DIETS OF COYOTES AND OTHER CARNIVORES IN OLYMPIC NATIONAL PARK

FINAL REPORT

FOR NPS PROJECT NUMBER UMT-141
Rocky Mountains CESU agreement

BY

DR. L. SCOTT MILLS
WILDLIFE BIOLOGY PROGRAM
UNIVERSITY OF MONTANA
MISSOULA, MT 59812
Phone 406 243-5552
Email: LScott.Mills@umontana.edu

October 2008

INTRODUCTION

As coyotes expand their range in the U.S., they are having effects on endemic species in Parks and natural areas. Coyotes first arrived on the Olympic Peninsula in the early 20th century, and in the high country around the 1980s. In Olympic National Park, an in-depth study by University of Montana researchers (supported by National Science Foundation funding) has definitively linked coyote predation to an alarming decline of the Olympic marmot, an endemic species found almost entirely within the park (Griffin et al. 2008).

An M.S. student from University of Montana, Julia Witzcuk, collected 958 carnivore scats across the alpine area of the Park during 2004-2006. The identity of the species leaving the scat was determined in the field, but field-based assessments of species identity are not without error. Furthermore, no detailed analysis of diet was conducted. Therefore, the purpose of this small project was to extend previous work to include a rigorous assessment of diet based on field-collected scats, including diagnostic determination of species identity through genetic analysis. Specifically, the goals of this project were to: a) determine species identification for a large subsample of the scats collected in the ONP high country; b) determine diets of carnivores, by species, based on the scats; c) for scats determined definitively to be coyotes, use additional DNA analysis to determine individual identity of minimum number of coyotes and how many of them were known to eat marmots.

METHODS

Scat Collection

Scats were collected in 2005 and 2006, systematically on sample transects and opportunistically throughout the park during other activities (marmot trapping, presence/absence surveys etc.) (see Witczuk 2007 for details). During the summer (May – Sept.), the period when marmots are not hibernating, we conducted systematic monthly collection along 12 transects of varying length placed along park hiking trails and roads mainly in areas with relatively high marmot densities. The 125 km of transects provided representative coverage of areas containing marmot colonies across the park. All transects were located within an elevation range of 1000-2000 m (except for transect Lena starting at 750 m). The majority of the total length of transect (~70%) traversed alpine meadows and mixed meadow/forest habitats, while the remainder led through forests. Three transects (Hurricane, Obstruction and Royal) were located in the areas containing intensively studied sites with marked marmots, annually monitored for various demographic rates by Griffin et al. (2007, 2008); these transects (plus Steeple) were traveled more frequently than once a month (usually twice a month) in the course of other marmot project activities, and scats were collected whenever they were encountered.

All carnivore scats (except mustelids and bear, whose scats are easily distinguishable from other carnivores and have never been reported – or observed in 4 years of intensive study (eg Griffin et al. 2007, 2008) – to prey on Olympic marmot) were collected from sample transects. For each scat, UTM coordinates were recorded and a 1-cm long segment of scat was placed into a plastic tube with silica gel for genetic analysis. Also, an assessment of species identity based on scat physical characteristics was made in the field; mid-sized carnivores in the Park include bobcat, cougar, fox and domestic dog (foxes are not native and unlikely to be in the Park alpine; pers. comm., Patti Happe, ONP Wildlife Branch Chief). The rest of the scat to be used for diet analysis was placed into a plastic zip-lock bag labeled with location and date and approximate age of the scat (fresh, medium and old).

Genetic Analysis of carnivore species and coyote individuals

Two different genetic analyses were performed to first determine species of carnivores responsible for each scat and second identify individual coyotes. Scat samples, stored with silica gel at room temperature prior to DNA extraction, had DNA extractions and amplifications

performed in separate buildings to reduce the risk of contaminations of low quantity/low quality fecal DNA with DNA from polymerase chain reaction (PCR) products. Approximately 0.20 g of material scraped from the scat surface with a scalpel was used for extraction, using the QIAampTM DNA Stool Mini Kits (QIAGEN). One negative control in each batch of extractions was used to test for contamination.

Species identification was based on an extension of the approach of Bidlack et al. (2007), amplifying via PCR a fragment of the cytochrome-*b* region of mtDNA. Reaction mixtures were 20 µL (4 µL of DNA extract, 0.5x reaction buffer, 8mM dNTPs, 10 mM primers:CanidL1 and HCarn200 [IDT], 15 mM $MgCl_2$, 0.8 units of Platinum Taq [INVITROGEN]). We ran PCR in PTC-100 thermocycler (MJ Research): initial denaturation at 95°C for 2 min., 40 cycles of 1 min. at 94 and 54°C, 40 cycles of 2 min at 72°C. We used two negative controls and four positive controls from tissue samples in each PCR. To distinguish between coyote, fox, cougar, bobcat and black bear we used three restriction digests; a double digest with *HpaII* and *DdeI* (Bidlack et al. 2007) followed by a digest with *MboI* to definitively distinguish coyote and black bear. All digests were run for 16 hours at 37°C and visualized by electrophoresis through 2% agarose gels post-stained with ethidium bromide.

Identification of individual coyotes was based on nuclear microsatellite markers. We used 6 microsatellite loci (FH2137, FH2159, FH2140, FH2235, FH2096, FH2001, Prugh et al. 2005) for coyote individual identification. We optimized two multiplex-PCRs for nuclear DNA amplification. The first mix included loci FH2096, FH2235, and FH2137 and contained: 2.5 µL of DNA extract, 1x QIA multi-plex mix (QIAGEN), 1x primer mix (each primer concentration of 0.2µM) , and 0.5x Q-solution. The second mix, for loci FH2140, FH2001, and FH2159, contained: 2.5 µL of DNA extract, 1x QIA multi-plex mix, and 1x primer mix. The final volume of reaction was 10µL in both cases. PCR was performed on a thermocycler using a touch-down profile: initial denaturation at 95°C for 5 minutes, followed by 20 cycles with 94°C denaturation for 30 seconds, 1 minute annealing starting at 62°C and stepping down 0.5°C per cycle, and 1 minute extension and then an additional 25 cycles at 52°C annealing temperature with an additional final extension cycle of 5 minutes. Genotypes were visualized using fragment analysis on a capillary automated DNA sequencer (Applied Biosystems) and analyzed with GeneMapper software v3.7 (Applied Biosystems). To minimize genotyping errors, PCR and analyses were performed at least twice for each sample (Frantz et al. 2003).

Diet Analysis

All collected scat samples were autoclaved, soaked in water for 24 hours, washed through a sieve and air dried (Arjo et al. 2002). Undigested prey items (hair, bone fragments, seeds etc.) were manually separated. Prey were distinguished by comparison with specimens housed at the University of Montana zoological museum and a hair identification key (Moore et al. 1974).

The frequency of occurrence (Corbett 1989, Hidalgo-Mihart et al. 2001) for prey species *j* was calculated separately for each month and sample transect by:

$$(\text{number of fecal samples with species } j \times 100) \; / \; \text{number of fecal samples} \qquad \text{(Equn. 1)}$$

For the monthly analysis, we assigned each sample to the most likely date of deposit based on approximate age of scat recorded. Fresh scats were always assigned to the month of collection. Scats recorded as old or medium were assigned to the month of collection if collected after the 10th day of the month, and to the previous month if collected in the first 10 days of the month. Old scats collected away from the sample transects were removed from monthly analysis because the date of deposit was impossible to determine.

RESULTS

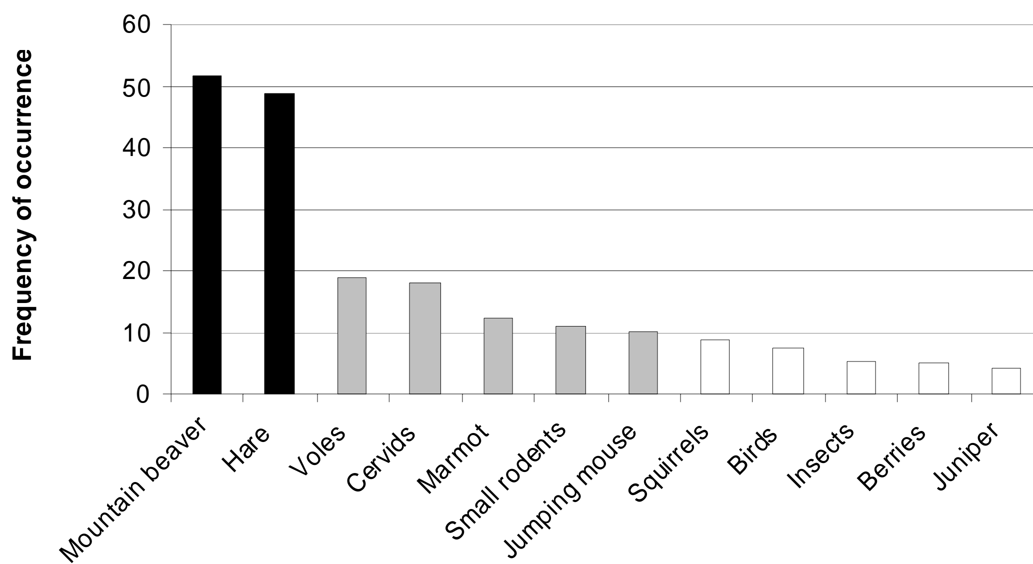
A total of 958 scats were collected during the 2 year study; 89% (857 scats) were collected on the sample transects, with an average rate of encounter of 3.4 scats per km per year. Of the 11% (101 scats) collected opportunistically, only 27 were collected more than 1 km from the sample transects. The number of scats collected was similar between years (428 in 2005 and 530 in 2006), and were pooled for all analyses.

Based on the identifications in the field, coyote scats constituted the vast majority of scats collected in all studied regions (84% percent on average). We checked for error rate in field-based scat identifications using 100 scats for which we had both field assignment of the species and the species confirmation with mtDNA. Overall accuracy in distinguishing coyote scats from scats of felids was 85%. More often felid scats were incorrectly assigned as coyote than coyote scats as felids (56% of 18 scats genetically identified as felids were assigned in the field to coyote and only 9% of 82 coyote scats were assigned as bobcat or cougar). Among scats for which species identification was confirmed by mtDNA, coyote scats (n = 85) were found mainly in the open areas of the alpine zone but also in the forest at lower elevations; in contrast bobcat scats (n = 12) were found exclusively in the forest, mainly along deep river valleys (Dosewallips and part of Cameron region). The lowest elevation where confirmed coyote scat was collected was approximately 1200 m and the highest 2000 m.

Prey Contained in All Carnivore Scats

For this project, complete diet analysis was conducted for a random selection of 397 of the collected scats. For this set of carnivore feces, collectively coming from coyotes, bobcat, and cougar, the primary prey were mountain beaver and hare, with voles, cervids, and marmot also having frequency of occurrence >10% (Figure 1).

Figure 1. Frequency of occurrence of prey items in all scats analyzed (from coyote, bobcat and cougar) (N=397). The black bars represent prey items occurring in >40% of all scats, grey bars in >10%, and white bars <10%. The "small rodents" category includes unidentified vole and mouse species.



Prey Consumed by Carnivore Species

Because there is some known error in the determination of carnivore species in the field, our analysis of prey by carnivore species is limited to samples for which we have definitively determined species through genetic testing (Table 1). Again, mountain beaver and hare are primary prey species for all 3 species, although not surprisingly, cervids are the dominant prey item for cougars. Because marmot predation was of pre-eminent importance, for our first round of analysis completed to date we intentionally emphasized DNA species identification of carnivores that ate marmots. For that reason, the frequency of marmots may be biased high. This will disappear when we complete ongoing genetic analyses of species identity for all of the samples whose diet was analyzed; the marmot % occurrence across all carnivores in Figure 1 does not suffer from this potential bias, because that graphic relies on all 397 diet samples pooled for all carnivore species.

Table 1. Frequency of occurrence of prey items in ONP, by confirmed predator species.

	COYOTE n=79		**BOBCAT[1]** n=31		**COUGAR** n=13	
PREY GROUP	**Count**	**Frequency of occurrence[2]**	**Count**	**Frequency of occurrence[2]**	**Count**	**Frequency of occurrence[2]**
Mountain beaver	39	**49.4**	12	**38.7**	4	**30.8**
Hare	33	**41.8**	16	**51.6**	3	**23.1**
Voles	18	**22.8**	3	**9.7**	0	**0.0**
Cervids	10	**12.7**	5	**16.1**	6	**46.2**
Marmot	31[3]	**39.2[3]**	10	**32.3[3]**	1	**7.7[3]**
Small rodents	5	**6.3**	5	**16.1**	0	**0.0**
Jumping mouse	5	**6.3**	2	**6.5**	0	**0.0**
Squirrels	6	**7.6**	4	**12.9**	1	**7.7**
Birds	8	**10.1**	2	**6.5**	0	**0.0**
Insects	3	**3.8**	1	**3.2**	1	**7.7**
Berries	5	**6.3**	0	**0.0**	0	**0.0**
Juniper	6	**7.6**	0	**0.0**	0	**0.0**
Grass	10	**12.7**	6	**19.4**	6	**46.2**

[1] 22 out of 31 samples genetically recognized as bobcat exhibit unusual pattern which seems to indicate some lynx introgression. These samples will undergo additional sequencing procedures.
[2] See equation 1 in text for Frequency of Occurrence.
[3] As noted in text, the frequency of marmots in coyotes is probably biased high until we finalize the follow-up carnivore species identification testing we are doing. No other diet items or other carnivore species are affected.

Diet of Individual Coyotes

Individual animals left multiple scats. For coyotes, we are interested in a minimum count of number of different coyotes sampled, and whether the majority of individual coyotes had marmot remains in their diet. When determining individuals from genotypes it is necessary to avoid both genotyping errors, which would inflate the number of different individuals (Kalinowski et al. 2006) and the shadow effect (or high probability of identity leading to different individuals being considered the same), which would bias low the number of individuals (Mills et al. 2000). After taking measures to minimize both issues, we find that there was a minimum of 14 different coyotes in the high country of Olympic National Park. Importantly, at least 12 of these consumed marmots. Table 2 shows a sample of the diets of 9 of the individual coyotes.

Table 2. A representative sample of the diets of 9 of the 14 individual coyotes identified in the ONP high country.

			PREY IN SCAT												
Individual #	Individual Coyote ID	Scats associated with that individual	Mt beaver	Hare	Voles	Cervids	Marmot	Small rodents	Jumping mouse	Squirrels	Birds	Insects	Berries	Juniper	Grass
1	7L1	1013					+								
2	7L2	590					+						+		
	GW1	543		+	+		+								
	GW1	1062	+				+								
3	GW1	1116					+								
	GW2	375	+	+						+					
	GW2	516	+			+							+		
4	GW2	521				+							+		
5	H1	589	+				+		+						
6	L1	1015					+								
	O1	267	+		+										
	O1	273		+										+	
	O1	289					+								
	O1	292	+												
	O1	306	+	+	+										
	O1	309					+								
	O1	322	+				+								
	O1	426	+												+
	O1	430		+	+						+				
	O1	475	+					+							
	O1	478		+					+		+				
	O1	709		+	+		+							+	+
	O1	787				+	+								
	O1	790				+	+								
7	O1	814		+			+								
	O2	800	+		+		+							+	+
	O2	1146			+		+								
8	O2	1151	+		+		+						+		+
	O3	264		+											
	O3	291		+			+								
	O3	317	+												
	O3	320	+												
	O3	584		+			+		+						
	O3	797		+			+								
9	O3	1137		+			+								

DISCUSSION AND CONCLUSIONS

We find that coyotes, cougars, and bobcat in the high country of ONP are collectively consuming as their main prey items mountain beaver and snowshoe hare, with voles, cervids and marmots also making up substantial portions of the diet. These trends roughly hold when considering these three carnivores by species. At the level of individual animals, nearly all individual coyotes (at least 12 out of 14 individuals) did consume marmots as part of their diet. Therefore, invasive coyotes in the high country of ONP are playing the role of a generalist predator, supported by a wide range of prey species, that also consume marmots. The fact that coyote numerical response is decoupled from the numbers of (declining) marmots could well lead to destabilizing positive density dependence, whereby predation by subsidized coyotes could decrease marmot numbers with no consequence for the coyotes. A more detailed understanding of coyote movements, numbers, and distribution in ONP is therefore urgent.

LITERATURE CITED

Bidlack, A. L., S. E. Reed, P. J. Palsboll, W. M. Getz. 2007. Characterization of a western North American carnivore community using PCR-RFLP of cytochrome b obtained from fecal samples. Conservation Genetics 8:1511-1513.

Corbett, L. K., 1989. Assessing the diet of dingoes from feces: a comparison of 3 methods. Journal of Wildlife Management, **53**:343-6.

Griffin, S. C., T. Valois, M. L. Taper, and L. S. Mills. 2007. The impact of tourism on Olympic marmot behavior and demography. Conservation Biology 21:1070-1081.

Frantz, A. C., L. C. Pope, P. J. Carpenter, T. J. Roper, G. J. Wilson, R. J. Delahay, and T. Burke, 2003. Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Molecular Ecology, **12:**1649–1661.

Griffin, S. C., M. L. Taper, R. Hoffman, and L. S. Mills. 2008. The case of the missing marmots: are metapopulation dynamics or range-wide declines responsible? Biological Conservation 141:1293-1309.

Hidalgo-Mihart, M. G., L. Cantú-Salazar, C. A. López- González, E. Martínez-Meyer, and A. González-Romero, 2001. Coyote (*Canis latrans*) food habits in a tropical deciduous forest of western Mexico. American Midland Naturalist, **146**:210–216.

Kalinowski, S. T., M. L. Taper, and S. Creel. 2006. Using DNA from non-invasive samples to identify individuals and census populations: an evidential approach tolerant of genotyping errors. Conservation Genetics 7:319-329.

MILLS, L. S. CONSERVATION OF WILDLIFE POPULATIONS: DEMOGRAPHY, GENETICS, AND MANAGEMENT. BLACKWELL PRESS, 2007. 407 PAGES.

Mills, L. S., J. J. Citta, K. Lair, M. Schwartz, D. Tallmon. 2000. Estimating animal abundance using non-invasive DNA sampling: Promise and Pitfalls. Ecological Applications 10:283-294.

Mills, L. S., K. L. Pilgrim, M. K. Schwartz, and K. McKelvey. 2000. Identifying lynx and other North American felids based on mtDNA analysis. Conservation Genetics 1:285-288.

Moore, T. D., L. E. Spence, and C. E. Dugnolle, 1974. Identification of the dorsal guard hairs of some mammals of Wyoming. Wyoming Game and Fish Department, Cheyenne, Wyoming.

PRUGH, L. R., C. E. RITLAND, S. M. ARTHUR, AND C, J. KREBS. 2005. MONITORING COYOTE POPULATION DYNAMICS BY GENOTYPING FAECES. MOLECULAR ECOLOGY 14:1585-1596.

Witczuk, J. 2007. Monitoring program and assessment of coyote predation for Olympic marmots. M.S. Thesis, University of Montana.