Comparison of hair and DNA-based approaches in dietary analysis of free-ranging wolves (*Canis lupus*) in Alberta, Canada

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Abstract

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Dietary information of free-ranging animals is essential for understanding their ecology, conservation and management. Carnivore diet is most frequently estimated using morphological analysis of prey remains found in scats. However, genetic methods are becoming increasingly common and may identify prey parts that are unidentifiable with morphological methods (Symondson 2002). We developed an easy and accurate molecular approach to assess occurrence of prey species in the diet of free-living wolves (*Canis lupus*) and compared the results to analyses of prey hair in the same samples. The occurrence of DNA and hair remains for moose (*Alces alces*), woodland caribou (*Rangifer tarandus caribou*), deer (*Odocoileus* sp.), snowshoe hare (*Lepus americanus*)

and American beaver (*Castor canadensis*) were compared in wolf scats from northeastern Alberta, Canada. Detection of any prey species was 1.34 times as likely with DNA analysis than with hair analysis. DNA analysis showed significantly higher occurrences of every prey species ($p<0.05$) except deer. These findings highlight the advantage of molecular dietary analysis in differentiating between taxonomically similar prey species and increased prey detection rates as compared to morphological analysis.

Introduction

Knowledge of species' dietary habits is crucial for the study of complex ecosystem processes, such as resource partitioning between species, as well as effective wildlife conservation and management (Treves & Karanth 2003; Razgour 2011). On the ecosystem level, the question "Who eats what?" is necessary for understanding predator-prey dynamics and trophic interactions (Symondson 2002; Sheppard & Harwood 2005). On the species level, food habits and predation risk contribute to understanding of resource selection, population change, and physiological health (Mills 1992; Deagle 2010). Diet is foundational in animal ecology and evolution, but is especially important in the study of large carnivore species (Gese 2001; Treves & Karanth 2003). As animals at or near the apex of food webs, predators can exert a disproportionate effect on ecosystem functioning relative to their biomass through predation (Paine 1974; Crooks and Soule 1999; Estes 2011). In addition, the marked worldwide decline of large carnivores in the last century (Palomares 1999; Ceballos *et al.* 2005; Schipper *et al.* 2008; Ripple et al. 2014) makes research on their diet and resource use urgent for effective conservation and management. Dietary studies identify the main prey of carnivores, as well as individual animals or

populations that depredate on livestock, creating human-wildlife conflict (Treves & Karanth 2003), both of which can be important for establishing conservation and management priorities. However, obtaining dietary information is often a challenging part of ecological studies, especially for cryptic or far-ranging species such as large carnivores (Gese 2001; Shehzad et. al. 2012).

The most widely used method to study carnivore diets is scat analysis (Leopold and Krausman 1986; Gamberg and Atkinson 1988) because sample collection can be planned over large spatial and temporal scales (Spaulding *et al.* 2000; Wasser et al 2004, 2011a). Traditionally, dietary scat analysis has relied upon morphological identification of indigestible prey remains, such as bone and hair (Symondson 2002, Sheppard & Harwood 2005). With rigorous training, keys to prey species, and standardized sampling protocols (Ciucci *et al.* 1996), morphological analysis can be used to gain reliable dietary information. Assignment keys for morphologically distinguishing prey hair, feathers, and bone are available for different ecoregions (Adorjan and Kolenosky 1969; Kennedy and Carbyn 1981; De Marinis and Asprea 2006). Morphological prey identification protocols developed in Europe (Ciucci *et al.* 1996) are used to certify observers and reduce bias. However, morphological analysis is labor intensive and may miss or underestimate prey (Casper *et al.* 2007), especially smaller bodied and/or short-haired organisms (Sheppard and Harwood 2005); it can also be affected by observer bias in the absence of rigorous sampling protocol and observer training (Spaulding *et al.* 2000). Digestive processes, hair length and prey size may also render some prey remains unidentifiable (Casper *et al.* 2007). Studies of both invertebrate and vertebrate predators show that morphological

dietary analyses miss many trophic relationships (Dennison and Hodkinson 1983; Feller *et al.* 1985; Jarman et al. 2013).

In recent years technological advances have made DNA dietary analysis a feasible alternate method to morphological analysis and many studies have advanced the technique in vertebrate dietary ecology. The use of the Polymerase Chain Reaction (PCR) enables the amplification of trace amounts of degraded prey DNA in predator feces (King *et al.* 2008). Short mtDNA amplicons (<200 base pairs) are targeted to increase amplification success of potentially degraded DNA. Many methods have been used to amplify and identify prey DNA. Sutherland (2000), was one of the first studies to use DNA analysis to study vertebrate diets. Amplification of the highly conserved 12S region of the vertebrate mitochondrial genome was followed by Restriction Fragment Length Polymorphism (RFLP) analysis to determine prey species. Sequencing technology is now an affordable alternative to RFLP. Recent studies have used next-generation or pyrosequencing to successfully identify a wide range of prey species in generalist or rarely studied predators (Clare 2009; Deagle et al. 2009; Pompanon et al., 2012, Valentini et al. 2009, Shehzad et al.2012, Jarman 2013), taking advantage of DNA sequence archives such as Genbank. These archives also facilitate development and use of taxon-specific primers that target prey by fragment analysis (Deagle *et al*. 2007, Casper *et al*. 2007; King *et al.* 2010).

Although genetic techniques have been applied successfully to dietary ecology, the preferred approach between DNA and morphological dietary methods has been an area of debate. Casper *et al.* (2007) compared DNA and morphological methods in captive *Arctocephalus* seals using group-specific primers and found that detection of prey items was up to 5.8 times higher with DNA analysis than hard parts analysis. These results

suggest that DNA analysis is more reproducible and accurate in identifying prey than traditional methods (Symondson 2002; Sheppard and Harwood 2005; Deagle *et al.* 2010; King *et al.* 2010). However, to date there have been few studies comparing these methods (Casper *et al.* 2007), and none comparing these approaches for a large free-ranging terrestrial carnivore. This study compares DNA- and hair-based diet analyses - two commonly used methods for detection of prey species in carnivores - using wolf (*Canis lupus*) scats from the oil sands of Northeastern Alberta.

The diet of wolves in northeastern Alberta is of special ecological and conservation interest. The woodland caribou (*Rangifer tarandus caribou*) in the oil sands is threatened by impacts from a combination of industrial development and predation pressure and the relative roles of these two pressures have generated substantial debate (Wasser et al 2011b, Boutin *et al.* 2011, Latham 2011b; Hervieux *et al.* 2013, 2014). Knowing the importance of various prey species in the wolf diet can help separate the relative impacts of predation and oil development on the population growth of caribou and other ungulates.

This study aimed to 1) develop a molecular approach based on species-specific DNA primers to determine the occurrence of prey species in the diet of wolves in the Alberta oil sands and 2) compare morphological (hair) and DNA-based methods to study the diet of wolves.

Materials and Methods

Study Site and Field Methods

Wolf scat samples were collected as part of a larger study on the effects of oil development and wolf predation on woodland caribou, moose, and deer in the Alberta oil sands (Wasser

et al. 2011). We analyzed 124 wolf scats collected in 2009 across a 2,500-km^2 study area of the Egg-Pony and Wiau caribou herd ranges on the East Side of the Athabasca River (ESAR) in Alberta, Canada. The study area was divided into 40 contiguous 8x8 km cells (Wasser *et al.* 2011). Trained dog teams sampled each 8 x 8 km cell for wolf scat in four non-overlapping transects per cell between 1 January and 15 March 2009. Each looped transect averaged 5 km in length. Point locations for each collected scat were recorded by a global positioning system (GPS). Subzero temperatures assured that scats were collected frozen and remained so until transferred to a -20°C freezer at camp. Samples were shipped to the lab on dry ice and stored at -20°C freezer until further processing. All samples in these analyses were confirmed to be wolf by mitochondrial DNA (mtDNA) analyses (Wasser et al. 2011).

Hair analysis

Prior to conducting the morphological analyses, one of us (CS) was trained in hair sample analysis by the USFWS National Forensic Laboratory in Ashland, OR. Hair reference samples were acquired from ventral, dorsal, and neck regions of the following possible wolf prey species (Fuller 1980; Latham 2009; Latham 2011b): caribou (*Rangifer tarandus*), moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), beaver (*Castor canadensis*) and snowshoe hare (*Lepus americanus*). Specimens were obtained from the University of Washington Burke Museum and the USFWS National Forensic Laboratory. Macroscopic features were described for the hair of each species such as average length, coloration and texture. Microscope photos of hair medullary characteristics were also taken for each species.

All frozen wolf feces were mixed thoroughly and lyophilized prior to prey hair analysis in a Labconco FreeZone Freeze Dry System at -50°C for a minimum of 48 hours. Lyophilized samples were sifted through a 1 mm stainless steel mesh to pulverize fecal matter and separate any indigestible prey remains such as hair, bone, feathers and scales. Macroscopic identifications were recorded based on comparisons to our hair reference collection and keys (e.g. cuticular scale patterns, coloration and average length of prey species' hair) (Adorjan and Kolenosky 1969; Moore *et al.* 1974; Kennedy and Carbyn 1981). A representative sample of each type of guard hair found in the scat was then removed for microscopic analysis (Moore *et al.* 1974). Hair was mounted on glass slides with Flo-Texx® liquid medium (Columbia Diagnostics Inc., Springfield, VA) to make permanent slides (Dove and Koch 2010; Wheeler and Wilson, 2008). Microscope photographs of the medullary cells were taken with a compound light microscope under low (10x) and high (100x) magnification (Dove and Koch 2010). These photographs were compared to the reference collection of hair medullary cell microscope photographs.

Developing the molecular approach for prey analysis

For the molecular identification of prey items, we designed species-specific mtDNA primers to identify the six most common prey species (see above) and any additional species constituting >1% of prey identified in our hair analyses. Complete mtDNA sequences of caribou, white-tailed deer, mule deer, moose and American beaver were collected from Genbank (Accession numbers: caribou- AB245426.1; white-tailed deer- JN632673.1; mule deer- JN632670.1; moose- JN632595.1; American beaver- FR691684.1). Multiple mtDNA regions of snowshoe hare were retrieved (Accession numbers: cytochrome b- HQ596459.1; 16S Ribosomal RNA-DQ334833.1; 12S Ribosomal

RNA- AY292707.1; D-loop- HM771306.1) because a complete genome sequence was not available. The sequences were aligned with MEGA ver. 5 (Tamura *et al.* 2011) and screened visually for unique species-specific variations. All primers were designed manually from the reference sequences, with the following criteria: 1) select amplicon size of < 250 bp to help assure amplification success of potentially degraded prey DNA present in predator feces; 2) design multiple primers from different widely-spaced mtDNA regions per prey species to ensure amplification and guard against false negatives from mutation at one of the primer binding sites; and 3) vary amplicon sizes for each species to optimize multiplexing capacity. Primers were tested for target specificity by running each primer against all other possible prey species in a PCR reaction. Results of the cross-species standardization were visualized on agarose gels (Supplementary Material Figure S1). We designed a total of 12 primer pairs for the six targeted species: two pairs each for deer (*Odocoileus* sp.), caribou, moose, beaver, and wolf and one pair each for mule deer and snowshoe hare (Table 1).

DNA Extraction

The outer surface of fecal samples tends to be richer in DNA and have less PCR inhibitors compared to the center of the sample (Ball et al 2007; Wasser et al 2011a). However, prey DNA might be more representative from well-homogenized samples. To determine the optimal method of DNA extraction from the wolf scats for prey analysis, we selected 28 samples (the maximum that could fit on a single PCR plate, run in triplicate) out of the total 124 wolf scats. Each sample was processed in two ways: the outer mucosal surface of the scat was swabbed for DNA (Ball et al 2007), and the freeze-dried fecal powder of the scat was homogenized and sifted. Swabs and sifted scats were each analyzed using the

Qiagen DNeasy kit (Wasser et al 2011a). However, sifted samples were additionally analyzed using the Qiagen stool kit to guard against potential PCR inhibitors (Wasser et al 2011a).

Amplification success of prey DNA was significantly higher using the fecal powder-Qiagen DNeasy kit (50%) compared to the fecal powder-Qiagen stool kit (30%) or the surface mucosal swabbing method (34%) in our initial comparisons ($p<0.05$ for both comparisons, Student's t-test, n=28). All subsequent DNA analyses were thus performed using the fecal powder-Qiagen DNeasy kit. In the modified tissue kit extraction, 0.2 g of thoroughly mixed lyophilized fecal powder was incubated overnight in duplicate with 300 µl ATL buffer and 30 µl Proteinase K. Following digestion, DNA extraction was performed with the Qiagen tissue spin column protocol. DNA was eluted twice with 100 µl of elution buffer and stored at -20°C until further processing. Each set of 12 extractions was accompanied by two negative extraction controls to monitor contamination.

PCR Amplification and Fragment Length Analyses

All standardizations with the species-specific primers were performed with a set of reference samples that included tissue DNA from the target prey species: caribou, moose, mule deer, white-tailed deer, American beaver and snowshoe hare. Reference samples from wolf and other sympatric species that could be misidentified or cause contamination in the field were also examined, such as coyote (*Canis latrans*), domestic dog (*Canis lupus familiaris*), and human. The primer standardization was conducted in three steps: 1) optimization of thermocycler profile; 2) cross-species amplification tests with reference DNA; and 3) multiplexing different combinations of the species-specific primers. All PCR

reactions were performed in 10 µl volume reactions with 3-5 µl of Qiagen multiplex mix (Qiagen), 4 µM of BSA, 0.2 µM of primers and 3 µl of fecal DNA extracts. The following conditions were used for moose, mule deer, beaver and snowshoe hare: initial denaturation (95°C for 15 min); 45 cycles of denaturation (95°C for 30 s), annealing (T_a for 30 s) and extension (72°C for 30 s), followed by a final extension (72°C for 20 min). The deer and caribou markers performed best with a touchdown PCR including an initial denaturation (95°C for 15 min), followed by a 10 cycle touchdown from 55°C-52°C (0.3°C decrease each cycle), further followed by 35 cycles of denaturation (95°C for 30 s), annealing (52°C for 30 s), extension (72°C for 30 s), and a final extension (72°C for 20 min). Negative controls were included to monitor possible contamination. Each primer was fluorescently labeled and one µl of the PCR product was analyzed using an ABI 3100 sequencer with ROX-400 size standard (Applied Biosystems). DNA fragment lengths of fluorescent peaks were visualized to assess the presence or absence of prey species with program GENEMARKER using a custom allelic bin created for each species. Fragment analysis using the more sensitive the ABI 3100 was chosen over the use of an agarose gel in order to separate products that were close in size.

Prey biomass correction and statistical analyses

Frequency of occurrence of prey items in the scats was recorded. Prey biomass corrections were done according to Weaver's (1993) linear regression model (Y=0.439 + 0.008X), where X = the average live weight of the prey species and Y = the estimated prey biomass consumed per scat. This linear regression model was derived from combined studies that span prey size from snowshoe hare to adult moose and is robust to variable field conditions. Bias may be introduced with biomass corrections when small prey (e.g. a

snowshoe hare) and very large prey (e.g. an adult moose) each comprise 20-80% of the diet (Weaver 1993). This does not apply to our study because the majority of prey (>80%) is made up of intermediate sized prey (e.g. deer and caribou), and the FO for the smallest prey species are both under <10% (but see discussion). Average weights for prey specific to Northeastern Alberta were used to accurately reflect prey size in our study area. These weights were 400 kg for adult moose, 80 kg for adult white-tailed deer, 20 kg for adult beaver, 140 kg for adult caribou, and 1.5 kg for snowshoe hare (Lancia and Hodgdon 1984; Renecker and Hudson 1993; Bubenik 1998). An adult mule deer average weight of 85 kg was obtained from the Alberta Environment and Sustainable Resources council (http://srd.alberta.ca/FishWildlife/WildSpecies). An average of the standard weights for both white-tailed and mule deer (82.5 kg) was used to approximate deer biomass for the since we were unable to separate the two deer species. Standard errors (SE) around percent occurrence and percent biomass of prey were estimated using a 95% binomial distribution (Zar 1999). Comparison of prey occurrences between the two methods was done using a Wilcoxon signed-rank test for paired samples (Zar 1999, McDonald 2009).

Results

Hair analyses of prey species in wolf scats

We found a total of 129 prey occurrences with hair analysis out of the 124 wolf scats analyzed, with an average of 1.04 prey species per scat. At least one prey species was found in every scat. Deer (*Odocoileus* sp.) were the dominant prey species by prey occurrence, comprising 74.4 % (± 7.4%) of the prey detected, and 59.3% of the biomass (± 6.0%) (Figure 1). We were unable to differentiate between white-tailed and mule deer with hair

analysis. Moose hair was detected in 10.1% (± 1.7%) of the scats, representing 26.6% (± 3.4%) of the biomass. Caribou hair was present in 10.9% (± 1.8) of the scats, representing 12.3% of the biomass (± 0.8%). Non-ungulate prey made up a much smaller proportion of the prey items. Beaver occurred in 3.1% (± 0.4%) and snowshoe hare 1.6% (± 0.5) of the scats and contributed little biomass with 1.3% (± 0.4) and 0.5% (± 0.2%), respectively.

DNA analyses of prey species in wolf scats

For the 124 wolf scats analyzed using the fecal powder-Qiagen DNeasy kit, we found a total of 173 prey occurrences, with an average of 1.4 prey species per scat. At least one prey species amplified in 85% of the scat extracts. DNA analyses showed the presence of up to four separate species in one scat (n=4). Deer (*Odocoileus* sp.) were the dominant prey detected in 42.7% (± 5.1%) of the scats, and made up 21.2% of the prey biomass (± 3.9). Moose mtDNA was detected in 26.0% (± 3.3%) of the scats and made up 42.6% (± 6.0%) of the biomass. Caribou was present in 16.2% (± 2.2) of the scats and made up 11.4% of the biomass (± 1.9%). Non-ungulate prey made up a much smaller proportion of the prey detections. Beaver occurred in 8.1% (± 1.3%) and snowshoe hare in 6.9% (± 0.8) of the scats and contributed very little biomass with 2.2% (± 0.1) and 1.4% (± 0.1%), respectively.

Differences in wolf diet composition with hair and DNA analysis

We directly compared the number of occurrences of the primary prey species found in wolf scats (n=124) using DNA and hair analysis. DNA yielded a significantly greater number of total prey occurrences (n=173) compared to the hair analysis (n=129) ($p<0.005$, $W=1057$, Wilcoxon signed rank-test; Figure 2). The average number of different prey species per scat was 36% higher using DNA (DNA method: 1.40; Hair method: 1.04).

DNA analyses also found a significantly greater number of occurrences compared to hair for all prey species except deer ($p<0.001$ for moose ($W=265$); $p<0.05$, for caribou ($W=203.5$); snowshoe hare ($W=15$) and beaver ($W=15$), Wilcoxon signed-rank test). Deer were the only prey species that had a significant decrease in occurrence with genetic analysis ($p<0.01$, $W=235$).

Hair versus DNA methods differed significantly in the proportion and frequency of occurrence (FO), for all species (based on non-overlapping 95% confidence intervals) in the wolf diet except for caribou (Figure 1). The ranking of both the dominant prey (deer) and least common prey (non-cervids) by FO was the same between methods. The proportion of moose in the wolf diet showed the most dramatic increase (16%) using DNA methods.

We found significant differences for prey biomass proportions under hair and DNA analysis. Moose had a significantly greater biomass proportion under DNA analysis (42.6%) compared to hair (26.6%). Deer represented significantly more biomass in hair (59.3%) compared to DNA (27.0%) analyses. With DNA methods, moose was ranked as the primary prey species in terms of biomass contributions, while with hair analysis deer contributed the most biomass. There were no significant differences in the biomass of caribou and non-cervid species between analysis methods.

Discussion

We developed a reliable and accurate molecular method to assess wolf diet and compared results to diet analyses of the same samples using hair analysis. The mitochondrial DNA primers developed in this study showed high specificity to the target species or genus, only

amplifying DNA from the target prey for which they were designed, and not from others in the reference species sample set of non-target potential prey (Supplementary Material, Figure S1). The short amplicon sizes and multiple primers from different regions of the mtDNA also resulted in a relatively high prey DNA amplification success of 85%.

DNA extraction

We found that the optimal method of DNA extraction for carnivore fecal diet research to be the use of well-mixed freeze-dried fecal powder using the Qiagen DNeasy kit. While the swab extraction method (Ball *et al.* 2007; Wasser *et al.* 2011) is effective in capturing host DNA, well-mixed fecal powder better captures prey DNA, and provides a more representative sample of multiple prey species present in scat. The fecal powder extracted with the Qiagen DNeasy kit also provides improved prey DNA amplification in wolf scat and is less expensive than the Qiagen stool kit.

Comparison of hair and DNA-based analysis

Frequency of Occurrence

Molecular dietary analysis fared significantly better than hair analyses in terms of prey detection and differentiation. We observed a significant increase in the frequency of occurrence for all prey species (except deer), as well as the total number of prey occurrences with DNA as compared to morphological methods. Casper *et al.* (2007) found that prey detection was significantly higher and less variable with DNA methods than hard parts analysis in captive fur seals. They suggested that excretion of prey DNA may occur with higher frequency and regularity than that of indigestible parts. The differences in detection rate for DNA vs. hair-based prey estimates in this study was greatest for moose,

our largest prey species. The increased detection rate for moose with DNA was 8.75 times higher than with hair. This was markedly greater than the next largest increase in prey detection with DNA, which was 3.8 times higher for the smaller non-cervid prey. As the largest animal in our study, moose also had the smallest ratio of surface area (hair): volume (organs, muscle). Thus in a single wolf scat, it is more likely that the fecal powder will contain moose DNA from tissue or muscle digestion, rather than hair remains, which would be unidentifiable with hair analysis. This would lead to an underestimation of moose occurrence with morphological analysis only. Our results suggest that the occurrence of large mammalian prey species is likely to be more accurately represented by genetic analyses than traditional methods.

Deer were the only prey species for which we observed a significant decrease in the frequency of occurrence with DNA analysis. The higher frequency of deer under hair analysis may have been due to observer misidentification because the hair of deer and caribou are strikingly similar, especially when only a fragment of the hair is available and/or the hair color is faded from digestion. Observer misidentification is an issue acknowledged with hair analysis between similar prey species (Spaulding et al. 2000). , The use of DNA analysis removes the issue of observer misidentification, which is especially important when working with morphologically similar prey species. Our results support the application of molecular diet methods (King *et al.* 2010; Sheehan 2012) to improve the detection of prey species, especially morphologically similar species or very large prey species that may be under represented by hair analysis.

Prey biomass consumed

Biomass proportions also differed significantly between hair and genetic methods of analysis. However, it is important to appreciate the limitations of the biomass calibrations when interpreting our results. Changes in biomass followed the same changes in frequency of occurrence between methods: deer biomass drastically decreased between hair and genetic methods from 59 to 21%, while moose biomass increased from 27 to 43%. Biomass estimation is an attempt to calibrate frequency of occurrence by accounting for disparity in prey body size. This correction is especially important when large prey species represent significant portions of the diet. However, biomass estimations cannot be used to estimate the number of individual prey animals eaten because there is a large weight range within ungulates according to the age class or nutritional state of the animal, especially during the winter season (Weaver 1993). This source of variation cannot be accounted for in any of our analyses since there are also overlapping hair traits for different ungulate age classes during winter. That said, the Weaver (1993) biomass estimation used has been widely and recently applied in other wolf dietary studies (Fuller 1980, James 2004, Latham 2013), and is an important component to accurately reflect the prey's contribution to the predator's nutrition. Based on the hair analysis results alone, we might have inferred that deer have replaced moose as the primary prey species in a system where wolves historically preyed mostly on moose (Fuller 1980, James 2004). However, the genetic method suggests that, based on biomass, moose still dominate the diet of wolves in the region, although deer represent an increased biomass proportion compared to previous studies where they were scarce (Fuller 1980, James 2004).

This study was not done as part of a captive feeding trial, so we do not definitively know which method most closely represents reality. The possibility of observer bias with

morphological analysis (Spaulding et al. 2000), and the higher prey detection rate found by Casper et al. (2007) with genetic methods in a captive feeding trial suggest that DNA analysis offers a more reproducible and accurate method for studying diet in wild animals. However, hair analysis still offers a legitimate alternative, especially in regions where peer-reviewed keys to identifying remains of prey species exist and standardized protocol is used (Ciucci 2004).

Wolf diet in the Alberta oil sands

The results of our study contribute to growing research that suggests a significant shift in the prey of wolves in the boreal forest of the Canadian Oil Sands. In the mid-1990's, James (2004) documented that wolf diet consisted primarily of moose in northern Alberta. Moose made up 43% of the wolf diet in winter, with deer comprising a scant 10%. However, in the past decade, Latham (2011b; 2013), found that deer were the primary winter prey species, occurring in 61%, of scats and moose in only 18%, although moose still contribute a significant proportion of the biomass. These results agree with our findings that moose still play an important role in terms of biomass but are no longer the most frequent prey of wolves. This change in wolf diet is hypothesized to be due to a 17.5 fold increase in the deer population in the Alberta Oil Sands (Latham 2011a), caused by a combination of the destruction of older boreal forest by industry and climate change, which has allowed deer to expand northwards (Dawe 2011). Further study and monitoring of this ecosystem is imperative due to the massive changes to the landscape and prey community.

Conclusion

The results of this study confirm significant ecological changes between wolves and their prey in the boreal forest of Canada, and suggest that DNA methods are advantageous over hair analysis in terms of increased prey detection rates and differentiating between closely related taxa. Forensic hair methods require a high amount of specialized individual training, may be prone to subjectivity and require access to hair identification guides that are often difficult to find or non-existent for certain regions. However, hair analysis may be a good choice in systems where detailed prey identification guides have been developed and when standardized sampling methods are followed (Ciucci 2004). Although DNA analysis is more costly, once developed the method is efficient, detects a higher number of prey items and can be easily repeated. Species-specific primer development and standardization is also becoming easier with new software and access to genetic databases such as GenBank. Other genetic approaches such as qPCR, next generation or high-throughput sequencing could further aid in dietary research, especially for generalist or elusive carnivores, as the technology becomes increasingly accessible (Shehzad *et al*. 2012a). It is our hope that future research on wolf diet derived from the molecular methods outlined in this study will inform management and conservation efforts in the oil sands and elsewhere in North America.

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Table 1: Mitochondrial DNA markers developed in this study.

Species	Primer name	Sequence	Amplicon size (bp)	Annealing temp. (T_a)	Multiplex*
American Beaver (*Castor canadensis*)	**Beaver 1F**	**ACTAAGCCTACATAAGCTGC**	**117**	**55**	**M1**
	Beaver 1R	**TAGGCCAGATGCTTTGTGGC**			
	Beaver 2F	**ATCACAAAAACTACCATGAC**	**200**	**55**	**M1**
	Beaver 2R	**TGTTGGGTCAACATTGCGTT**			
Caribou (*Rangifer tarandus*)	**Caribou 1F**	**AATGCCCGTTATAGCTGTTC**	**130**	**52-54**	**M2/single**
	Caribou 1R	**AGGTAGGTAAGATTAAGCTG**			
	Caribou 2F	**TATTGGATCAACAAATCTTC**	**184**	**52-54**	**M2/single**
	Caribou 2R	**CATGGGAATTAATGGAGCGG**			
Deer (*Odocoileus sp.*)	**Deer 1F**	**TGCCAAACAGATAATCTGAC**	**78**	**52-54**	**M2/single**

	Deer 1R	**ATTCATAGGCTAGACTTACG**			
	Deer 2F	**TTGATTACTCATCAAATGCC**	**150**	**52-54**	**M2/single**
	Deer 2R	**TTTGGTAAAATATTCTCAAG**			
Moose (*Alces alces*)	**Moose 1F**	**TACTCTTTTAATCCCTATGC**	**155**	**55**	**M1**
	Moose 1R	**GTGTTGCTAATACTTATCAG**			
	Moose 2F	**GAATGAACCGAATATGGTAC**	**135**	**55**	**M1**
	Moose 2R	**AGTCCTGTAAGGGATACTGC**			
Snowshoe hare (*Lepus americanus*)	**SS Hare F**	**AAGCTATGCTACTCCAGGGT**	**98**	**55**	**M1**
	SS Hare R	**TTTGATTTTAAATCACGCTT**			

* Describes different primer multiplex combinations M1, M2, M3 and single. Beaver, moose and snowshoe hare primers perform equally well singly or multiplexed together in Multiplex 1 (M1). Caribou and WT deer primer work better, although not significantly better (Student's t-test, $p>0.05$), in single PCR reactions. Caribou and WT Deer may also be multiplexed together in reaction M2.

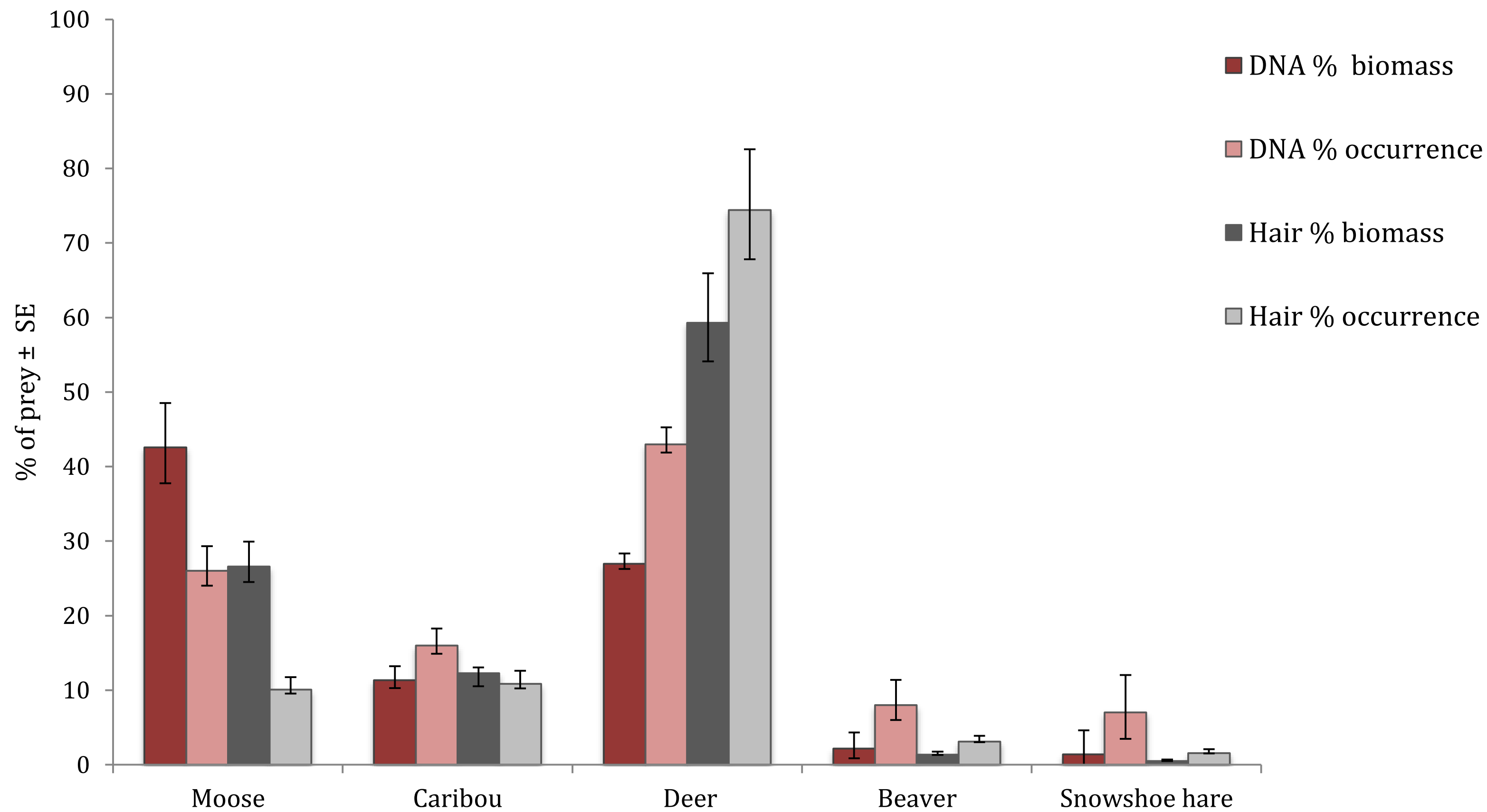

Figure 1: Wolf diet based on DNA and hair analysis of wolf scats (n=124) on the East Sides of the Athabasca River in northeastern Alberta, Canada, 2009. Percent biomass was calculated following Weaver 1993. Standard errors were estimated based on binomial 95% confidence intervals (Zar 1999). Values for white-tailed deer and mule deer are represented only for the DNA analysis, since we were unable to separate the two deer species with hair analysis.