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Enhanced understanding of predator–prey relationships using molecular methods to identify predator species, individual and sex

MATTHEW A. MUMMA,* COLLEEN E. SOULLIERE,† SHANE P. MAHONEY† and LISETTE P. WAITS*
*Department of Fish and Wildlife Sciences, College of Natural Resources, University of Idaho, Moscow, ID 83844, USA,
†Sustainable Development and Strategic Science, Department of Environment and Conservation, Government of Newfoundland and Labrador, P.O. Box 8700, 2 Canada Drive, Suite 306, St. John's, Newfoundland

Abstract

Predator species identification is an important step in understanding predator-prey interactions, but predator identifications using kill site observations are often unreliable. We used molecular tools to analyse predator saliva, scat and hair from caribou calf kills in Newfoundland, Canada to identify the predator species, individual and sex. We sampled DNA from 32 carcasses using cotton swabs to collect predator saliva. We used fragment length analysis and sequencing of mitochondrial DNA to distinguish between coyote, black bear, Canada lynx and red fox and used nuclear DNA microsatellite analysis to identify individuals. We compared predator species detected using molecular tools to those assigned via field observations at each kill. We identified a predator species at 94% of carcasses using molecular methods, while observational methods assigned a predator species to 62.5% of kills. Molecular methods attributed 66.7% of kills to coyote and 33.3% to black bear, while observations assigned 40%, 45%, 10% and 5% to coyote, bear, lynx and fox, respectively. Individual identification was successful at 70% of kills where a predator species was identified. Only one individual was identified at each kill, but some individuals were found at multiple kills. Predator sex was predominantly male. We demonstrate the first large-scale evaluation of predator species, individual and sex identification using molecular techniques to extract DNA from swabs of wild prey carcasses. Our results indicate that kill site swabs (i) can be highly successful in identifying the predator species and individual responsible; and (ii) serve to inform and complement traditional methods.

Keywords: molecular techniques, Newfoundland, predator identification, saliva swab

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Introduction

Predation is a central process in ecological communities, and the assemblages of predator and prey species can create an array of complex interactions (Prugh *et al.* 2005; Zager & Beecham 2006; Barnowe-Meyer *et al.* 2010). Accurately determining the predator species responsible for prey mortality is an important first step to understanding predator-specific roles in predator–prey systems. Directly observing predation is ideal, but such events are generally rare and only possible to observe for diurnal predators in open habitats (Blejwas *et al.* 2006). Alternatively, monitoring the survival of prey species or the predation habits of predator species via radio-collared individuals and performing site investigations can be an effective means of evaluating predator–prey interactions. The former requires identification of predator species from kill site observations using predator-specific kill site evidence, such as hair, scat or species-specific killing or feeding characteristics (Onorato *et al.* 2006). However, there is often overlap between the killing and feeding characteristics between different predator species and variability in experience among field technicians making it difficult to ensure accurate and consistent predator species identification (Cozza *et al.* 1996).

Molecular methods present a promising alternative approach that could decrease the uncertainty of predator species identification. Molecular methods have been implemented extensively in wildlife research to answer questions regarding gene flow, social structure, hybridization and population viability (DeYoung & Honeycutt 2005), but have only recently been used to identify predator species at kill sites. Predator scat and hair collected at elk (*Cervus elaphus*) kill sites were used to identify predator species (Onorato *et al.* 2006), and predator

Correspondence: Matthew A. Mumma, Fax: 208 885-9080;
E-mail: mttmmm@hotmail.com

saliva collected from killing wounds on a threatened marsupial was used to identify feral cat predation (Glen *et al.* 2009). In addition, cotton swabs were used to sample predator saliva from domestic sheep carcasses to differentiate between wild canid and feral dog predation (Williams *et al.* 2003; Sundquist *et al.* 2008; Caniglia *et al.* 2012).

Molecular predator species identification could also inform predator–prey dynamics and management actions through the identification and sex of individual predators. For example, molecular methods were used to determine that two mountain lions (*Puma concolor*) preyed more frequently on an endangered bighorn sheep (*Ovis canadensis*) population, and the authors suggested targeted removal of individual predators could decrease predation while not jeopardizing predator populations (Ernest *et al.* 2002). Targeted control efforts were also recommended by a study that used kill site swabs and telemetry data to identify specific male coyotes and breeding pairs as domestic sheep killers (Blejwas *et al.* 2006).

To further evaluate the potential of molecular methods for generating valuable data on predation at a large spatial scale, we applied these methods at caribou (*Rangifer tarandus*) calf kill sites in Newfoundland, Canada. The predator–prey system on the island of Newfoundland is an ideal model because of a changing multipredator system, a dramatic increase in neonate predation, and a large proportion of unassigned kills (26%) using traditional field methods (Mahoney & Weir 2009). The Newfoundland caribou population has decreased by >66% since the late 1990s (Mahoney & Weir 2009) and an increase in calf predation, partially the result of a changing predator guild, contributed to the decline. In previous studies, the major predator of Newfoundland caribou calves was black bear (*Ursus armericanus*), but Canada lynx (*Lynx canadensis*) accounted for additional predation, and occasionally mortalities were attributed to red fox (*Vulpes vulpes*) and bald eagle (*Haliaeetus leucocephalus*) (Mahoney & Weir 2009). However, coyotes (*Canis latrans*) have become a significant predator of caribou calves following their colonization of Newfoundland from mainland North America via sea ice in the 1980s (Trindade *et al.* 2011), and their impact on the caribou population may be underestimated given the large number of unassigned calf kills.

Our goal was to evaluate the power of molecular methods to study predation using the caribou predator–prey system in Newfoundland as our model. We were interested in the following research questions: (i) are molecular methods able to identify the predator species at more kill sites than field observation methods; (ii) is there a difference in the proportion of predation attributed to each predator species between molecular and field observation methods; (iii) are a majority of kills attributed to a small number of individual predators; and (iv) do male predators prey on caribou calves more frequently than female predators? Based on the success of previous studies that used molecular methods to evaluate predator species at kill sites (Williams *et al.* 2003; Blejwas *et al.* 2006; Sundquist *et al.* 2008) and the large proportion of unassigned caribou calf kills in previous Newfoundland studies (Mahoney & Weir 2009), we predicted that molecular methods would identify the predator species at more kill sites than field observation methods and that the proportion of predation attributed to each predator species would differ between molecular and field observation methods. We also expected that male coyotes would be detected more frequently at kill sites than females based on studies of coyotes depredating domestic sheep (Blejwas *et al.* 2006).

Material and methods

Study site

The island of Newfoundland (111 390 km^2) has a cool maritime climate and consists of coniferous forest interspersed by windswept barrens and peatland (McManus & Wood 1991). Caribou are the only native ungulate on Newfoundland and are widely distributed across the island. The calving grounds of three caribou herds (La Poile, Middle Ridge, and Northern Peninsula) (Fig. 1) ranging from 500 to 1500 km^2 were selected for study (Rayl 2012).

Capture and monitoring of caribou calves

From 27th May through 1st June 2010, we hand-captured 92 1-to-3 day old caribou calves across the three study sites (Fig. 1). Each calf was fitted with a 200 g expandable, breakaway very high frequency (VHF) radio-collar containing a motion sensitive transmitter (Lotek Wireless Inc., New Market, ON, Canada; Telemetry Solutions, Concord, CA). Transmitter pulse rates increase for collars that are stationary for >4 h signalling calf mortality or a slipped collar. Collar pulse rates were checked daily via fixed-wing and/or helicopter flights from the date of capture until June 11th and monitored every other day from June 12th until June 25th. From June 26th through July, calves were checked weekly.

When a collar indicated calf mortality, we investigated the location for caribou calf remains and predator sign. For each calf mortality, trained field personnel evaluated kill site observations and assigned a black bear, coyote, Canada lynx, red fox, bald eagle or unknown

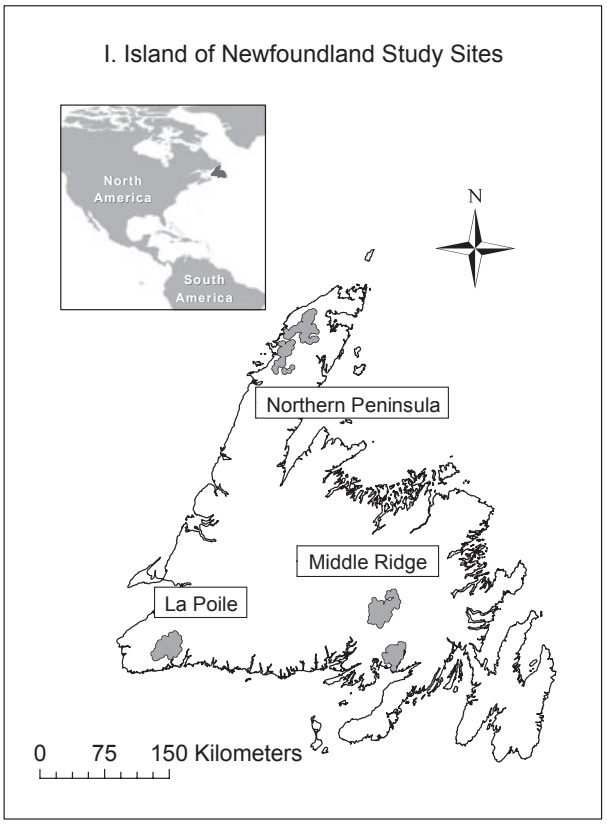

Fig. 1 The location of our three study sites (shaded in grey) on the island of Newfoundland, Canada.

predator. Personnel experience varied between the nine biologists that evaluated kill sites from ≥30 years to 1 year, but all calf mortalities were assessed by multiple biologists both in the field and at a later date through the review of kill site images and site observations recorded using standard field protocols (Fig. S1 Supplementary Information). When present, predator scat and hair samples were collected, and carcass remains were sampled for predator saliva containing predator DNA using cotton swabs.

Predator species assignment using field observations

In Newfoundland, caribou calf kills attributed to black bear typically consist of a skinned hide with a few bone fragments and chewed hoof tips. Distinguishing between coyote and lynx kills is less clear. Throat trauma and a highly variable amount of calf remains are commonly seen for both predators, but calf remains at coyote kills are often pulled apart and spread over a larger area. The size and spacing of canine punctures provide another method of differentiating between the two predators. In addition, claw punctures on the dorsal surface of calf carcasses are suggested as evidence of a lynx kill, but the potential for talon punctures from bald eagles prevents the use of punctures alone as identifying characteristics. Characteristics of a fox kill are unclear, and predator species assignments for kill sites consisting of buried front halves and decapitated heads remain uncertain.

Sample collection for molecular analysis

Field personnel searched for predator scat and hair in the vicinity of the collar location out to roughly 25 m. Approximately 0.5 mL of total faecal material was collected from multiple locations on the lateral surface of each predator scat using small sticks collected from woody shrubs in the field (Stenglein *et al.* 2010) and placed in 2-mL-collection tubes containing DETS buffer (Frantzen *et al.* 1998). Predator hair samples were placed in individual paper envelopes and stored collectively in sealed plastic bags containing silica desiccant (Roon *et al.* 2005). Field personnel wore sterile latex gloves for both scat and hair collection to prevent cross-contamination between kill sites and samples.

Sterile, cotton swabs were used to sample remains for residual predator DNA from saliva (Williams *et al.* 2003; Blejwas *et al.* 2006; Sundquist *et al.* 2008; Glen *et al.* 2009; Caniglia *et al.* 2012). We swabbed haemorrhaged and nonhaemorrhaged wounds while wearing sterile latex gloves and avoided touching multiple wounds with the same gloves to prevent cross-contamination between carcasses and wounds. We considered haemorrhaged wounds to be caused by the predator species, because haemorrhaging is an indication that the wound was inflicted, while the prey species was still alive. Nonhaemorrhaged wounds were labelled as feeding wounds and recognized as potentially attributable to the predator or scavenger species. The collar, bones, hide and other remaining tissues were also swabbed and labelled as feeding wounds when the majority of the carcass was consumed or when we found only the collar. Up to four different areas or tissues from each carcass were swabbed.

Swab technique

We conducted a literature search and pilot study to determine the most effective swabbing and preservation method (Supplementary Information). We chose ethanol as our wetting agent to assist in lifting dried cells from tissues and to promote rapid drying for DNA preservation. We collected two ethanol-soaked swabs (A and B) from each area or tissue to provide a back-up sample in case of laboratory error. Immediately following collection, all swabs were placed in individual paper envelopes that were collectively

stored in sealed plastic bags containing silica desiccant and stored at room temperature.

DNA extraction and species identification

We extracted all samples in a laboratory dedicated to low-quantity DNA samples and used the QIAGEN QIAamp DNA stool mini kit (QIAGEN Inc., Valencia, CA) for scat samples and the Qiagen DNeasy tissue kit for hair and swab samples. When available, 10 follicles were used in each hair extraction, and for all extraction batches, a negative control was used to monitor for contamination. B swabs were only processed when we wanted to verify an A swab species identification or when all A swabs from a carcass failed to provide a species identification.

Species identification for all samples was conducted using a mitochondrial DNA (mtDNA) control region fragment analysis method. This test uses primers previously reported for differentiating black bear, coyote and two nontarget species: brown bears (*Ursus arctos*) and wolves (*Canis lupis*) (Murphy *et al.* 2000; Onorato *et al.* 2006), plus an additional primer (H3R) designed to differentiate red fox (Dalén *et al.* 2004). This test identifies all species, with the exception of the lynx, via species-specific fragment size (black bear 158–164.5 base pairs (bp) and 396–401 bp, coyote 115–120 bp and 362.5–364 bp, wolf/dog 123–128 bp and 367–369 bp, and red fox 342.9–344.5 bp only). PCR conditions are under supplemental information. We tested swabs from carcasses that failed to identify a predator species after initial testing using species-specific mtDNA primers developed for the Iberian lynx (*Lynx pardinus*; Palomares *et al.* 2002) that we documented to work on known Canada lynx samples from Newfoundland. Additional details and PCR conditions are provided in Supplemental Information.

Any samples that failed the two previous analyses were amplified and sequenced using mtDNA cytochrome B primers that amplify most carnivores (Farrel *et al.* 2000) using conditions described in Onorato *et al.* (2006). These primers were effective in identifying black bears, Canada lynx and red foxes, but not coyotes. Samples that failed to amplify with the Farrell primers were amplified and sequenced using the canid-specific mtDNA control region ScatID primers using conditions and primers described in Adams *et al.* (2003) to identify coyote samples that failed the initial species ID screening.

A predator species was assigned when detected from a haemorrhaged wound swab or from a feeding wound swab when a carcass did not contain a haemorrhaged wound. We did not use molecular tools to test for the presence of bald eagles, because they frequently scavenge kill sites and are rarely the predator of caribou calves (O'Gara 1994).

Nuclear DNA individual and sex determination

We only detected black bear and coyote DNA at kill sites, and therefore amplified successful samples with black bear or canid-specific microsatellite loci to identify individual predators. When both the A and B swabs from a single wound positively identified the species, we analysed the swab for individual identification that amplified best for species identification. Due to the low genetic diversity of Newfoundland black bears, we screened 18 loci and then developed two PCR multiplexes using the most polymorphic loci. Black bear multiplex one includes six microsatellite loci (G10C, G10M, G10P, G10X, CXX20 and Mu23 – Paetkau *et al.* 1998; Taberlet *et al.* 1997; DeBarba *et al.* 2010 and Ostrander *et al.* 1993) and one sex-determining locus (Ennis & Gallagher 1994). Black bear multiplex two includes five microsatellite loci (G1A, G10B, Mu15, Mu50 and Mu59 – Paetkau *et al.* 1998; Taberlet *et al.* 1997 and Bellemain & Taberlet 2004). The PCR conditions are provided in Supplementary Information.

For coyotes, nine microsatellite loci (FH2001, FH2054, FH2088, FH2137, FH2611, FH2670, FH3725, C09.173 and Cxx.119 – Breen *et al.* 2001; Guyon *et al.* 2003; Holmes *et al.* 1994) based on the methods of Stenglein *et al.* (2010) and two sex-determining loci (DBX6 and DBY7 – Seddon 2005) were combined in one canid PCR multiplex. For PCR conditions, see Supplemental Information.

Black bear and coyote samples were tested in duplicate for their respective PCR multiplexes. Samples that failed to amplify at ≥4 loci were dropped from the analysis. We ran up to six PCR replicates for each multiplex and each multiplex replicate included all primers. To obtain a consensus genotype at each locus, we required each allele to be detected twice for heterozygotes and an allele to be detected three times for homozygotes. Samples that failed to achieve a consensus for ≥9 loci in black bears and ≥6 loci in coyotes were dropped from the analysis. Our consensus genotype thresholds were chosen to meet a probability of identity siblings (PID_{sibs}) (Waits *et al.* 2001) value of <0.003. This means that <1/333 comparisons of first-degree relatives would have identical genotypes at the loci analysed and were used to avoid including false recaptures in the data set. PID_{sibs} values for coyotes ranged from 0.0023 to 0.00025 and for black bears from 0.00012 to 0.0000027 depending on the loci that amplified.

We matched completed genotypes using the software GenAlEx6 (Peakall & Smouse 2005). Replicate PCRs for samples that matched at all but one or two loci were also evaluated to determine whether mismatches could be attributed to allelic dropouts or false alleles. Individuals that were only detected once were analysed using the software RELIOTYPE (Miller *et al.* 2002) to estimate the

genotyping error rate and evaluate the reliability of the final consensus genotype. We required consensus genotypes to be ≥95% reliable and retested samples until our threshold was achieved.

Results

We investigated 32 caribou calf mortalities between May 28th and July 22nd. Six of these carcasses were not from our sample of collared individuals, but were found opportunistically on the landscape. There was a large amount of variation in the quantity of caribou calf remains. At 12 kill sites, we found a mostly intact carcass impacted by various degrees of consumption. A dismembered carcass and significant remains were found at an additional five sites, while a severed head was found buried at three other sites. Scant remains of bones, hoof and hide were found at eight sites, and the collar alone was found at four sites. Blood or bite marks were found on three of the four collars.

Molecular species identification success rates

We collected 12 scat, 3 hair and 157 swab samples for molecular analysis. None of our extraction negatives elicited a positive result in our species identification test. Sixty-seven per cent, 0% and 54% of scat, hair and swab samples were successful for species identification (Table 1), and no result was obtained from all negative controls. If the A swab from a wound was successful, we did not always test the B swab, and therefore only tested 139 of 157 swab samples. The success rate for killing wound swabs was 86% and 46% for feeding wound swabs (Table 1). Because multiple swabs were collected from each carcass, we identified a predator species at 100% of carcasses that had a killing wound (10) and 94% of carcasses overall. We only found a collar at one of the sites where a predator species was not identified using molecular tools, and it was unclear whether the calf was preyed upon or had slipped its collar because there was no blood or tooth marks on the collar. Only a single predator species was detected at each kill site using molecular tools. Predator scats were only present at a small proportion of kill sites, but predator species identified by scat samples confirmed the predator species identified via swab samples.

Table 1 Molecular species identification success rates by sample

Sample type	# Samples	% Success /sample	# Carcasses	% Success /carcass
Scat	12	67	5	80
Hair	3	0	2	0
Killing wound swab	28	86	10	100
Feeding wound swab	111	46	22	90
Total swab	139	54	32	94

Molecular and field observation method comparison

Molecular methods detected a predator species at 30 of 32 kill sites (94%), while the field observation method assigned a predator species at 20 of 32 kill sites (62.5%). Predator species were assigned for 11 kill sites where the field observation method failed in comparison with one kill site where the field observation method alone assigned a predator species. Molecular methods identified a predator species at three kill sites despite locating only a collar during field investigations. The molecular and field observation methods both failed for the site where the collar was potentially slipped.

Twelve kills (63%) had a molecular and field observation predator species assignment that agreed, but assignments differed at another seven kills (37%) where a predator species was assigned by both methods. At three kill sites, molecular methods detected coyote DNA when field observation methods assigned Canada lynx or red fox. There was also one kill assigned to coyote and three kills assigned to black bear via the field observation method that the molecular methods assigned to the opposite species.

Molecular methods assigned 20 caribou calf kills (66.7%) to coyote, including all three severed heads, and 10 to black bear (33.3%), while red fox and Canada lynx were not detected using molecular tools (Fig. 2B). Coyote DNA was detected at 70% of carcasses containing a killing wound with black bears accounting for the remaining proportion. Kill sites assigned using field observation methods (n = 20) attributed 8 (40%), 9 (45%), 2 (10%) and 1 (5%) to coyote, black bear, Canada lynx and red fox, respectively (Fig. 2A). The field observation method did not attribute any kills to bald eagles, but site investigations inferred the occurrence of eagle scavenging at several sites. Field observation and/or molecular methods assigned a mammalian predator species to each of these sites.

Individual and sex identification

Swabs that were successful for species identification were also analysed for individual identification. Overall an individual predator was identified from 62% of swabs and at 70% of carcasses (Table 2). Molecular methods

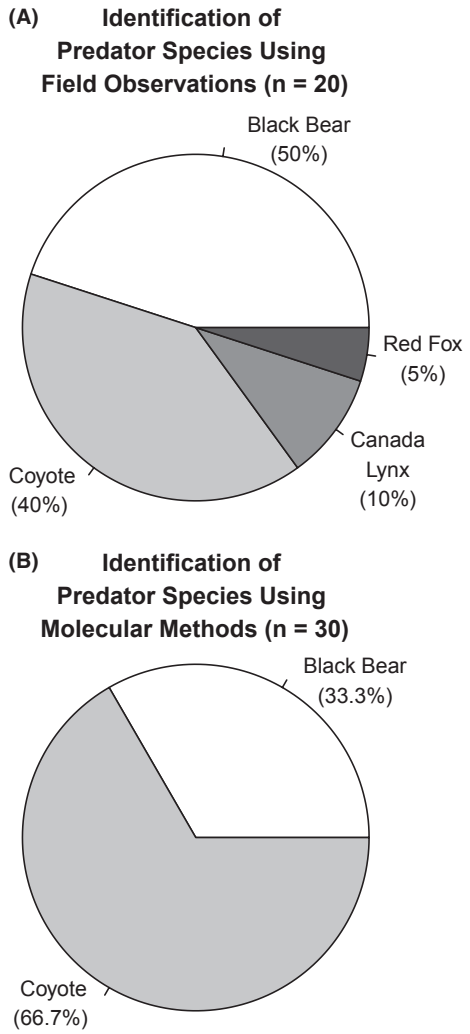

Fig. 2 The proportion of predation attributed to each predator species via field and molecular methods.

Table 2 Swab sample individual success rates by species

Sample type	# Samples	% Success /swab	# Carcasses	% Success /carcass
Black bear swab	21	52	10	70
Coyote swab	26	69	20	70
Total swab	47	62	30	70

only detected a single individual at each kill, although one swab recovered from a kill site where we visually detected two coyotes was mixed as evidenced by the existence of >2 alleles for multiple loci. Individual identification for black bear swabs was slightly lower (52%) than the success rates for coyote swabs (69%) (Table 2). We detected five unique, individual black bears, one of which was found at three caribou calf kill sites. Four of these bears were male, including the individual detected three times. We detected 11 individual coyotes, and all but two were male. In addition to nine single captures, we detected one male coyote two times and another male three times.

Discussion

Our pilot study using DNA analysis to evaluate predation of Newfoundland caribou calves confirmed the utility of molecular methods for improving kill site predator species identification. Species identification was highly successful and could be assigned to 30 of 32 kill sites (94%). Two striking benefits of using molecular methods to study predation are the reduction in mortalities attributed to unknown predators and the increased accuracy of predator species assignments. Molecular methods decreased the proportion of calf mortalities attributed to unknown predators by 31.5% (10 kills), and at seven kill sites (37%), molecular methods detected a different predator species than was assigned using field observation methods. Molecular methods also provide a means to determine predator-specific kill site observations, which should improve the accuracy of predator species identification using field observations.

In our system, molecular methods changed our understanding of the proportion of calf predation attributed to each predator species. The field observation method determined that black bear (45%) were the primary predator of caribou calves followed by coyote (40%) and attributed a small amount of predation to Canada lynx and red fox (Fig. 2A). However, coyotes were detected nearly twice as frequently (66.7%) as black bears (33.3%) according to the molecular results, while Canada lynx and red fox were not detected (Fig. 2B). The increase in the proportion of coyote kills estimated using molecular methods can be explained by additional DNA-based predator species identifications at kills with nondescript predator killing and feeding characteristics and the assignment of coyotes to several kill sites that were assigned to Canada lynx and red fox using field observation. Although the proportion of predation attributed to coyote (66.7%) and black bear (33.3%) in our study (Fig. 2B) using molecular methods was similar to a caribou calf study in Quebec (Crete & Desrosiers 1995) and dissimilar to studies in Alaska (Jenkins & Barten 2005) and British Columbia (Gustine *et al.* 2006), the value of these comparisons is limited because our results are based on 1 year of research in comparison with the other studies, which spanned 2–7 years. Furthermore, preliminary data from our second year of research indicate there may be a more equal proportion of kills attributed to coyote and black bear.

Field biologist experience, uncertainty between species-specific killing and feeding characteristics, and scavenging may explain why field observation methods assigned a different predator species than molecular methods. Less experienced biologists were less likely to leave a kill site unassigned to a predator species and were more likely to have incongruent molecular and field identifications, which may indicate a failure to recognize similar kill site characteristics between predator species. Overlapping kill site characteristics could explain why coyote DNA was detected from the cervical killing wounds of three carcasses where field observations assigned Canada lynx (two kills) and red fox (one kill). Coyote DNA was also detected at two kill sites with skinned hides that were assigned to black bear via field observations. Detecting coyote DNA at kill sites with skinned remains of intact hide suggests this handling behaviour is not specific to black bear, but could result from coyote scavenging a bear kill. Scavenging could also explain the incongruence between molecular and field identifications for three carcasses discovered opportunistically that may have been on the landscape longer and were therefore more prone to scavenging than calves that were regularly monitored.

Inadvertently attributing calf predation to a scavenging species is a potential weakness of both molecular and field methods. Ideally, molecular methods would only use killing wound swabs to determine the predator species. However, we felt that using all of the collected samples was justified, because of the potential for a negative bias in the proportion of black bear kills because black bears tend to consume the majority of the carcass leaving only a few remains and eliminating any evidence of the killing bite wound. In fact, we potentially detected a bias because the proportion of coyote to black bear kills decreased from 2.3 for killing wounds to 1.8 for killing and feeding wounds combined. Furthermore, we think that scavenging was limited overall because 22 of the 26 (85%) collared calf kill sites were recovered early in the study when monitoring was frequent, and molecular methods only detected one individual predator per kill site for both collared calves and calves discovered opportunistically with the exception of the kill site with the mixed swab.

The application of molecular methods to identify individual predators and their sex is an underexplored resource that could help inform predator–prey management. Other studies have shown that one or more specialist predators can have a large impact on prey populations (Ross *et al.* 1997; Ernest *et al.* 2002; Festa-Bianchet *et al.* 2006). Our sample sizes were too small to draw any conclusions regarding individual specialization, but the skewed number of male coyotes detected at kill sites supported our hypothesis that male predators prey on caribou calves more frequently than female predators. This is consistent with Blejwas *et al.* (2006) attributing most domestic sheep kills to territorial, male coyotes. It is possible that the propensity of male predation is related to differences in home range size between males and females and/or the constraints placed on the female during cub- and pup-rearing (Harrison & Gilbert 1985), which coincides with caribou calving in the Newfoundland system. Alternatively, differences in nutritional requirements between sexes have been proposed as an explanation of sex-biased predation for other species (Barboza & Bowyer 2000).

Our per sample species identification success rates for hair, scat and swabs (0–67%) were lower than other studies (85–97%) (Blejwas *et al.* 2006; Onorato *et al.* 2006), but may have been affected by the damp Newfoundland climate, which would lead to increased DNA degradation (Piggott 2005; Murphy *et al.* 2007; Brinkman *et al.* 2010). Furthermore, our swabs were collected from killing and feeding wounds and from carcasses that were likely 1–2 days old in contrast to other studies that sampled carcasses within 24 h (Blejwas *et al.* 2006; Sundquist *et al.* 2008). However, our species identification success rates per carcass (94%) were similar to other studies as a result of collecting multiple swabs from every carcass as recommended by Sundquist *et al.* (2008). Our individual identification success rates for swabs (62%) were slightly higher than other studies (50–58%) (Blejwas *et al.* 2006; Sundquist *et al.* 2008; Caniglia *et al.* 2012). We affirm the suggestion by Sundquist *et al.* (2008) to collect multiple swabs and further recommend sampling multiple locations of each carcass to increase per carcass success rates.

Additional research is necessary to determine the length of time predator DNA stays viable on a carcass. Multiple studies demonstrated amplification success rates for scats decrease with time since deposition (Piggott 2005; Murphy *et al.* 2007; Santini *et al.* 2007; Panasci *et al.* 2011), and we anticipated a similar relationship for swab success rates. However, we did not see a change in molecular identification success rates between carcasses of collared individuals that were monitored every other day ($n = 22$) and once a week ($n = 4$) or for carcasses discovered opportunistically ($n = 6$). This may suggest that once a week monitoring is sufficient, but the increased likelihood of scavenging must also be considered by researchers hoping to balance amplification success rates with the costs of monitoring.

In summary, we have demonstrated the effectiveness of DNA-based methods for identifying predator species, individual and sex at caribou calf kill sites in Newfoundland. Molecular methods can increase the reliability and accuracy of predator species identifications and could be

particularly informative in sparsely studied, multipredator systems. We feel that molecular methods are underutilized in the study of predation and recommend their application across a wide range of studies. However, we do not think that molecular methods should replace field observation methods, but must instead be viewed as complementary because both methods inform understanding of predator–prey relationships.

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Data Accessibility

Microsatellite data: DRYAD entry doi:10.5061/dryad.sd871.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. The field observation sheet for caribou calf kill site MR-2010-016.

Data S1. Material and Methods.