magnification. We examined the structures of the cuticle, medulla, and cross sections under a microscope and compared those to a reference collection of hairs representing various regions of the body from all local, potential prey species. To aid in identification of other food items, we collected reference samples of ants, as well as potential vegetative food items (including leaves, berries, and seeds) at different phenological stages from our study sites. We also identified these and other remains (e.g., feathers) using relevant taxonomic keys and manuals. For comparisons between morphological and molecular techniques, we simply tallied whether or not a given target prey species (caribou, moose, or snowshoe hare) occurred in a given scat.

*Comparison of morphological and molecular methods.*—We evaluated if a ranking of the relative prey frequencies was consistent between methods and used 2-tailed McNemar's chi-squared tests with continuity corrections (Agresti 1990) in R (R Development Core Team 2014) to evaluate if there were differences for each prey species (caribou, moose, or snowshoe hare) between the paired morphological and molecular results. To assess the potential for false positives and negatives, we tabulated the number of scats that agreed and disagreed between methods for each prey species.

We also compared the cost/sample associated with each method, which includes supply costs and technician costs assuming a pay rate of US\$15/h. Since the composition of a predator community dictates if a molecular predator species identification test is necessary to distinguish between morphologically similar predator scats, we do not present the costs of the predator species identification test because it would be the same for both diet analyses. However, we report the costs of the molecular diet analysis with and without the costs of extraction, since extraction would be an additional cost for the molecular analysis in systems that do not require a molecular test to identify the predator species. Our estimates for the molecular analysis are based on processing samples in batches of ~20 during extraction and ~96 during PCR. We also provide the cost/sample with and without the cost of developing the molecular prey species identification test. Because development costs per sample decrease as the number of samples increases, we present the cost/sample for 100, 500, and 1,000 samples. All cost estimates

presume that the cost differential for field collections is negligible and assume access to a government or University facility containing specialized equipment for molecular analyses.

**Testing of scat uniformity.**—In 2012, scat detection dogs were transported via helicopter to remote locations within the Middle Ridge to locate black bear and coyote scats as part of a larger predator abundance survey. Five black bear and 5 coyote scats were selected to evaluate if prey DNA is spread uniformly throughout a scat. We sampled each scat from 2 exterior locations and 2 interior locations and then tested the 4 samples of each scat in duplicate. We evaluated the differences in prey species detection from the 4 samples of each individual scat.

## RESULTS

**DNA sequencing and validation of molecular prey species identification test.**—A 210-bp fragment of the *Cytb* region of mtDNA was successfully amplified and sequenced for the 4 caribou and 3 moose reference samples. We found a total of 2 unique haplotypes for both caribou and moose. The 8 caribou sequences (accession numbers AY726672–8) and 4 moose sequences (accession numbers AY090099, AY245520, EF077657, and M98484) represented 4 distinct haplotypes for each species. Thus, we used a total of 6 haplotypes from each species to design the conserved forward primer and the species-specific reverse primers. We amplified and sequenced a 370-bp fragment of the *Cytb* region of mtDNA for the 3 snowshoe hare reference samples and found 2 unique haplotypes. The 3 snowshoe hare sequences (accession numbers AF010152, AY292733, and U58932.1) represented 2 haplotypes, 1 of which matched a haplotype from the reference samples. Therefore, 3 unique snowshoe hare haplotypes were used to design the species-specific primer pair.

All validation samples that produced PCR product (caribou  $n = 20$ , moose  $n = 16$ , and snowshoe hare  $n = 20$ ) showed bands at the correct species-specific size (Table 2). In addition, there was no cross-species amplification in the validation samples. All samples that failed to produce PCR product were fecal pellets, which is likely due to the DNA being degraded in these samples rather than a failure of the prey species identification test.

**Comparison of morphological and molecular results.**—We tested 140 black bear scats and 156 coyote scats for prey remains. All extraction negative controls and PCR controls were negative. Both methods identified moose as the most common prey item for black bears followed by caribou and snowshoe hare, although differences in the percentages of scats containing moose and caribou were not significant

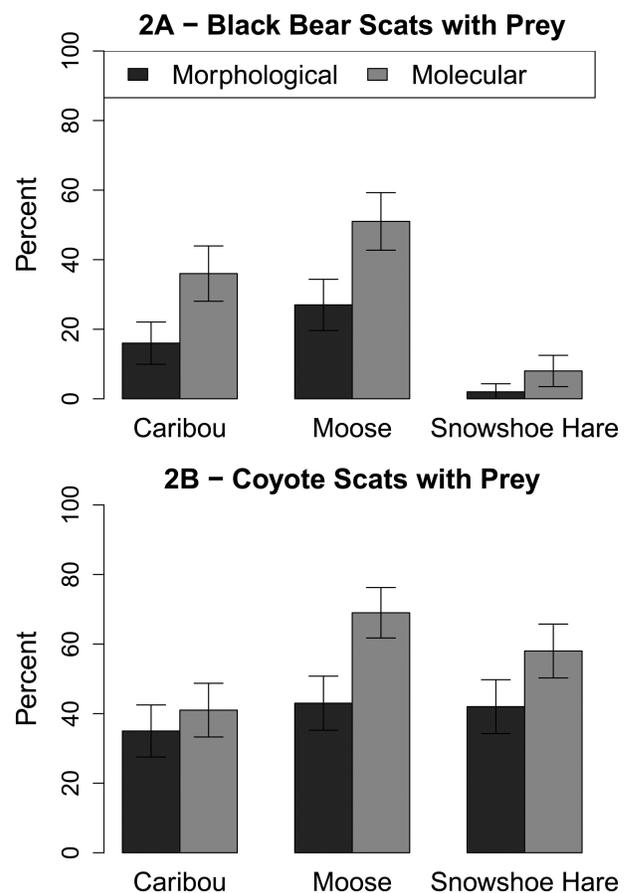
**Table 2.**—Known samples used during the development of the molecular prey identification test.

Species	Sample type	Number of samples	Number of success
Caribou	Plucked hair	20	20
Moose	Tissue	3	3
	Fecal pellet	17	13
Snowshoe hare	Tissue	3	3
	Fecal pellet	19	19

within methods (Fig. 2A). Moose was also the most common item for coyotes followed by snowshoe hares and caribou, but none of the percentages estimated via morphological methods were significantly different and moose and snowshoe hare were not significantly different for the molecular analysis (Fig. 2B).

However, we did find differences in the percentages of scats containing prey species between morphological and molecular methods for both coyotes and black bears scats (Figs. 2A and B). Overall, morphological analyses found one or more prey species in 34% of black bear scats and 78% of coyote scats, while molecular analyses detected one or more prey species in 70% and 90% of black bear and coyote scats, respectively. We found significant differences between methods for the percentages of black bear scats containing caribou ( $\chi^2 = 14.58_{df=1}$ ,  $P < 0.001$ ), moose ( $\chi^2 = 20.94_{df=1}$ ,  $P < 0.001$ ), and snowshoe hare ( $\chi^2 = 4.9_{df=1}$ ,  $P < 0.027$ ). For coyotes, molecular methods found significantly higher percentages of scats containing moose ( $\chi^2 = 27.12_{df=1}$ ,  $P < 0.001$ ) and snowshoe hares ( $\chi^2 = 11.5_{df=1}$ ,  $P < 0.001$ ) but not caribou ( $\chi^2 = 1.62_{df=1}$ ,  $P > 0.203$ ) in comparison to morphological methods.

Although the majority of scats agreed between methods with regards to the presence of a specific prey species, a substantial



**Fig. 2.**—The percentage (and 95% CI) of A) black bear and B) coyote scats containing caribou, moose, and snowshoe hare as determined by morphological and molecular analyses.

number of scats differed between methods. Morphological and molecular analyses were in agreement for 64%, 63%, and 92% of black bear scats regarding the presence or absence of caribou, moose, and snowshoe hare, respectively (Table 3). Morphological and molecular analyses of coyote scats agreed for 68%, 62%, and 70% for caribou, moose, and snowshoe hare, respectively (Table 3). Positive molecular and negative morphological results were the more common disagreement (6–31%) for scats from either predator, but there were also disagreements (< 1–13%) for negative molecular and positive morphological analyses (Table 3).

*PCR consistency and scat uniformity.*—We found that 16% (caribou), 11% (moose), and 3% (snowshoe hare) of duplicate PCR tests of black bear scats failed to produce the same prey species result. Duplicate PCR tests of coyote scats for caribou, moose, and snowshoe hare failed to produce the same result in 8%, 11%, and 6%, respectively. We also found that 0/5, 1/5, and 0/5 of black bear scats and 0/5, 1/5, and 1/5 of coyote scats failed to produce the same prey species result (caribou, moose, or snowshoe hare, respectively) across the 4 subsamples taken from different locations of the same scat.

*Cost comparison.*—We estimated the cost of morphological analysis at \$16.10/sample (Table 4). The molecular analysis cost/sample, excluding the costs of development, DNA extraction, and predator species identification, was \$2.48 (Table 4). When we include the molecular prey species development cost (~\$2,000), the per sample rate for analyzing 100, 500, and 1,000 scat samples was \$22.48, \$6.48, and \$4.48, respectively (Table 4). If we assume that a molecular test was not required to identify the predator species, the cost of the molecular diet analysis increases to \$17.65/sample, and once development costs are included, the cost per sample increases to \$37.65 for 100 samples, \$21.65 for 500 samples, and \$19.65 for 1,000 samples (Table 4).

**Table 3.**—Percentage of scats that agree or disagree between molecular and morphological methods with regards to the presence (+) or absence (–) of caribou, moose, or snowshoe hare (e.g., Caribou was detected by both methods (+/+) in 8% of black bear scats). Methods agree equals the combined percentage of scats where both methods detected (+/+) or did not detect (–/–) the prey species.

Predator	Analysis method		Morphological		Methods agree
		----- >			
Black bear ( <i>n</i> = 140)	Molecular	Caribou	(+)	(–)	(64%)
		(+)	(8%)	(28%)	
		(–)	(8%)	(56%)	
Black bear ( <i>n</i> = 140)	Molecular	Moose	(+)	(–)	(63%)
		(+)	(42%)	(31%)	
		(–)	(6%)	(21%)	
Black bear ( <i>n</i> = 140)	Molecular	Snowshoe Hare	(+)	(–)	(92%)
		(+)	(1%)	(6%)	
		(–)	(> 1%)	(91%)	
Coyote ( <i>n</i> = 156)	Molecular	Caribou	(+)	(–)	(68%)
		(+)	(22%)	(19%)	
		(–)	(13%)	(46%)	
Coyote ( <i>n</i> = 156)	Molecular	Moose	(+)	(–)	(62%)
		(+)	(37%)	(32%)	
		(–)	(6%)	(25%)	
Coyote ( <i>n</i> = 156)	Molecular	Snowshoe Hare	(+)	(–)	(70%)
		(+)	(35%)	(22%)	
		(–)	(7%)	(35%)	

## DISCUSSION

Although morphological and molecular methods indicated identical trends in prey species rankings within the diet of black bears and coyotes, there were a large number of discrepancies between the prey species detected for individual scats. Approximately one-third of comparisons differed between morphological and molecular results across all prey species (Table 3), and McNemar's chi-squared tests (Agresti 1990) indicated that the pairwise scat results were different for all prey species except caribou in coyote scats.

The most common disagreement between methods was positive molecular and negative morphological results, which may indicate that molecular methods had higher rates of detection, although we also observed scats with negative molecular and positive morphological results (Table 3). Molecular methods found a significantly higher proportion of black bear and coyote scats containing prey for 2 out of 3 prey species. Casper et al. (2007a) found higher rates of prey detection with molecular methods, but another study reported lower rates of detection for molecular methods in comparison to morphological methods (Tollit et al. 2009). Tollit et al. (2009) attributed decreased detections to old scats with degraded DNA, which was likely less prevalent in our study since we only tested scats that were successfully identified as black bear or coyote via molecular analyses.

Several factors could contribute to false positives and negatives for both methods. Both false positives and negatives for morphological analysis of scats could be the result of assigning hard parts to the wrong species via technician error (Spaulding et al. 2000). This is especially problematic in systems with closely related species and often results in researchers grouping species by genus or higher taxonomic levels (Zabala et al. 2003; Tollit et al. 2009; Zeale et al. 2011). In this study, 1 person worked diligently to correctly classify

**Table 4.**—The cost (US\$) of morphological diet analysis and molecular diet analysis of scats broken down by development cost, supply cost, technician (Tech) time, technician cost per sample, and cost/sample for 100, 500, and 1,000 samples.

Analysis method	Itemized costs				Totals		
	Development cost	Supply cost	Tech time (min)	Tech cost (\$15.00/h)	Cost/sample (100)	Cost/sample (500)	Cost/sample (1,000)
Morphological prey ID <sup>a</sup>	0	\$1.10	60	\$15.00	\$16.10	\$16.10	\$16.10
Molecular prey ID <sup>a,b</sup>	Not included	\$1.60	3.5	\$0.88	\$2.48	\$2.48	\$2.48
Molecular prey ID <sup>a</sup>	\$2,000	\$1.60	3.5	\$0.88	\$22.48	\$6.48	\$4.48
Molecular extraction and prey ID <sup>b,c</sup>	Not included	\$6.40	45	\$11.25	\$17.65	\$17.65	\$17.65
Molecular extraction and prey ID <sup>c</sup>	\$2,000	\$6.40	45	\$11.25	\$37.65	\$21.65	\$19.65

<sup>a</sup> Does *not* include the cost of DNA extraction and molecular *predator* species identification.

<sup>b</sup> Does *not* include the cost of *development* for the molecular *prey* species identification test.

<sup>c</sup> Does *not* include the cost of molecular *predator* species identification, but does include the cost of DNA extraction.

the 10 morphological samples from each scat; still, errors were possible. Alternatively, false negatives could result from the absence of hair, bone, or other hard parts in scats. This may be less likely to occur for small mammals where the entire carcass is consumed (Hewitt and Robbins 1996) but is more probable when portions of large mammals, such as the viscera, are consumed that include little or no hair or bone. False positives could also occur for molecular analyses as a result of cross-contamination or via the occurrence of nonspecific peaks on electropherograms. We limited the probability of false positives as a result of cross-contamination using negative controls during DNA extractions and PCRs. We also were conservative in assigning prey species by retesting unclear samples and requiring strong (> 200 florescent units) and clear peaks (only included peaks that had a similar shape exhibited by positive controls and distinguished themselves from nonspecific noise). False negatives could be the result of prey DNA not being uniformly distributed throughout a scat or PCR inconsistency both of which we demonstrated by comparing duplicate results and testing different locations of black bear and coyote scats. We tried to limit these sources of error in our study by sampling fecal material from multiple locations on each scat and by testing samples in duplicate.

In our study, the per sample cost comparison between morphological and molecular analyses was dependent upon whether or not a molecular method was needed to identify scats to the predator species, the cost of developing molecular methods, and the number of samples being processed. The cost of morphological diet analysis exceeded molecular analysis if we assumed that a molecular predator identification test was necessary and development costs were excluded (Table 4). When development costs were included, morphological methods exceeded the cost of molecular methods if  $\geq 147$  samples were processed. However, if scats did not need to be identified to the predator species using molecular methods, the additional cost of extraction raised the cost of molecular diet analysis above the cost of morphological methods, and the difference was further increased by the inclusion of the development costs for the molecular test. It should also be noted that the instrumentation necessary to perform molecular diet analyses is considerably more expensive than equipment needed for morphological analyses.

In addition to the low cost of equipment, morphological diet analyses present several other advantages. The straightforward nature of hard part analysis makes it readily accessible to a wide range of researchers (Casper et al. 2007b), and morphological analyses are also capable of analyzing highly degraded scats that would be unlikely to have viable DNA (Tollit et al. 2009). Perhaps most important is the potential of morphological analyses to provide a means of quantifying the proportion of hard parts to specific prey species (Klare et al. 2011). The quantification of prey consumed allows an assessment of the relative contribution of different food items to a species' diet, along with the potential to determine the total biomass consumed, evaluate prey selection when availability is known, and consider the population-level impacts on a prey species. Another advantage of morphological analyses could be the ability to determine the age of individuals being consumed via differences in hard part size (Latham et al. 2013). Although our goal was to compare the presence of 3 main prey species in black bear and coyote diets, it warrants mentioning that the morphological technique identified additional food items not reported in the manuscript.

Despite certain advantages of morphological methods, we think our study provides evidence that molecular methods likely identify prey species more consistently, thereby presenting the potential for increased accuracy and precision of diet assessments. Our molecular test was only designed for caribou, moose, and snowshoe hare; however, the advances in next generation sequencing and DNA barcoding approaches show great promise by permitting a single test for many prey and/or plant species, which could be transferred across systems (Valentini et al. 2008; De Barba et al. 2014a). This approach would be particularly useful in systems where little is known about the diet of a study organism and would likely increase the probability of detecting infrequent food items. Other molecular studies have shown great promise using real-time PCR to quantify the proportion of prey DNA in scats as an index of the proportion of prey in predator diets (Matejusova et al. 2008; Bowles et al. 2011), which requires extracting the entire scat or assuring that fecal material is homogenized prior to removing a subsample. Although morphological analyses have a similar approach by evaluating the proportion of prey items in scats, they can be compromised by differences in hard part digestibility, which creates the need for both predator- and prey-specific

correction factors in order to estimate true proportions (Hewitt and Robbins 1996; Tollit et al. 1997). Ultimately molecular techniques have the potential to improve the accuracy of diet analyses and provide increased insight into how species utilize and compete for food resources, but further research using controlled feeding studies with a large number of prey items will be essential to validate molecular methods.

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