FECUNDITY OF FEMALE WOLVERINE IN MONTANA

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ABSTRACT

We collected carcasses from trapper-harvested wolverine (*Gulo gulo*) in Montana from 1984 through 2005 to evaluate pregnancy rates and corpora lutea production as an estimate of wolverine fecundity in eco-regions and subpopulations of western Montana. Pregnancy rates in the northwest eco-region were 100 percent for adults (n = 11) and 30 percent for subadults (n = 15). Pregnancy rates within the southwest eco-region were less than observed in the northwest (67.8 % for adults, n = 28; and 12.5 % for subadults, n = 16). We observed similar results in subpopulations from northwestern and southwestern Montana. Median corpora lutea counts for pooled adult and subadult females also differed among eco-regions being greater in northwest (median = 3.0, n = 26) than the southwest (median = 0.0, n = 44). Litter sizes and measurements of recovered fetuses are also presented. Comparisons of our data to similar studies in North America suggested adult pregnancy rates and mean litter sizes observed in the southwestern eco-region of our study area are the lowest reported in the literature.

Key words: corpora lutea, *Gulo gulo*, in-utero, Montana, pregnancy, reproduction, wolverine.

INTRODUCTION

Wolverines (*Gulo gulo*) were once widely distributed across the North American continent. However, recent findings suggest that this distribution may have been disjunct in the Pacific and Rocky Mountain states, being limited to high elevation habitats in the mountain west (Aubry et al. 2007). Habitat loss and over harvest have been cited as causes for the reduction in occupied range observed in the 1800s and early 1900s (Newby and Wright 1955, Wilson 1982, Hash 1987, Aubry et al. 2007). Currently the wolverine's range is believed to be limited to Alaska, northern and western Canada, and the mountainous regions of the northwestern contiguous United States (Wilson 1982, Hash 1987, Aubry et al. 2007). Although once considered to be near extinction in Montana (Newby and Wright 1955), the Rocky Mountain states of Idaho and Montana are considered to have the largest and most stable populations south of Canada, partially due to close proximity to Canada and availability of suitable habitat (Hash 1987, Aubry et al. 2007, Brock et al. 2007). Legal harvest has been eliminated in the lower 48 states with the exception of Montana, which still maintains a limited trapping season.

Information regarding population parameters such as age, sex structure, and reproduction is limited for populations in the wolverine's southern range due to their low relative abundance and secretive nature. The majority of information available on population parameters in North America has come from Alaska and northern Canada where wolverine abundance has been maintained (Rausch and Pearson 1972, Liskot et al. 1981, and Banci and Harestad 1988). Studies conducted in the lower 48 states focused on basic ecology, movements, habitat use, and genetics with limited information regarding reproduction and age structure (Hornocker and Hash 1981, Copeland 1996, Cegelski et al. 2003, Cegelski et al. 2006, Aubry et al. 2007, Brock et al. 2007, Copeland et al. 2007, Inman et al. 2007*a*, Inman et al. 2007*b*,

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Inman et al. 2007*c*). Lack of information about southern wolverine population structure led to management decisions based primarily on data extrapolated from populations in Alaska and Canada. Reproductive information from these studies demonstrates variation in reproductive parameters, especially pregnancy rates (Rausch and Pearson 1972, Liskop et al. 1981, and Banci and Harestad 1988). However, management decisions based on information obtained through these studies may not be applicable to wolverine populations occupying habitats in the southern portion of their range.

The objectives of this study were to 1) evaluate the reproductive potential of Montana wolverines based on *a priori* boundaries and for three genetically isolated subpopulations suggested by Cegelski et al. (2003), 2) provide basic information on reproductive and fetal development rates, and 3) evaluate the ability of corpora lutea (CL) counts to estimate number of in-utero fetuses in wolverine.

STUDY AREA

The study area consisted of the mountainous region of western Montana. Montana Fish, Wildlife and Parks (MFWP) manages wildlife populations based on seven administrative regions throughout the state. Wolverine harvest occurs in five of those regions with the majority occurring in regions one, two and three all of which occur in western Montana (Fig. 1).

Climates and habitats vary within the study area across a northwest-to-southeast moisture gradient. Meridian weather patterns influence the climate of the northwestern region resulting in less variation in temperatures and higher levels of precipitation, ranging from approximately 35.5 cm to 55.9 cm annually in the valleys, up to 86.4 cm in the mountain foothills, and 215.9 cm at the upper elevations of the mountain ranges (Montana Natural Resource Information System 1971-2000). Precipitation typically increases with elevation that ranges from ~ 621 m to 1200 m in the valleys to about 1800 m to 2700 m in the mountains. Habitats generally consist of valley forests comprised of *Thuja*, *Picea,* and *Tsuga* species intermixed with grassland openings. *Abies* dominates the mountainous forests of the northwest.

Broad valleys and prairie ecotones interrupt mountain ranges and characterize

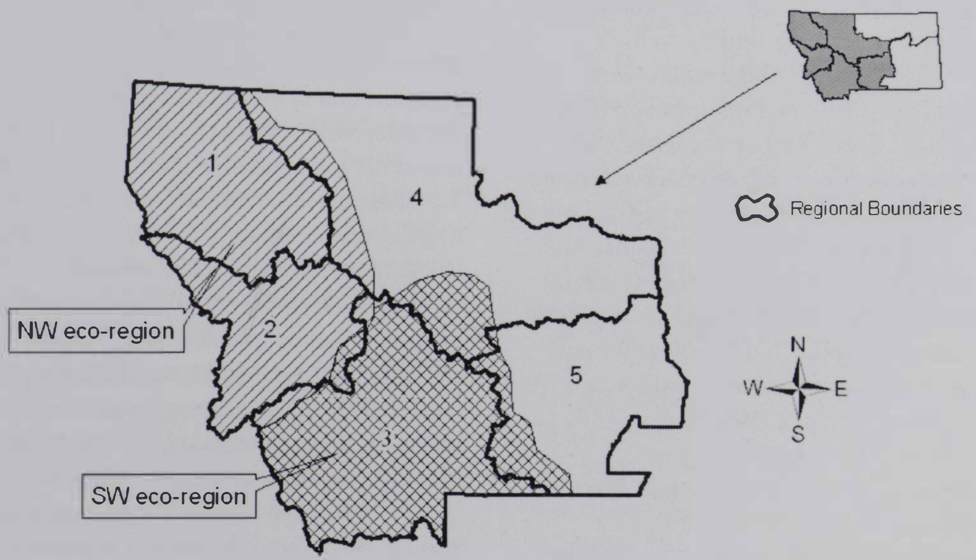

Figure 1. Study area delineating eco-regions of western Montana (inset). The eco-region boundaries were determined using Montana Fish, Wildlife and Parks administrative regions boundaries and ecological considerations. Administrative regions are numbered and eco-regions are identified by cross-hatching.

the southwest and southeast portion of the study area. Valley habitats and prairie ecotones consist primarily of grass and sagebrush habitats containing river and creek riparian areas dominated by willow (*Salix* spp.) and cottonwood (*Populus* spp.). The forested regions of the southern mountains consist primarily of lodgepole pine (*Pinus contorta*), Douglas fir (*Psuedostuga menziesii*) and white pine (*Pinus albicaulis*) interspersed with grass and sagebrush (*Artemisia* spp.) parks. Annual precipitation varies from ~ 15.24 cm in the dryer valleys to 35.6 cm in the wetter mountain valleys, increasing to > 216 cm in isolated locations of the Absaroka and Beartooth ranges (Montana Natural Resource Information System 1971-2000). As with the northwestern region of the study area precipitation generally increases with elevation that ranges from about 1370 m in the lower valleys to 3350 m in the higher mountains.

The study area was divided into two eco-regions, northwestern (NW) and southwestern (SW) based on general ecological difference and management boundaries. The NW eco-region consisted of MFWP administrative regions one, two and the portion of region four containing the Rocky Mountain front. The SW eco-region consisted of MFWP administrative regions three, five and the southern half of region four, including the Little Belt, Big Belt, and Crazy Mountains (Fig. 1). Higher precipitation rates, moderate temperatures, and connected habitats generally characterize the NW eco-region. Habitat within the SW unit generally exhibits greater temperature variation, lower precipitation rates and isolated mountain chains separated by open grassland or sagebrush valleys.

The study area was further divided into subpopulations based on the findings of Cegelski et al. (2003) in a study that assessed and evaluated genetic structure producing three subpopulations of wolverine in Montana. The subpopulations were designated as the Rocky Mountain Front (RMF), Gallatin (GAL) and the Crazy/Belts (CB) (Fig. 2). The RMF subpopulation comprises a majority of the mountainous portion of northwestern Montana excluding the extreme western

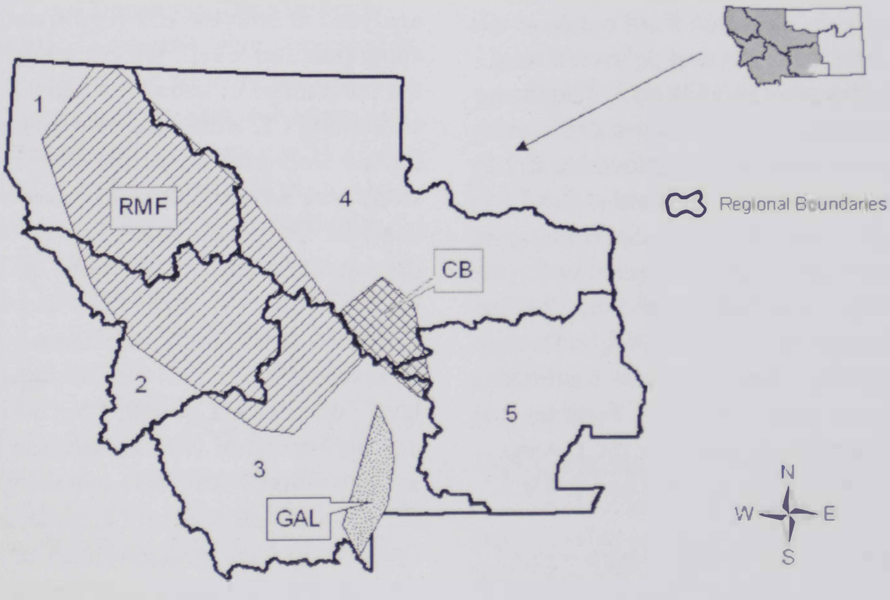

Figure 2. Subpopulation delineations based on home kernel estimates established by Cegelski et al. (2003) for western Montana (inset). Subpopulations are designated as RMF, CB and GAL for the Rocky Mountain Front, Crazy/Belts and Gallatin subpopulations, respectively. Montana Fish, Wildlife and Parks administrative are delineated and identified numerically.

edge. The GAL subpopulation resides within the mountainous regions of southwestern Montana and consists of the Bridger, Gallatin, and eastern portions of the Madison Ranges. The CB subpopulation consists of the Little Belt, Big Belt and Crazy Mountain Ranges in south-central Montana.

METHODS

Carcasses were collected from trapper-harvested wolverines from the 1984-85 through the 2004-05 trapping seasons. Trapping seasons ran from early December through mid February. Trappers were required to submit skinned wolverine carcasses to a MFWP official after harvest and complete a harvest form stating harvest date and location as part of a required registration process. Carcasses were frozen at regional collection points and transported to the MFWP Wildlife Research Laboratory in Bozeman, Montana, for examination.

Carcasses were defrosted and examined at the MFWP Wildlife Research Laboratory during which female reproductive tracts and an upper canine were collected. Reproductive tracts were collected and preserved in 10 percent buffered formalin. Ovary pairs were dissected from bursa, packed in distilled water and delivered to Matson's Laboratory in Milltown, Montana, for sectioning and mounting on slides. Ovaries were then serially sectioned at a thickness of 10 microns collected at 0.4-mm intervals across the ovary. Sections were mounted on slides and stained with an aniline blue, acid fuchsin, and orange G solution. We examined slides using a four-power dissecting microscope and identified and counted corpora lutea (CL). Females were considered to be pregnant if CL were present. We totaled the number of CL for both ovaries in the pair.

Attempts to recover blastocysts were not conducted based on difficulties in recovery rates observed in similar studies and the effects of freezing on recovery (Rausch and Pearson 1972, Liskop et al. 1981, Banci and Harestad 1988). Macroscopically visible fetuses were removed from pregnant wolverines during the examination process. Weight in grams, recorded to the nearest 0.1 g, and crown-rump length, measured in millimeters, were obtained for individual fetuses and, when discernable, sex was determined and recorded. Due to the small number of fetuses collected, crown-rump length, weight, and sex data were pooled for the entire study area. Body measurements were not taken during early fetal development, i.e., embryonic, when gross structure was not apparent.

We collected upper canines for aging using cementum analysis by warming the skull in hot water. The canine was removed and ~ 5 mm of the root tip was cut from the tooth and sent to Matson's Laboratory, Milltown, Montana, for aging. Because harvest occurred from December through mid February, young of the year (juveniles) would have been ~ 0.5 years old when harvested. Therefore, we recorded cementum ages in yearly intervals starting at 0.5 for juveniles. Wolverines were placed into three age classes depending on cementum age, juvenile (0.5 yrs), subadult (1.5 yrs) and adult (> 1.5 yrs).

Corpora lutea (CL) counts were analyzed at both the eco-region and subpopulation level. We compared the percentage of subadults and adults containing CL within the two levels. All female wolverine harvested within the study area were included in the eco-region analysis. Only wolverines harvested within the minimum convex polygons established for the RMF, GAL and CB subpopulations (Cegelski et al. 2003) were considered when conducting analysis at the subpopulation level. We used CL counts from pregnant and non-pregnant females in mean and median comparisons between study areas. CL counts from pregnant females only were used to calculate potential litter sizes. Data for age and CL counts were non-normally distributed requiring non-parametric statistical evaluation at both the eco-region and subpopulation levels.

The Mann-Whitney W test was used to compare differences in median values of age and CL counts between eco-regions. We determined statistical difference in median age and CL counts among subpopulations with the Kruskal-Wallis test. Two-sample hypothesis analysis was conducted to determine significance in the difference of pregnancy rate between similar age classes of the two eco-regions. Significant differences in pregnancy rates among similar age classes of each subpopulation were determined by Chi-square analysis. A sign test for paired samples was used to test the hypothesis that the difference between the median number of CL and the median number of fetuses did not equal zero based on the number of values above and below the hypothesized median for individuals where fetuses were macroscopically visible. We used a P-value ≤ 0.05 to determine significance for all tests. Mean values ± one standard deviation are presented for comparison to previous studies although statistical analysis was not conducted.

RESULTS

Eco-region Level Analysis

We collected a tooth and reproductive tracts from 83 female wolverines harvested by trappers from December 1985 through February 2005. Thirty-one were harvested in the NW eco-region and 52 were harvested in the SW eco-region. Juveniles, subadults and adults comprised 16.1 percent ($n = 5$), 48.4 percent ($n = 15$) and 35.5 percent ($n = 11$) of the NW eco-region harvests, respectively. Within the SW eco-region juveniles comprised 15.4 percent ($n = 8$), subadults 30.8 percent ($n = 16$) and adults 53.8 percent ($n = 28$) of the harvest. Ages based on cementum analysis ranged from 0.5 to 10.5 in the NW eco-region and 0.5 to 9.5 in the SW eco-region. Although median ages were 1.5 and 2.5 for the NW and SW eco-regions, respectively, they did not differ ($P = 0.2002$, W = 132.5).

We observed no CL in ovaries from wolverine aged as juveniles by cementum analysis. Based on these findings and those of other authors (Wright and Rausch 1955, Rausch and Pearson 1972 and Banci and Harestad 1988), we did not consider juvenile wolverines to be sexually mature and did not include them in analysis of reproductive parameters. As a result, ovaries from 26 NW eco-region wolverines and 44 SW eco-region wolverines > 1 year old were used in analysis of reproductive parameters.

Pregnancy rates based on the presence of CL were higher in the NW eco-region compared to the SW eco-region for subadult ($P = 0.0011$, z statistic = 3.269), adult ($P =$ 0.0318, z statistic = 2.146) and pooled age classes ($P = 0.0139$, z statistic = 2.2.458). Likewise, median CL counts were also greater for NW eco-region wolverine when both age classes were pooled ($P = 0.0233$, W = -175) ranging from 0.0 in SW eco-region subadults to 3.0 in both subadults and adults from the NW eco-region. Mean CL counts ranged from a low of 0.4 ± 1.0 in SW eco-region subadults to a high of 3.2 ± 0.6 for NW eco-region adults (Table 1).

CL were present in nine NW eco-region subadult wolverines of which seven (77.8 %) contained three CL and two (22.2 %) contained four CL. The number of CL observed in adult ovaries of wolverine harvested in the NW eco-region ranged from two (9.1 %) to four (27.3 %) with the majority (63.6 %) containing three CL. Three CL were present in the ovaries of both pregnant SW eco-region subadults. CL were present in 21 adult ovaries ranging from one (9.1 %) to four (36.4 %), with the majority (54.5 %) containing three. When only pregnant females were evaluated mean litter sizes were 3.2 ± 0.4 ($n = 9$) for subadults and 3.2 ± 0.6 for adults in the NW eco-region and 3.0 ± 0.0 ($n = 2$) for subadults and 3.1 ± 0.8 ($n = 19$) for adults in the SW eco-region.

Subpopulation Level Analysis

We evaluated age composition and reproductive parameters for 69 wolverine harvested within the 95-percent home-range kernel delineation established by Cegelski et al. (2003) (Fig. 2). Number of female wolverine carcasses examined in the RMF, GAL and CB subpopulations were 27, 30 and 12, respectively. Median age did not

Table 1. Reproduction of female wolverine for eco-regions of western Montana, 1984-2005. Pooled samples combine both subadult (age=1.5) and adult (age>1.5) samples. Calculations of mean and median corpora lutea counts include pregnant and non-pregnant animals.

	NW eco-region					SW eco-region				
			CL Count					CL Count		
Age Class	n	% Pregnant	Median	Mean	SD	n	% Pregnant	Median	Mean	SD
Subadult	15	60.0	3.0	1.9	1.7	16	12.5	0.0	0.4	1.0
Adult	11	100.0	3.0	3.2	0.6	28	67.8	3.0	2.1	1.6
Pooled	26	76.9	3.0	2.5	1.4	44	47.7	0.0	1.5	1.6

differ among subpopulations ($P = 0.8055$, test statistic = 0.4327) being 1.5 for RMF, 1.5 for GAL and 2.5 for CB and ranging from 0.5 - 9.5 for both the RMF and GAL and 0.5 - 5.5 for CB.

Pregnancy rates and CL counts were evaluated for subadult and adult wolverine within each subpopulation (Table 2). Juveniles were excluded resulting in a total of 25, 24 and 10 ovary pairs available for analysis from the RMF, GAL and CB populations, respectively. Pregnancy rates did not differ when similar age classes of subadult ($P = 0.1995$, $X^2 = 3.22$, df = 2), adult ($P = 0.1080$, $X^2 = 4.45$, df = 2) and pooled age classes ($P = 0.1381$, $X^2 = 3.96$, df = 2) of all subpopulations were compared. Median CL counts for pooled age classes were 3.0 for the RMF, 1.0 for the GAL and 0.0 for the CB subpopulations, but did not differ ($P = 0.1559$, test statistic = 3.7164). Although not tested, mean CL counts for pregnant adults were 3.0 ± 0.5, 2.2 ± 1.6 and 1.6 ± 1.7 for the RMF, GAL and CB subpopulations, respectively. When CL were present, counts ranged from 2 to 4 in the RMF and GAL and 1 to 4 in the CB.

Fetal analysis

Due to the small number of females with macroscopically visible fetuses or embryos, fetus measurements and comparisons of CL to fetuses from all study areas were pooled. Fetuses were observed in 21 female wolverine harvested during the study period that ranged in age from 1.5-9.5 years. We detected CL in ovaries in all instances where fetuses were observed. A total of 60 fetuses were collected resulting in an average of 2.8 fetuses/pregnant female with litter sizes ranging from 1 - 4. We obtained crown-rump length and weight measurements for 35 fetuses from 13 females. Twenty-five fetuses from eight females were in early stages of development and were thus classified as embryonic and not measured or weighed. Crown-rump lengths ranged from 24-132 mm and weights varied from 1.0-113.2g. The first date that we observed macroscopically visible fetuses occurred on 5 January in a single female, pregnant with three embryonic fetuses. Sex was determined for 19 fetuses collected from the reproductive tracts of six female wolverines resulting in an in-utero fetal sex

Table 2. Age class composition and pregnancy rates of female wolverines within the RMF, GAL and CB subpopulations of western Montana, 1984-2005. Pregnancy rates were determined by the presence of corpora lutea.

Age	RMF		GAL		CB	
Class	n	Pregnant (%)	n	Pregnant (%)	n	Pregnant (%)
Subadult	16	9 (56.2)	10	2 (20.0)	3	0 (0)
Adult	9	9 (100)	14	10 (71.4)	7	4 (57.1)
Pooled	25	18 (72.0)	24	12 (50.0)	10	4 (40.0)

Table 3. Date of death, litter size, fetus measurements and corresponding numbers of corpora lutea (CL) in ovaries from pregnant wolverine harvested in Montana, 1985-2005. Fetuses without macroscopically apparent features were considered embryonic and measurements were not obtained.

Date of Harvest (mm/dd/yy)	Litter Size	Mean crown-rump length (mm)	Mean weight (g) of fetuses	Sex Ratio	Number of CL
1/5/95	3	Embryo	Embryo	Unknown	3
1/13/94	3	Embryo	Embryo	Unknown	3
1/17/96	2	71.5	15	Unknown	3
1/19/02	1	Embryo	Embryo	Unknown	3
1/19/03	3	Embryo	Embryo	Unknown	3
1/20/01	2	Embryo	Embryo	Unknown	2
1/21/87	2	92.5	43.5	1M:1F	3
1/23/01	2	88.0	28.0	0M:2F	2
1/23/97	2	Embryo	Embryo	Unknown	3
1/26/92	3	29.7	1.2	Unknown	3
1/29/05	3	Embryo	Embryo	Unknown	3
2/02/97	4	80.3	19.5	1M:3F	4
2/03/96	2	37.5	3.0	Unknown	3
2/03/02	4	Embryo	Embryo	Unknown	4
2/04/96	2	26.5	1.0	Unknown	3
2/04/98	4	Embryo	Embryo	Unknown	4
2/07/04	3	40.0	2.6	Unknown	3
2/08/98	4	43.5	2.7	Unknown	4
2/12/93	4	130.2	100.0	2M:2F	4
2/12/04	4	101.5	45.2	3M:1F	4
2/14/03	3	74.0	18.2	0M:3F	4

ratio of 7 males:12 females. Mean crown-rump lengths, mean weights, litter size, date the pregnant female was trapped and the number of CL detected in ovaries for individual females appear in Table 3.

CL counts were consistent with the number of fetuses present in 66.7 percent (14/21) of pregnant females. In six cases, number of CL exceeded number of fetuses by one, and in one case number of CL exceeded number of fetuses observed by two. Median number of CL and fetuses observed was 3.0, however, the number of CL and fetuses above and below 3.0 differed ($P = 0.0133$, test statistic = 2.4749). Although not evaluated statistically, mean litter size based on the number of fetuses was 2.8 whereas mean number of CL present was 3.2. A direct linear relationship suggested that potential litter size was 87.5 percent of the CL counted/individual.

Estimating mean number of fetuses based on linear interpolation of a relationship between number of CL and fetuses yielded an average litter size in the NW eco-region of 2.6 ($n = 9$) for pregnant subadults and 2.8 ($n = 11$) for pregnant adults. Estimated litter size for the SW eco-region was 2.6 ($n = 2$) for pregnant subadults and 2.7 ($n = 19$) for pregnant adults. At the subpopulation level, estimated mean litter size for pregnant subadults was 2.6 and 2.8 for the GAL ($n = 2$) and the RMF ($n = 9$), respectively. We observed no pregnant subadults in the CB subpopulation. Estimated litter sizes for pregnant adult females were 2.6 ($n = 9$) for the RMF, 2.7 ($n = 10$) for the GAL and 2.5 ($n = 4$) for the CB.

DISCUSSION

We did not detect evidence of breeding activity based on presence of CL among juvenile wolverine during our study, which was consistent with observations in previous studies of North American wolverine (Rausch and Pearson 1972, Liskop et al. 1981, and Banci and Harestad 1988). Fecundity differed significantly between the eco-regions of western Montana with higher pregnancy rates and CL production in the NW eco-region.

Nutritional status is often theorized as the main reason for differences in age of first reproduction (Mean and Wright 1983, Banci and Harestad 1988), but habitat quality and food resources may also influence overall productivity. In our study the NW eco-region generally receives more precipitation and has moderate temperature fluctuations compared the SW eco-region. Mountain goats (*Oreamnos americanus*) occupy alpine habitats and moose (*Alces alces*) are present in habitats ranging from riparian to alpine throughout much of both the NW and SW eco-regions offering scavenging opportunities for wolverines. Food sources, such as marmots (*Marmota* spp.) and other small prey, are well distributed across the study area (Foresman 2001) although densities may vary. The NW eco-region is dominated by smaller ungulates (white-tailed deer [*Odocoileus virginianus*] and mule deer [*O. hemionus*]), while elk (*Cervus elaphus*) represent less of the ungulate biomass. Conversely, elk represent a higher percentage of the ungulate biomass in the SW eco-region.

Availability of food during winter may be directly related to reproductive success. Persson (2005) observed increased birth rates and reproductive frequency in females provided with food supplement during mid-winter as compared to non-supplemented females. Ungulate carrion is believed to be an important food source for wolverines with live prey, small mammals and vegetation of less significance particularly during winter (Hornocker and Hash1981, Rausch ad Pearson 1972, Banci 1994, Copeland 1996, Packila et al. 2007). Although precipitation, temperatures, and some food resources differed between the two eco-regions, a relationship of reproduction to overall habitat quality is not well understood in our study area.

Perhaps related to habitat quality, wolverine density within a given area may also have influenced reproductive success. Although wolverines in mountainous areas of western North America typically occupy large home ranges (Hornocker and Hash 1981, Copeland 1996, Inman et al. 2007*a*) and are capable of long-range movements (Gardner et al. 1986), low densities may result in a reduced likelihood of contact between sexually mature individuals. Adequate density projections for wolverines throughout our study area were not available, but we generally assumed that densities may have been higher in the NW eco-region than the SW eco-region. If our assumption proves true, it may partially explain some differences observed in pregnancy rates between the two eco-regions.

In our study, pregnancy rates in the SW eco-region were lower for both subadult and adults. Assuming that age groups have similar patterns of sexual maturity within both the NW and SW eco-regions, the difference in reproduction suggests reduced access to sexually mature males during breeding season in the SW eco-region. Squires et al. (2007) suggested that harvest of reproductive-aged adults may have suppressed observed reproduction in western Montana wolverines. The number of wolverines harvested during this study was only slightly greater in the SW eco-region (n =101) than in the NW eco-region (n = 97) (Montana Fish, Wildlife and Parks, unpublished data). Adult females comprised a larger proportion of the harvest in the SW eco-region (n = 28) compared to the NW eco-region (n = 11). The higher percentage of adult females harvested in the SW eco-region suggested possible increased vulnerability and perhaps contributed to differences in reproduction.

Dispersal of wolverines from source populations may be important in maintaining populations in harvested regions (Krebs et al. 2004, Lofroth and Ott 2007). Information from recent studies indicates that populations in southwestern Montana, i.e. the SW eco-region and the CB and GAL subpopulations, demonstrated significant genetic differentiation compared to populations in northwestern Montana, i.e. the NW eco-region and the RMF subpopulation, (Cegelski et al. 2003, Cegelski et al. 2006). Increased genetic differentiation observed in subpopulations of the SW eco-region suggested limited dispersal of wolverines from source populations into southwestern Montana. The effect harvest and apparent limited dispersal into the SW eco-region had on pregnancy rates and overall reproduction is unknown. If winter harvest can reduce male/female interactions during mating season, then a reduction in pregnancy rates may be expected. However, Inman et al. (2007*a)* observed that wolverine territories quickly refilled when the occupant was removed due to death. This suggested that wolverines are able to refill suitable unoccupied habitat from adjacent areas and are present during mating season, but did not indicate that mating occurs. Other social factors may influence mating behavior.

Genetic variability in the wolverine populations of North America has been addressed in numerous papers (Wilson et al. 2000, Kyle and Strobeck 2001, Chappell et al. 2004, Tomasik and Cook 2005, Cegelski et al. 2006). Cegeski et al. (2003) described the three genetically differentiated subpopulations in Montana used in this study. Of the subpopulations, the CB was the most genetically isolated, followed by the GAL and the RMF was least (Cegelski et al. 2003). Percentage of pregnant subadults and adults and mean CL counts, although not analyzed statistically, were lowest in the CB, higher in the GAL and highest in the RMF. Differences in habitat, food availability, harvest effects, connectivity or some combination of these factors may have influenced reproduction in the GAL and CB subpopulations. Median CL production and pregnancy rates of all subadult and older wolverine within these subpopulations were evaluated and the differences observed were not considered to be significant at the $P < 0.05$ level. However, this result may be influence by small sample sizes, particularly in the CB subpopulation.

Subpopulation boundaries were based on the genetic evaluation of Montana wolverines and not a priori values. However, subpopulations were subunits of the eco-regions: the RMF was a subunit of the NW eco-region and the GAL and CB subpopulations were subunits of the SW eco-region. Reproductive differences at the subpopulation level likely influenced differences in fecundity observed at the eco-region level. Factors that influence gene flow and levels of genetic isolation observed by Cegelski et al. (2003) may also have attributed to reduced reproduction observed in the CB and GAL subpopulations and subsequently the SW eco-region. Further investigation is needed to understand variables related to the limited reproductive capabilities of the CB and GAL subpopulations and the SW eco-region and the role of reduced genetic variability.

Differences in wolverine reproduction may occur at different spatial scales. Information presented in studies on wolverine in Alaska (Rausch and Pearson 1972), British Columbia (Liskop et al. 1981) and the Yukon (Banci and Harestad 1988) suggest that difference in reproduction occurs between widely separated wolverine populations at northern latitudes. Our data suggested such differences may also occur on a finer scale as observed between eco-regions and possibly subpopulations of western Montana. Our study also showed that adult pregnancy rate and mean litter size observed in the SW eco-region, the periphery of this species' range, is the lowest reported for North American wolverine. Our findings were consistent with the relatively low reproductive rates observed in a study of wolverines in the greater Yellowstone area although sample size for that study was small (Inman et al. 2007*c*).

Age composition of a population may contribute to reproductive differences. Banci and Harestad (1988) found the percentage of pregnant or post partum females was highest for 3- and 4-year-old females, and mean number of CL increased with age, which was highest (4.4 ± 1.1) in females ≥ 6 years old. Pregnancy rates and CL production in the subadult age class were critical factors in reproductive differences observed between eco-regions in this study. Nearly 58 percent of the wolverines from the NW eco-region were subadults, whereas only 36 percent were subadults in the SW eco-region. Despite a predominance of subadults in the NW eco-region, pregnancy rates for wolverines ≥ 1 year old were much higher (76.9 %) than those observed in the SW (47.7 %). We also observed higher median CL counts for the NW eco-region despite the disparity in subadults. We included both pregnant and non-pregnant females in our analysis of median CL counts and pregnancy rates between the two eco-regions. If age was a primary factor influencing reproduction, the predominance of subadults in the NW eco-region should produce lower CL production than observed in the SW eco-region, provided that harvested wolverines and cementum age analysis are unbiased estimators of fecundity and age structure for each eco-region. Our data did not support the conclusion that age structure of the wolverines used in this study was a primary factor influencing difference in reproductive performance between eco-regions in Montana.

Comparisons among our study and other studies of North American wolverines may provide insight into the fecundity of wolverine populations in the southern reaches of their distribution. However, differences in aging techniques and age classification methodologies made direct comparison of age-related reproductive parameters difficult. A completely accurate aging technique has not been developed for wolverine to date. Although cementum analysis has been used in prior studies (Rausch and Pearson 1972, Liskop et al. 1981 and Banci and Harestad 1988), some error using this method may occur (Banci 1982). Rausch and Pearson (1972) and Liskop et al. (1981) used cementum analysis as an age estimate, whereas Banci and Harestad (1988) used cementum annuli to determine ages but established age class based on skull characteristics. Rausch and Pearson (1972) defined subadults as individuals of 16-28 months, and we defined a subadult as a yearling ~ 1.5 years old. Despite the assumption that some misclassification of age class likely occurred in prior studies as well as ours, and differences in methodology used for determining age classes, comparison of our data to similar studies still yields insight into potential differences in reproductive parameters. We observed CL in 60 percent of the subadults in the NW eco-region and only 12.5 percent in the SW eco-region, whereas all ovaries from adult females in the NW contained CL as opposed to 67.8 percent in the SW. Results similar to our NW eco-region were found in Alaska with 50 percent ($n = 40$) of female wolverines 16-28 months of age being pregnant and ~ 91.8 percent ($n = 98$) of adults ≥ 29 months being pregnant (Rausch and Pearson 1972). Liskop et al. (1981) observed that 84.6 percent ($n = 13$) of subadults and 88.5 percent ($n = 26$) of adults ≥ 2 years old were pregnant in British Columbia. However, Banci and Harestad (1988) observed that only 7.4 percent ($n = 27$) of subadult and ~ 73.4 percent ($n = 79$) of adults classified as ≥ 2 years old were pregnant in the Canadian Yukon. Although all the wolverines examined for our study from the NW eco-region were considered pregnant based on CL presence in ovaries, this was likely an overestimation of true pregnancy rates. Hornocker and Hash (1981) found that only two of eight wolverines they tested within our NW eco-region boundaries appeared pregnant when first captured. However, the method of determining pregnancy and age of the wolverines examined in their study was not discussed and may have underestimated true pregnancy rates. They also reported a mean CL production of 2.93 in 15 reproductive tracts in pre-implantation condition from northwestern Montana, whereas the mean number of fetuses from six visibly pregnant females was 2.17. These findings are similar to those observed in our study, but Hornocker and Hash (1981) did not provide the age composition of their sample.

Litter sizes based on CL counts from this study were only compared to results presented by Banci and Harestad (1988) for Canadian Yukon wolverine because researchers conducting similar studies did not state whether they included ovaries containing no CL in calculations. Mean CL counts for pregnant adult wolverine in our study were consistent between eco-regions (3.2 ± 0.6 for the NW, 3.1 ± 0.8 for the SW) and varied slightly by subpopulation. The lowest adult mean CL counts were in the CB subpopulation (2.8 ± 1.2) and the greatest were in the RMF subpopulation (3.1 ± 0.4), but both were less than those observed in the Yukon for similar ages. Banci and Harestad (1988) reported mean CL counts for pregnant females of ages ≥ 2 years that ranged from 3.1 to 4.4. However, both Banci and Harestad (1988) and our data demonstrated that CL counts tend to overestimate the number of in-utero fetuses in paired studies. Our findings indicated that in paired samples, the number of fetuses present were 87.5 percent of the total number CL counted, a higher percentage than observed in the Yukon (82.1%, Banci and Harestad 1988). Estimating the potential number of fetuses using linear interpolation produced an average potential litter size of 2.7 for adult wolverine in the SW eco-region, which was lower than true litter sizes observed in Alaska (3.5, Rausch and Pearson 1972) and the Canadian Yukon (3.2, Banci and Harestad 1988), but greater than the average reported in British Columbia (2.6) by Liskop et al. (1981). However, only five pregnant adult females were examined from British Columbia and the reported average may not be representative of the population due to small sample size. Comparisons of wolverine reproductive data from various studies across the North American are challenged by small sample size and some of the observed variation may be artifacts of such limitations.

Corpora lutea counts and other variables including placental scars, the presence of blastocysts, and presence of fetuses have been used to determine potential reproductive activity for wolverine (Rausch and Pearson 1972, Liskop et al. 1981, Banci and Harestad 1988). We detected CL in ovaries from all of the females harvested from 5 January-14 February in which in-utero fetuses were present. We found no evidence of macroscopically apparent fetuses prior to 5 January, although variation may exist that was not detected in our sample. Rausch and Pearson (1972) observed nidation in two of 41 females killed in November and December. Macroscopically visible fetuses were present as early as 5 January with no evidence of parturition occurring prior to 14 February. Variation in fetal crown rump measurements and fetal weights occurred temporally suggesting variation in implantation and parturition as observed by Rausch and Pearson (1972) and Banci and Harestad (1988) although fetuses were recovered as early as November in Yukon wolverine (Banci and Harestad 1988). Embryonic fetuses were observed in reproductive tracts from 5 January to 4 February in our study that further demonstrated variation in the timing of implantation. Rapid regression of CL postpartum (Wright and Rausch 1955, Rauch and Pearson 1972), presence of CL in ovaries of females harvested in December, and presence of CL in ovaries from all females with macroscopically visible fetuses in our study suggested that CL serve as an adequate indicator of pregnancy from December through mid-February in Montana wolverine. Based on data from this study, parturition dates of Montana wolverine generally occur after mid-February and are temporally variable, but less so than observed in wolverine occupying northern latitudes of their range in North America.

Prior studies of wolverine reproduction in Alaska, the Canadian Yukon, and British Columbia have demonstrated differences in pregnancy rates in subadults and adults and variation in litter size (Rausch and Pearson 1972, Liskop et al. 1981, Banci and Harestad 1988). Although CL and in-utero fetal counts tend to overestimate actual reproduction, these data can provide managers with baseline information for wolverine reproduction. Our results indicated that significant differences in pregnancy rates and CL counts existed between wolverines in northwestern and southwestern Montana; differences in reproduction parameters can occur at fairly small geographical scales. Habitat quality, food availability, population density, harvest, and population genetics have all been suggested as possible influences on reproductive parameters evaluated in this and other studies. A combination of these elements likely contributed to differences in fecundity observed in this study. More information is needed to understand the relationships and effects these parameters have on wolverine populations.

Reproductive capability should be a primary consideration when modeling wolverine populations or making decisions affecting management. Within the southern portion of the wolverines range, basic information on reproduction is lacking, yet critical for management decisions. This study provided insight into basic fecundity parameters of wolverines in Montana and the southern extension of their range.

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