

**MONITORING PROGRAM
AND ASSESSMENT OF COYOTE PREDATION
FOR OLYMPIC MARMOTS**

By

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The Olympic marmot (*Marmota olympus*) is an endemic species to the Olympic Peninsula, Washington State. Although nearly all of its range is enclosed within Olympic National Park, declines and local extirpations of the species have been documented. The most plausible driver of the decline appears to be an increase in predator pressure. My thesis had two main objectives. First, I investigated the role of non-native coyotes (*Canis latrans*) in causing marmot mortality. Through park-wide carnivore scat analysis I determined the spatial extent of coyote predation on Olympic marmots and the magnitude of coyote predation relative to other carnivore species. I used mtDNA analysis of scats to determine carnivore species and microsatellite markers for individual coyote identification. Out of 958 carnivore scats collected, 84% came from coyotes and 10.3% contained marmots. The proportion of scats containing marmots was highly variable across studied regions, ranging from 3% to 34%. Among 79 scats with marmot remains for which predator species identification with mtDNA was successful, 85% arose from coyote, 10% from bobcat (*Lynx rufus*) and 5% from cougar (*Puma concolor*). Twelve out of 13 coyote individuals identified with genetic markers included marmots in their diet. Overall, occurrence of marmot remains in coyote scats observed could be considered high, especially if relatively low marmot densities are taken into account, supporting the potential for coyote predation to be the main driving factor of the observed marmot declines and extinctions. For my second objective, I designed a large scale, long-term monitoring program for marmot populations in Olympic National Park accounting for financial constraints. The monitoring program is designed to reflect extinction-recolonisation dynamics via park-wide occupancy sampling. The sampling design is based on annual surveys of a set of at least 25 randomly selected clusters (closely located groups of polygons with record of current or historical occupancy by marmots), and 15 additional polygons to test for colonisations.

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CHAPTER 1

INTRODUCTION

The Olympic marmot (*Marmota olympus*), endemic to the Olympic Peninsula, Washington State, is charismatic, easily seen by visitors, and a species of conservation concern. The species is found exclusively in high-elevation alpine meadows, with nearly all of its range enclosed within Olympic National Park. Its habitat is characterized by a short growing season and high year-to-year variability in temperature, winter length and snowpack. Abundance per site is 2-30 animals, with many colonies containing only 1 or 2 family groups and few colonies with over 20 animals; these small colonies occur on scattered habitat patches of grass-forb meadows within a matrix of unsuitable habitat (deep forested valleys, rocks and snow fields).

In 1998 Olympic National Park listed the marmot as a high priority in their Natural Resource Management Plan, based on anecdotal reports of historical colony disappearances. A study begun in 2002 by Griffin et al. has documented declines and local extirpations throughout the park, without colonization of new areas.

There are several possible hypotheses explaining the current decline of the Olympic marmot. Among these are climate change, increase in predation pressure, disease and inbreeding (Griffin et al. unpubl. data). There are already existing reports of climate change suspected to negatively influence alpine species (Beever et al. 2003, Floyd 2004, Krajick 2004). Reduced snowpack could affect hibernation conditions, availability of water resources and forage quality. Inouye et al. (2000) and Blumstein et al. (2004) report changes in yellow bellied marmot (*M. flaviventris*) activity patterns

(earlier timing of emergence from hibernation) possibly caused by increase of spring temperatures. Marmots could also be influenced by tree encroachment in the alpine zone (Schreiner and Burger 1994, Woodward et al. 1995). Although it is difficult to entirely rule out indirect and subtle influences of climate change, there is no evidence of deleterious climate-based effects on hibernation condition, and demographic vital rates (body condition, weaning success, litter size, juvenile survival) are comparable to historic levels, providing no evidence for depreciated forage quality, disease or inbreeding (Griffin et al. 2007).

Among the hypotheses for Olympic marmot decline, an increase in predator pressure on non-juvenile marmots seems most strongly supported by field data (Griffin et al. unpubl. data), with evidence of spatial and temporal correlation of high mortality events. Survival rate of adult Olympic marmots is considerably lower than that reported by Barash (1973) from the 1960s. It is generally recognized that for long-lived, slow-reproducing animals, such as marmots, population growth is highly sensitive to changes in adult survival (Fujiwara and Caswell 2001).

My thesis has two parts. First, I investigated the role of coyotes (*Canis latrans*) – a recent invader into the Olympic high country – in causing marmot mortality. Through park-wide carnivore scat analysis, I determined the distribution of coyotes and their marmot prey, addressing both the spatial extent of coyote predation on Olympic marmots throughout their range, as well as the magnitude of coyote predation relative to other carnivore species.

The second objective for my thesis was to design a large scale, long-term monitoring of marmot populations in Olympic National Park. The monitoring plan

provides a framework that park managers can use for assessing changes over time in occupancy, or distribution, thus providing a method to track the response of Olympic marmots to management actions.

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CHAPTER 2

COYOTE PREDATION ON THE OLYMPIC MARMOTS

2.1 Abstract

I investigated predation by non-native coyotes (*Canis latrans*) on the Olympic marmot (*Marmota olympus*), endemic to the Olympic Peninsula, Washington State. Although nearly all of the Olympic marmot habitat is protected within Olympic National Park, declines and local extirpations of the species have been documented. Through park-wide carnivore scat analysis I determined the distribution and relative density of coyotes associated with trail networks in Olympic highlands, the spatial extent of coyote predation on Olympic marmots, and the magnitude of coyote predation relative to other carnivore species. I used mtDNA analysis of scats to determine carnivore species, and microsatellite markers for individual coyote identification. Out of 958 carnivore scats collected, 84% came from coyotes and 10.3% contained marmots. The proportion of scats containing marmots was highly variable across studied regions, ranging from 3% to 34%. Among 79 scats with marmot remains for which predator species identification with mtDNA was successful, 85% arose from coyote, 10% from bobcat (*Lynx rufus*) and 5% from cougar (*Puma concolor*). Twelve out of 13 coyote individuals identified with genetic markers included marmots in their diet. Scats containing marmots arose predominantly from coyotes in all months and in all except one studied region. Overall, occurrence of marmot remains in coyote scats observed could be considered high, especially if relatively low marmot densities are taken into account, supporting the potential for coyote predation to be the main driving factor of the observed marmot declines and extinctions.

2.2 Introduction

The Olympic marmot (*Marmota olympus*), endemic to the Olympic Peninsula, Washington State, has the most restricted range and limited numbers among all U.S. marmots, with nearly all (~90%) of the habitat of the species protected within Olympic National Park. However it appears that the Olympic marmot has suffered severe declines and local extirpations in recent years, with over half of the 30 colonies periodically documented since the 1950s now extinct, no known colonizations of new areas, and total numbers reduced by perhaps half from the estimates in the late 1960s (Barash 1989, Griffin et al. unpubl. data).

Several possible hypotheses explaining the current decline – including climate change, disease and inbreeding – are being considered for the Olympic marmot based on historical data coupled with an ongoing 5-year field study in Olympic National Park (Griffin et al. unpubl. data). It appears likely that an increase in predator pressure is driving the decline. Non-juvenile survival is considerably lower than that reported by Barash (1973) for Olympic marmots in the 1960s, with little evidence of marmots dying from causes other than predation (Griffin et al. unpubl. data). Predation has also apparently been a crucial factor in the decline of the critically endangered Vancouver Island marmot (*M. vancouverensis*), a geographically neighboring species closely related to the Olympic marmot also endemic to a relatively small area (Bryant and Page 2005).

Several lines of evidence suggest that coyote (*Canis latrans*) predation might be the main cause of Olympic marmot mortality. First, Griffin et al. (unpubl. data) documented high mortality of non-juvenile marmots, with coyotes the dominant cause of deaths. Second, several (>10) killings, attempted killings or stalking of marmots by

coyotes have been observed (Griffin et al. unpubl. data). These observed coyote predations on Olympic marmot are unusual, given the extreme rarity with which these events are typically witnessed. During an intensive 20-year behavioral study of yellow-bellied marmots (*M. flaviventris*) in Colorado, only two instances of predation on marmots were witnessed (Armitage 1982). Similarly, a large telemetry study in the same area (Van Vuren 2001) indicated predation as the primary source of active season mortality (98%), and 10-38% coyote scats found contained marmot remains, but none of these predation events were observed despite frequent observations over 12 years of study (Van Vuren 1991, 2001).

A third reason supporting coyotes as the primary marmot predators in the Olympics is that there is relatively little evidence suggesting that other predators are killing many marmots. Other than coyote, predators directly observed foraging on Olympic marmots include cougar (*Felis concolor*) and golden eagle (*Aquila chrysaetos*) (Barash 1973, Janis Burger, pers. comm.). These species have been recognized as frequent predators on other North American marmots (Barash 1989, Bryant and Page 2005, Marr and Knight 1983). Bobcat (*Lynx rufus*) often preys on woodchuck (*M. monax*, Kwiecinski 1998) and Barash (1973) observed bobcat stalking Olympic marmots and eliciting alarm calls. Black bears (*Ursus americanus*) occasionally predate on yellow-bellied marmots (Van Vuren 2001) and are present on the Olympic Peninsula, although predation on Olympic marmots has not been observed and marmots and bears appear indifferent to each others' presence (personal observation). Mustelids and raptors smaller than golden eagles could possibly kill marmot infants (Barash 1989). Red-tailed hawks (*Buteo jamaicensis*) are common in the Olympics, often elicit alarm calls and were

sometimes chased by female marmot with a recently weaned litter (personal observation). Such behavior could be explained as maternal hypersensitivity due to offspring loss to particular predator (Berger et al. 2001).

Despite the fact that eagles are highly visible diurnal animals seen regularly in the study area and known to prey on marmots elsewhere (Marr and Knight 1983), few (<5) cases of eagle predation on marmots have been confirmed using telemetry (Griffin et al. unpubl. data), Cougar and bobcat are nocturnal, and so less likely to forage frequently on marmots, which are exclusively diurnal; furthermore cougar and bobcat are typically secretive and avoid people, whereas marmots live in open habitat with an activity season coinciding with extensive visitor use, and many of the colonies with documented declines and extinctions are located in the vicinity of heavily used trails. By contrast, coyotes are often diurnal where diurnal prey is abundant, even in areas with high human activity (List and Macdonald 2001). It is probable that eagles and other raptors do kill a number of marmots and cats take an occasional marmot, but Olympic marmots evolved with these predators and there is no reason to think that pressure from either is higher than it has been historically.

The possibility that coyotes are the primary predators on marmots is complicated by the fact that the coyote is almost certainly not native to Olympic National Park. Although historical absence is difficult to prove, available data (Scheffer 1995) suggest the coyote first appeared on the peninsula early in the 20th century, initially at low-elevation, logged areas. Its subsequent rapid increase in numbers closely paralleled a dramatic decrease and eventual extinction of the wolf population. However, as wolves usually forage on large ungulates below the subalpine zone (Arjo et al. 2002, Mech

1970), it is likely that before extirpation wolves only occasionally fed on Olympic marmots. The coyote is an opportunistic predator that often relies on small mammals, lagomorphs and rodents, including ground squirrel species (Arjo et al. 2002, Bowyer et al. 1983, Gese et al. 1996, Wells and Bekoff 1982). Coyote predation is the most important mortality factor for yellow-bellied marmot in Colorado (at least 47% of mortality; Van Vuren 2001) and are known to predate on *M. caligata* and *M. monax* (Barash 1989). Increased coyote numbers or range expansion has decreased the effectiveness of several endangered species recovery efforts including black-footed ferrets (*Mustela nigripes*), San Joaquin kit foxes (*Vulpes macrotis mutica*) and whooping cranes (*Grus americana*) (Goodrich and Buskirk 1995, Soulé et al. 2005).

Thus, coyotes are known to be able to reduce prey populations, especially naïve ones, and have been documented killing marmots on intensively studied sites in Olympic National Park, where marmots are undergoing a severe decline. My objective in this study was to assess coyote predation on marmots across the Olympic alpine. I did so by collecting scats, determining species and identity of coyotes using DNA analysis, and quantifying the presence of marmots in the scats.

2.3 Study Area

The study was conducted in Washington State, within Olympic National Park (Fig. 1). Terrain of the Olympic Mountains is rugged with the highest peak reaching an elevation of 2427 m. The maritime climate of the peninsula is characterized by wet winters and dry summers. The western side of the peninsula is one of the wettest places in the U.S. south of Alaska, with an average of about 360 cm of rainfall per year, while

the eastern part is relatively dry as it lies in a rain shadow (Houston and Schreiner 1994). Low-elevation areas are predominated by lush coniferous forest with Sitka spruce (*Picea sitchensis*), western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Douglas-fir (*Pseudotsuga menziesii*). Patches of forests at higher elevation are composed of subalpine fir (*Abies lasiocarpa*) and mountain hemlock (*Tsuga mertensiana*). Alpine meadows occur above 1500 m and are dominated by showy sedge (*Carex spectabilis*), pink mountain heather (*Phyllodoce empetriformis*), and blueberry (*Vaccinium deliciosum*) on wet sites and spreading phlox (*Phlox diffusa*) on dry sites (Houston and Schreiner 1994).

2.4 Methods

2.4.1 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.). I 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 (Fig. 1). 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. (unpubl. data); 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 to predate on Olympic marmot) were collected from sample transects monthly from May to September, the period when marmots are not hibernating. When snow conditions prevented access to some transects in the spring, the first sampling period was postponed until June. 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. The rest of the scat to be used for diet analysis was placed into a plastic zip-lock bag labeled with location and date as well as estimated species identification and approximate age of the scat (fresh, medium and old).

2.4.2. Genetic analysis of carnivore species and coyote individuals

Coyote scats can be confused with those of bobcat, cougar, juvenile bear, fox and domestic dog, all possibly present in the Olympic alpine (although foxes are not native and unlikely to be in the Park alpine; pers. comm., Patti Happe, ONP Wildlife Branch Chief). Thus species identification by physical characteristics of scats (Danner and Dodd 1982, Murie 1954, Weaver and Fritts 1979) was verified by genetic analysis using mtDNA for all scats containing marmot remains, and for an additional sample of carnivore scats without marmots.

Individual coyote identification by microsatellite markers was conducted on all coyote scats with marmot remains, as well as a sample of scats which did not contain

marmots. Scats without marmot remains were randomly selected from two separated focal areas highly differing in human use level: the Gray Wolf focal region is in the remote backcountry, with very low visitor use, while the Obstruction, Badger and Grand focal region is near the road and experiences high visitor use. To avoid confounding factors associated with population turnover, I chose scats collected in both focal areas during one year of the study (2005, the year with the highest number of scats with marmots collected). I sampled approximately 50% of all samples not containing marmots from each of the focal areas (18 and 45 samples selected from the low and high use areas respectively). Microsatellite analysis for individual identification was used to index the total number of coyote individuals responsible for detected marmot deaths across all studied regions and to obtain minimum population size of coyotes using trails in the two selected focal areas.

Scat samples were stored with silica gel at room temperature prior to DNA extraction. Extractions and amplifications of DNA were processed in separate buildings to reduce the risk of contaminations of low quantity/low quality fecal DNA with DNA from PCR products. Approximately 0.20 g of material scraped from the scat surface with a scalpel was used for extraction. We used the QIAampTM DNA Stool Mini Kits (QIAGEN) to extract DNA from the samples. One negative control in each batch of extractions was used to test for contamination.

We amplified using polymerase chain reaction (PCR) a fragment of the cytochrome-*b* region of mtDNA for species identification, extending the approach of Bidlack et al. (2007) in 20 μ L reactions (4 μ L of DNA extract, 0.5x reaction buffer, 8 mM dNTPs, 10 mM primers: CanidL1 and HCarn200 [IDT], 15 mM MgCl₂, 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 in the Park 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. To visualize the digested products we used electrophoresis through 2% agarose gels post-stained with ethidium bromide.

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 10 µL 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 (Appendix A, Frantz et al. 2003).

2.4.3 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. Marmot remains were distinguished from other prey species using comparison with specimens housed at the University of Montana zoological museum and a hair identification key (Moore et al. 1974). All scat samples were first searched for marmot teeth and bone fragments. Second, the hair mass constituting each sample was examined macroscopically for the presence of potential marmot hairs (using such characteristics as length, thickness and color). On samples potentially containing marmot hair I conducted microscopic examination to determine if it was marmot.

To assess the proportion of carnivore feces with marmot I calculated frequency of occurrence ($\text{number of fecal samples with marmot} \times 100 / \text{number of fecal samples}$; Corbett 1989, Hidalgo-Mihart et al. 2001) separately for each month and sample transect. For the monthly analysis, I 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. I did not quantify the presence of other prey in the scats.

2.5 RESULTS

2.5.1 Carnivore scat densities and distribution

Of the total of 958 scats, 89% (857 scats) were collected on the sample transects. The average rate of encounter per year was 3.4 scats per km of transect. Of the 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.

I observed considerable differences in density of scats among different transects, ranging from 2.4 to 13.2 scats/km (Fig. 2). Although four transects (Hurricane, Steeple, Obstruction and Royal) were traveled more frequently than once a month (usually twice a month) in the course of other marmot project activities, differences in sampling effort are not likely to have substantially influenced numbers of scats collected (Appendix B). Among the transects surveyed once a month, high to moderate scat densities were observed on Klahhane, in Badger and Grand Valleys and along the transects constituting Gray Wolf Loop. The lowest scat densities were observed on transects Lena and Seven Lakes.

In addition to sampling effort, the number of scats per kilometer of transect could be affected by number of tourists, if foot or vehicle traffic destroys the scats. However I did not observe an inverse relationship between tourist use intensity and scat number. For example, the Klahhane transect had a high rate of scats discovered and also is one of the

most popular trails in the park. Similarly three transects with approximately equally heavy tourist traffic had vastly different scat densities (Seven Lakes and Lena had the lowest number of scats and Grand much higher).

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 scat identifications made by collectors 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.

2.5.2 Predation on marmots

Marmot remains were found in 10.3% of all 958 scats collected, with the proportion similar for both years (12.1% in 2005 vs. 8.9% in 2006). Frequency of marmot occurrence varied across the season (Fig. 3), with a minimum in May (1.1%) and maximums in June (14.9%) and September (14.2%).

Marmot remains were found in scats from all regions studied except Steeple and Klahhane, an expected result as there are no remaining colonies along or near the Steeple transect and the three remnant marmot colonies at Klahhane are extremely small (~2-3 marmots; personal observation). The proportion of scats containing marmot remains in the other 10 regions across the park ranged from 3% to 34% (Fig. 4).

2.5.3 Genetic species identification

Species identification with mtDNA was conducted on 82 of the 99 scats determined to contain marmot remains (no genetic sample was collected for the other 17 scats). Amplification success for species identification was 96% (79/82). For 85% (67/79) of the samples with marmots, genetic analysis confirmed coyote as the predator. The remainder arose from bobcat (10%; 8 scats) and cougar (5%, 4 scats). Scats with marmots arising from coyotes predominate across all months (Fig. 5) and in all regions, with the only exception being Dosewallips, where all 3 scats with marmots were bobcats.

2.5.4 Individual identification of coyotes with genetic markers

Among all 130 scats selected for individual coyote identification by microsatellite marker analysis 61% of the samples were successfully genotyped. The first stage of the individual identification targeted the 67 confirmed coyote scat samples containing marmot remains and revealed 12 unique genotypes originated from different coyote individuals. In the main complex of adjacent regions with marmot colonies (Obstruction through Gray Wolf; Fig. 6) scats of the same coyote individual were sometimes found in 2 or 3 neighboring regions (the longest straight line distance observed between two scats

of the same coyote was 9.6 km). In general, scats from 1 to 3 coyote individuals were detected at each of the 8 regions where coyote/marmot samples were available for analysis.

The second stage of individual identification using microsatellite analysis was conducted on scats from 2005 collected in two focal areas with high or low human visitation levels. In addition to all scats containing marmots collected at a given year from each area, a random sample (approximately 50%) of the remaining scats (without marmot) was included. In total I analyzed 74 samples from the high use area (29 scats with and 45 scats without marmot) and 22 samples from low use area (4 scats with and 18 scats without marmot). Four coyote individuals were detected in the high use area (1.5 coyote individuals per 10 km of transect), while in the low use area three coyotes were found (2.5 coyote individuals per 10 km). I observed high predominance of scats from a single coyote individual in the high use area, where 32 scats from one coyote constituted 76% of all successfully genotyped scats, while for the remaining three individuals only 7, 2 and 1 scats each were assigned. In the low use area, the three coyote individuals were represented by 5, 3 and 1 successfully genotyped scat samples, respectively.

Marmot remains were found in scats of all 7 coyote individuals identified within the focal areas, except for one individual in the high use area represented by one scat. Except for this individual, all coyotes identified within the focal areas were detected in both years of study.

2.6 Discussion

The coyote, an exotic predator to the Olympic Mountains, is a major predator on the Olympic marmot. Coyote scats were the most frequent carnivore scats found on transects, and 85% of the carnivore scats containing marmot remains were from coyotes. By contrast, native carnivores (bobcat and cougar) kill marmots much less frequently (10% and 5% of scats with marmots, respectively).

I also found that coyotes are widespread throughout the Olympic highlands. Scats of coyotes were collected in all parts of the park studied, with the only discontinuity in coyote scats along two deep forested valleys where bobcat and cougar scats predominated. The considerable differences in scat densities among different regions of the park (Fig. 2) represent either differences in relative abundance of coyotes or differential use of trails. The highest number of coyote scats was observed on the Obstruction transect, where almost all of the intensively studied marmot colonies have been experiencing very low survival rates and declines (Griffin et al. unpubl. data). Similarly the high number of scats along the Klahhane transect may well be linked to the high marmot colony extinctions (at least 5 colonies) that occurred 2-15 years before this study. The third region with a high number of coyote scats observed was Steeple, where the marmot colony disappeared in 2001. On the other hand the lowest density of scats was observed at Seven Lakes region where marmot colonies are widespread and few abandoned sites have been detected (Griffin et al. unpubl. data).

Overall, my collection and analysis of carnivore scats across Olympic National Park complements the demographic and telemetry data collected since 2002 at intensively studied Olympic marmot colonies (Griffin et al. unpubl. data). The Royal

region had a low number of scats observed and just one scat containing marmot; marmots in this region have high estimated annual survival. The Obstruction region, where numerous scats were collected and much a higher proportion of scats contained marmot, have much lower estimated survival. The third intensively monitored marmot population, Hurricane, also had low estimates of survival and is a special case where complete isolation and very low current abundance of the remaining marmot colony (~20 individuals), likely led to the low number of scats containing marmot. There are however indications of high coyote impact in this region based on telemetry data (Griffin et al. unpubl. data).

In addition to the correspondence between results of this study and those from the intensively studied demography sites (Griffin et al. unpubl. data), scat study also extends the inference about coyote effects beyond findings at those locations. Before this study, instances of coyote predation on the Olympic marmot were recorded mainly at the trapping sites in the Hurricane and Obstruction regions. These sites are clustered in one corner of the park and differ from the other park areas because of the road access to the alpine zone and much higher, year-round visitor use. Because coyotes are known to be opportunistic generalists that thrive at the human interface (Arjo and Pletscher 2004; Gompper 2002), it might be expected that coyote predation on marmots would be limited to that area. However, my park-wide scat analysis shows that levels of coyote predation (proportion of scats with marmot) observed at intensively studied sites is similar to the levels detected in several remote regions throughout the park, often deep into backcountry wilderness (Fig. 4). Thus, coyote predation is widespread across the range of Olympic marmots. Individual identification of coyotes by genetic markers reveal similar

coyote use patterns (~2 coyotes per 10 km) for two focal areas that are highly different in location, relative access and visitor use.

Different proportions of marmots in the diet of coyotes inhabiting different regions can be caused by differences in prey density (more marmots in the area would contribute to more scats with marmots likely to be encountered) or behavioral differences between individual coyotes. Several authors reported coyote individuals differing in their diet preferences (Bekoff and Wells 1980, Fedriani and Kohn 2001). I found that, although certain coyotes are more likely to eat marmots than others, most individuals included marmots in their diet.

My scat studies indicate that coyote predation on marmots is present through the whole period of marmot emergence above ground, with two peaks in June and September (Fig. 3). The seasonal changes were consistent with patterns of carnivore predation on radio-telemetred Olympic marmots (Griffin et al. unpubl. data). Low predation is expected in May, due to a low availability of marmots, as most animals are active above ground no earlier than the second half of May, and some postpone emergence till June (see also Van Vuren 1991). June is the month of the highest number of confirmations of coyote predation from telemetry, and similarly high proportion of scats with marmot remains was observed in this study. In August and September scats still show high predation levels by coyotes, indicating that the relatively high unknown carnivore or unknown predation mortality from radio-telemetry (Griffin et al. unpubl. data) may likely be attributed to coyotes.

Overall, occurrence of marmot remains in carnivore (mainly coyote) scats observed in Olympic National Park (over 10% in many of the studied regions, up to 34%

in Badger region where all identified scats were assigned to coyote) could be considered high, especially if relatively low marmot densities are taken into account. In Colorado (Van Vuren 2001) at least 47% of 97 radio-telemetered marmots confirmed dead were killed by coyotes with a similar proportion of coyote scats with marmots as I observed (from 10 to 38% in different years of study; Van Vuren 1991). Being relatively rare and difficult to catch, marmots are not the primary prey of Olympic coyotes, although their fixed location and high energy value make them a valuable prey. Van Vuren (1991) reports intensity of coyote predation on yellow-bellied marmot to be independent of marmot densities. In Olympic Mountains where coyotes are subsidized by abundant multiple prey, we can expect no numerical response of coyotes to a decrease in marmot numbers. The coyote also is not limited by seasonal unavailability of marmots due to hibernation. Fixed predator density independent of prey densities can create a situation where predation rate increases with declining prey density, leading to the Allee effect (destabilizing positive density dependence, Mills 2007). Consequently we can expect that predation by a subsidized predator can reduce marmot population growth, driving whole colonies to extinction, with no consequence for the coyote population. This system is similar to those observed in many other areas where invasive predators supported by an abundant prey species drive alternate native prey to extinction (Mack et al. 2000, Prugh 2005).

In the case of highly fragmented marmot populations, local extinctions would cause a further decrease in connectivity between colonies and increase the overall risk to the species' persistence. In addition to direct effects on survival, increased predation pressure could increase the time marmots devote to vigilance and consequently reduce

time devoted to foraging. If so, reduced energy intake can cause higher mortality or decreased reproductive output (Armitage 2004, Carey and Moore 1986, Holmes 1984), although these effects have not been detected to date (Griffin et al. 2007). Furthermore, lower densities of marmots could also lead to lower frequency of warning calls and inadequate expansion of refuge burrows.

The results of the scat analysis support and extend ongoing demographic studies (Griffin et al. unpubl. data) in showing a high magnitude of predation pressure from coyotes on Olympic marmots. The fact that coyote predation is widespread underscores that reducing the problem through targeted coyote removal will not be easy. However, removal of the invasive coyote population in the Olympic Park high country may be the only solution to maintain the endemic marmot population.

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Figures

Figure 1. Sample transects for systematic scat collection: 1 – Hurricane, 2 – Klahhane, 3 – Steeple, 4 – Obstruction, 5 – Badger, 6 – Grand, 7 – Cameron, 8 – Dosewallips, 9 – Gray Wolf, 10 – Royal, 11 – Seven Lakes, 12 – Lena. Transects 7, 8 and 9 constitute “Gray Wolf Loop”; circles – locations of active marmot colonies in Olympic National Park recorded during the surveys in 2002-2006 by Griffin, Mills and Taper (unpubl. data); crosses - locations of extinct marmot colonies.

Figure 2. Number of scats (total for 2 years) with (black bars) and without marmot (gray bars) remains per kilometer of transect at each studied region.

Figure 3. Proportion of scats with marmot remains by month of scat deposit (n=913, $\chi^2=24.9$, df=4, $P<0.0001$). Numbers above the bars indicate sample size for each month.

Figure 4. Proportion of scats containing marmot remains in different regions of Olympic National Park (on and off transects). Numbers after the bar indicate sample size.

Figure 5. Number of scats with marmot remains by month of scat deposit (n=94). Different colors within the bars represent predator species identified with mtDNA: gray – coyote, black – felids (bobcat and cougar), white – predator species not identified.

Figure 6. Locations of scats from 7 coyote individuals identified using microsatellites in an area of Olympic National Park encompassing two focal areas: high visitor use (Obstruction, Badger and Grand transects) and low visitor use (Gray Wolf transect). Different symbols represent different coyote individuals detected across two years of study.

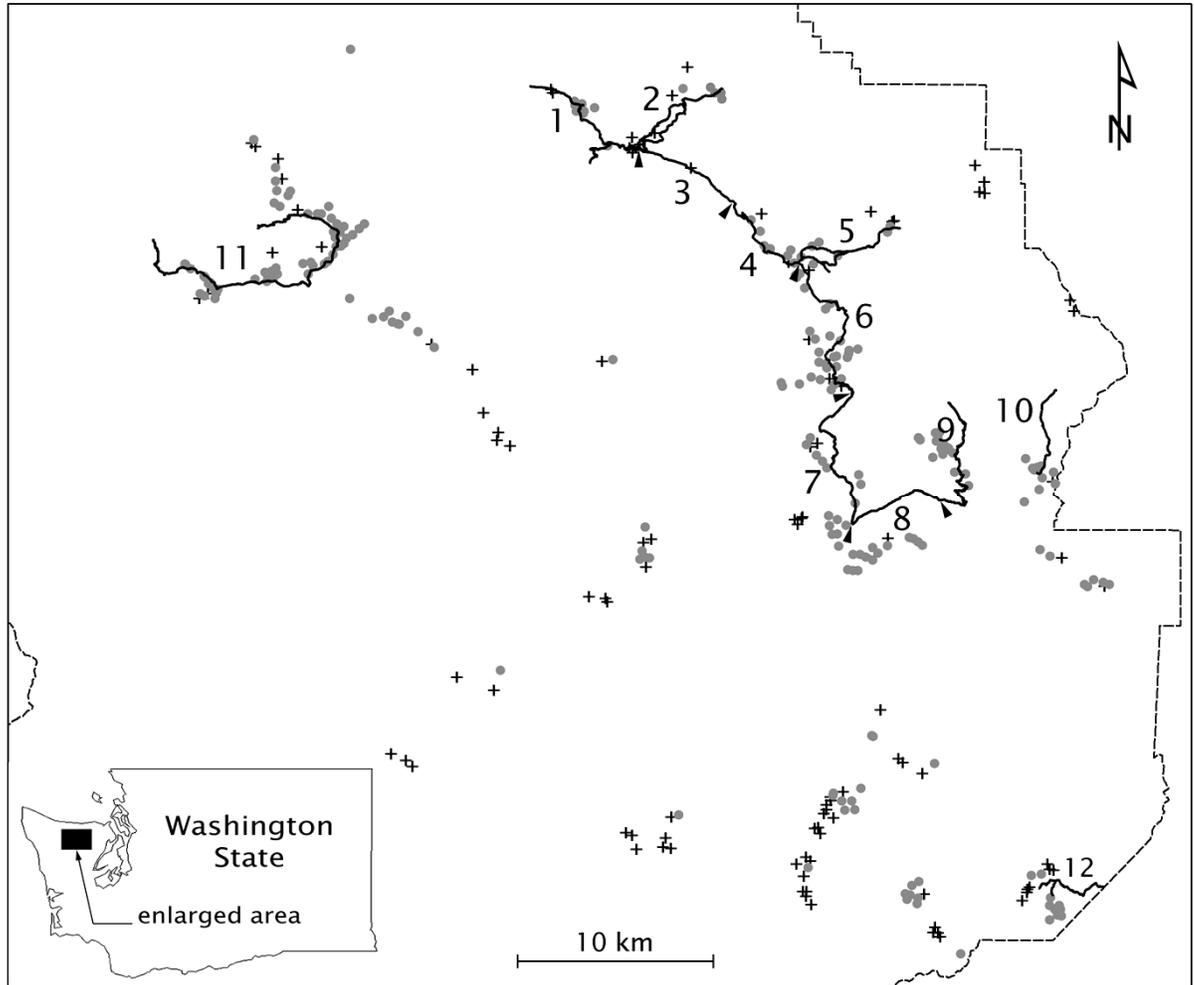


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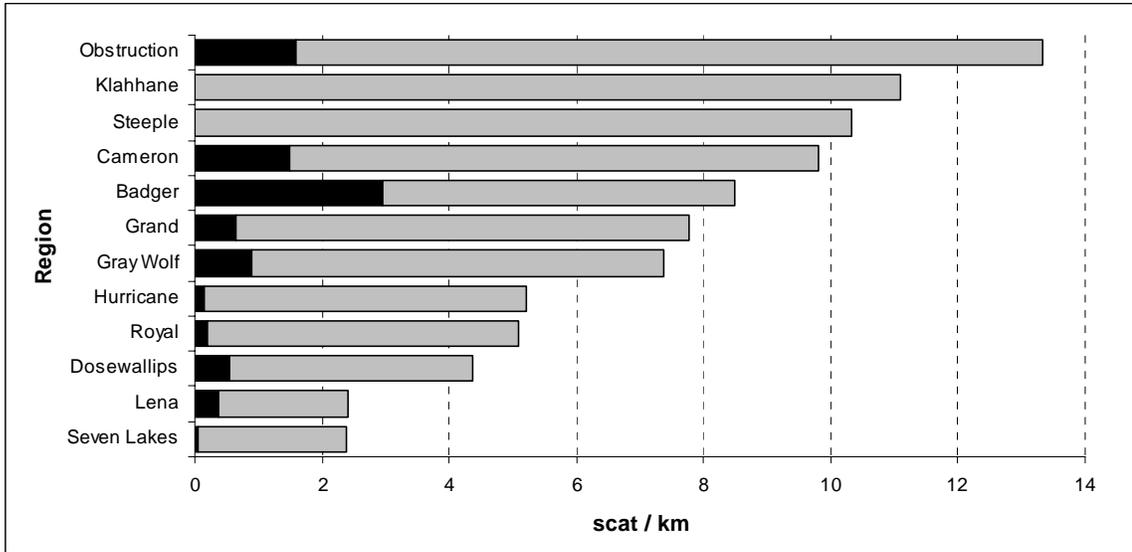


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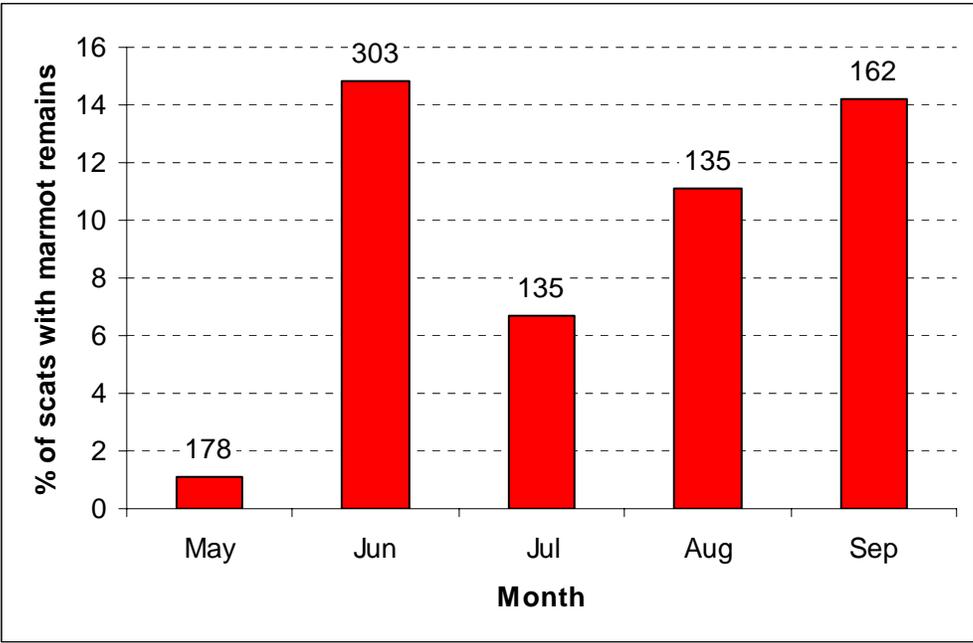


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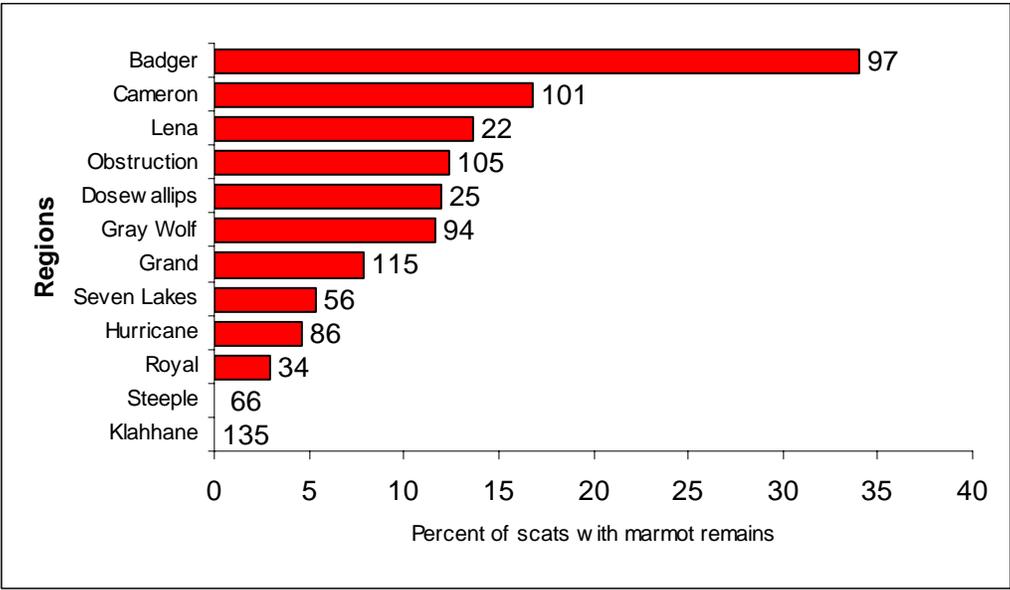


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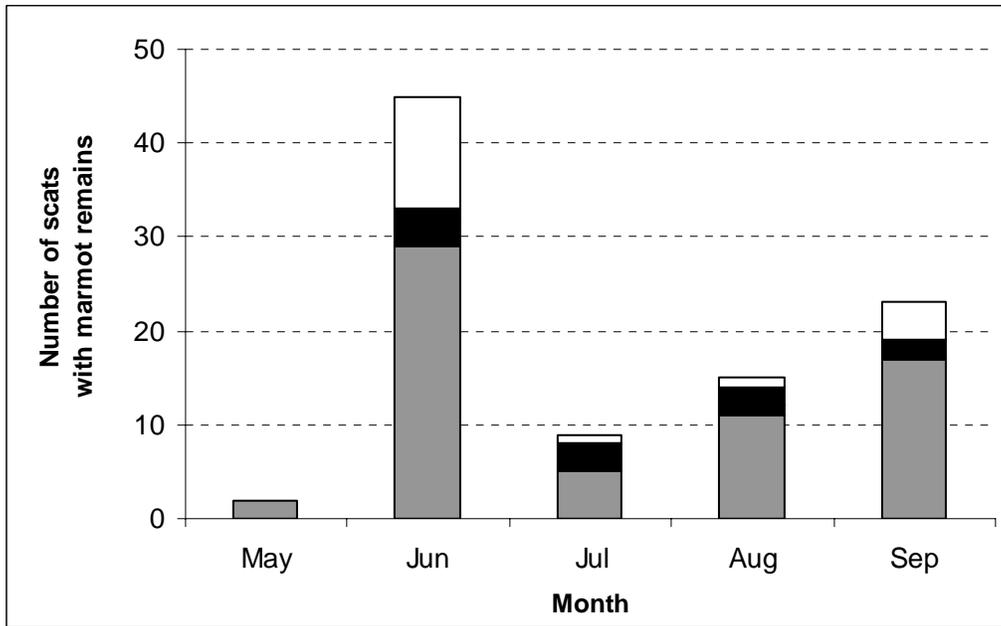


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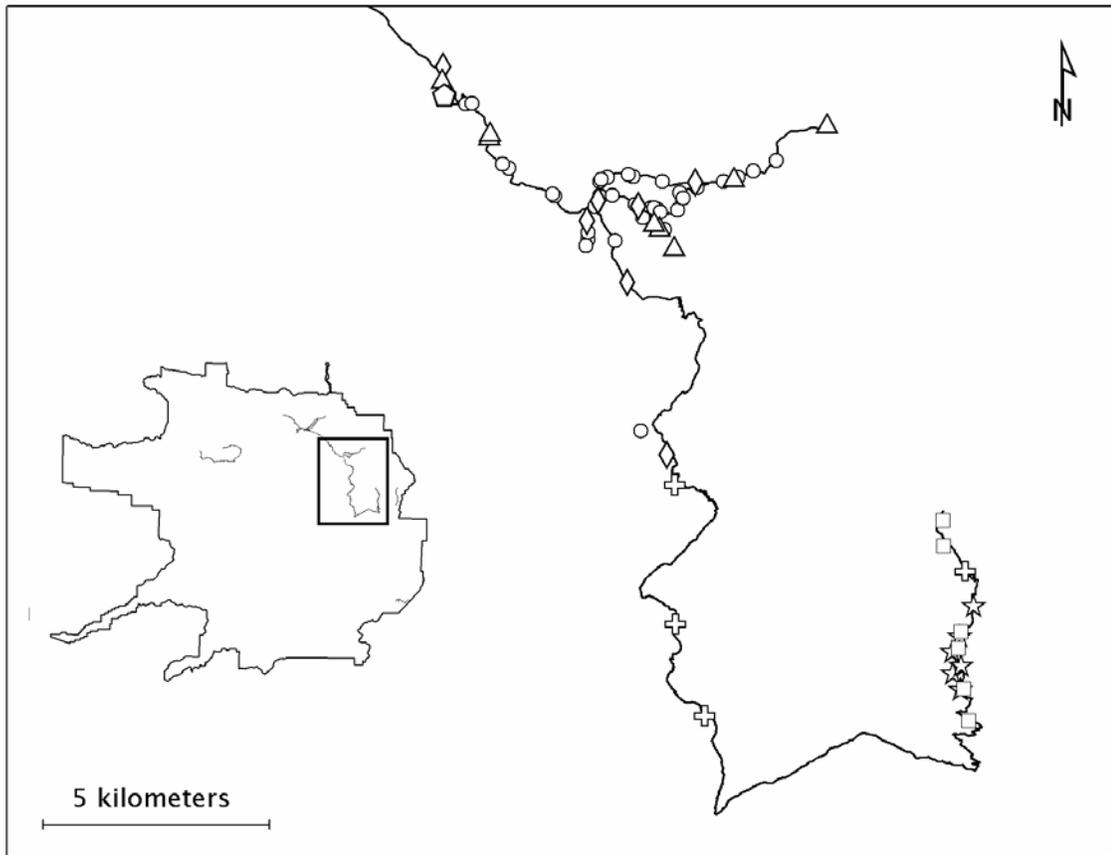


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CHAPTER 3
OPTIMIZING METHODS FOR OLYMPIC MARMOT
MONITORING PROGRAM

3.1 Introduction

Monitoring is one of the main tools of species conservation and management. Appropriate design and implementation of monitoring programs is of particular importance in the case of rare and declining species.

As an example of a large-scale, long-term monitoring program accounting for financial constraints, I present the multiple-stage process of survey design for Olympic marmots throughout its range in Olympic National Park. The Olympic marmot (*Marmota olympus*), endemic to the Olympic Peninsula, Washington State, has the most restricted range and limited numbers among all U.S. marmots. Although ~90% of its habitat is protected within Olympic National Park, it appears that the Olympic marmot has suffered severe declines and local extirpations in recent years (Griffin et al. unpubl. data). Effective management plans for this endemic species require quantitative information about population status, trends and distribution.

Although surveillance monitoring – the simple collection of measures of abundance or distribution over time to evaluate trend – has been criticized as being inefficient compared to more targeted adaptive hypothesis-testing (Nichols and Williams 2006) the monitoring program described here will provide essential baseline data across a logistically challenging National Park. Because it can easily be conducted by volunteers and park interns, surveillance monitoring of marmots would be an efficient and effective

method for confirmation of park-wide population declines in the future, thereby directing park management for the endemic marmot.

3.2. Monitoring Methods

There are several possible techniques that could be used to obtain abundance or distribution data over time for Olympic marmots: 1) Estimates of abundance using Capture-Mark-Recapture: by live trapping (Mark-Resight method) or by non-invasive hair sampling; 2) Population Indices of abundance (visual counts of marmots, burrow counts, pellet counts, hibernacula counts); 3) Estimates of distribution using presence-absence measures (occupancy estimation). In this section I will describe why abundance-based methods are less efficient for the Olympic marmot monitoring program than the third method, presence-absence occupancy estimation.

Trend detection based on formal estimates of abundance incorporating adjustments for incomplete detectability via Capture-Mark-Recapture (CMR) would likely be more precise and statistically powerful for tracking population size changes than indices or a presence-absence method (Pollock et al. 2002). However these techniques are much more expensive, labor-intensive and demanding in terms of crew experience. Use of live trapping is logistically difficult and may be inefficient, due to low trap success (Griffin et al. unpubl. data). Therefore, live trapping is feasible only on a few polygons relative to the size of the study area, which would result in low precision for park-wide estimation. Furthermore, not many areas in the park provide reasonable access for trapping; thus sites chosen for mainly logistic reasons will likely not be representative. Genetic techniques using DNA extracted from hair samples, although also costly, provide

the possibility of sampling remote populations without trapping and handling the animals (Morin and Woodruff 1996, Taberlet et al. 1999). Efficiency of the non-invasive sampling has proven to be much higher than live-trapping.

Visual counts of unmarked individuals could be used as an index of relative abundance of marmots. Unadjusted incomplete counts are almost certainly less accurate and reliable than formal estimates by CMR, but in the case of marmots likely much more useful for monitoring than sign indices, e.g: burrow counts (Severson and Plumb 1998). Marmots are excellent subjects for direct visual counts. They are sedentary, inhabit open habitats, highly visible, diurnal, and tolerant of close observation. Counting methods were developed for the Alpine marmots (*M. marmota*) in the Alps (Cortot et al. 1996, Lenti Boero 1999) and Vancouver Island marmots (*M. vancouverensis*, Bryant 1998). Repeated counts of Vancouver Island marmots initiated in 1972 were the main tool of the long-term population monitoring which eventually revealed catastrophic decline of the species. Also numerous authors have tested visual counts for indexing density of prairie dogs and ground squirrels (Fagerstone and Biggins 1986, Powell et al. 1994, Severson and Plumb 1998, Zegers 1981) usually with positive results. However, the method relies on the critical assumption that the number of animals observed during repeated visual counts constitutes a constant proportion of the true abundance (Thompson et al. 1998). As season, time of day and weather conditions substantially influence the number of animals active above ground, changes in detectability across time will substantially decrease accuracy of counts.

Likewise, sign indices such as burrow, pellet and hibernacula counts are less expensive and more time-efficient alternatives (Karels et al. 2004), but are unlikely to

accurately reflect true population size differences between habitats or over time, particularly for Olympic marmots or other alpine-dwelling marmots. These marmots are highly social and family groups usually share large, main burrows in the center of the territory (Armitage and Downhower 1974, Arnold 1990, Barash 1973, Blumstein and Arnold 1998). Thus the number of burrows is likely independent of the number of animals. Furthermore burrows are usually permanent constructions, lasting several years, and their number does not reflect year-to-year changes in density (Ramousse et al. 1997, Van Horne et al. 1997). Finally, burrow persistence and the number of burrows used by marmot colonies of similar size could vary with habitat type (Van Horne et al. 1997), e.g. a different number of shelters could be used by marmots inhabiting rocky outcrops compared to those digging on the meadows.

Similarly, the use of fecal pellet counts for monitoring abundance (Karels et al. 2004) is problematic for Olympic marmots for several reasons. First, scats randomly scattered in vegetation among the numerous burrows in the home range are rare and difficult to find; those on porches (mounds by the burrow entrance) are often destroyed by animals' movements and digging activity. In some colonies I did observe latrines but in many others scats are extremely rare. Number of scats is likely site dependent - latrines in rock crevices lasted longer than those on porches. These factors will likely cause the relationship between abundance of pellets and marmots to not be linear.

Although all count-based abundance-based methods are problematic for Olympic marmots, recent developments in presence-absence occupancy estimation provide an ideal approach for Olympic marmot monitoring. Presence-absence data could be used to monitor population size trends if there is a direct relationship between the proportion of

occupied habitat patches and abundance (MacKenzie et al. 2005). More importantly, presence-absence indicates species distribution, an important characteristic by itself that under some conditions may provide more information for conservation decisionmaking than trend in abundance (Finley et al. 2005, Joseph et al. 2006). Well-designed presence-absence monitoring should capture a general reduction in site occupancy as a result of constrictions of spatial distribution and population decline.

Marmots could be easily monitored by presence-absence techniques as they are diurnal, visible, and dig multiple burrows that are relatively easy to detect (Bryant 1998). A monitoring program should ensure constant effort of polygon searching in consecutive periods to reduce observer bias. Standardized polygon surveys require a detailed protocol (Appendix C) for searching and recording animal sightings and presence indices (calls, burrows, pellets). Detection of pellets constitutes a useful addition to the more subjective burrow categorization while determining site occupancy status (discriminating between active and recently abandoned sites). Scattered scats are unlikely to last longer than one season (Karels et al. 2004, Ramousse et al. 1997); thus presence of scats usually confirms current site occupancy.

For the purposes of a marmot distribution monitoring program, surveyed polygons are categorized as: Active, Abandoned (historical presence now extinct), or Null (no signs of marmot activity).

The presence-absence method could be compromised by false negatives (undetected presence) and its variability across time and space (Field et al. 2005, MacKenzie et al. 2005). In the case of imperfect detectability, MacKenzie et al. (2006) incorporate repeated surveys of sites within the season, allowing estimation of detection

probabilities to facilitate unbiased estimates of occupancy. However, I have found that for Olympic marmots, detectability is very high, 92% or greater, even with a naïve observer (Appendix D); Griffin et al. (unpubl. data) found a similar detectability using an independent estimate in the same system. Therefore, an efficient solution to be used here is a “removal design” (MacKenzie et al. 2006) whereby a second survey within the season is made only for the polygons where marmots were not detected. With such a high detectability, one additional survey will likely be sufficient for complete removal of the non-detection bias.

Given financial constraints while designing the monitoring program, I sought to balance the collection of precise information from intensive sampling over a small part of the entire population against less precise large-scale sampling (Bryant 1998). The monitoring program proposed relies on park-wide distribution assessment with presence-absence methodology. In effect, it targets detection of changes in occupancy measured as the proportion of the sampling units where the species is present during the sampling.

3.3 Sampling Design

3.3.1 Sampling frame

Determination of the monitoring sampling frame constitutes a crucial step of the design and highly influences the inference scope of the results. A representative sampling network of sites across the park should provide adequate coverage of marmot habitat, with focus on the areas of known (recent and historical) marmot distribution in order to detect extinction and recolonization events.

The Olympic marmot monitoring primary sampling frame is based on polygons delineated by breaks in aspect and encompassing marmot habitat determined to be occupied or abandoned during 2002-2005 field surveys (Griffin et al. unpubl. data) or for which other historical records of previous occupancy exist (Barash 1973, Wood 1973). I further excluded from the sampling frame 19 polygons inadequate for frequent monitoring activities because of inaccessibility. The resulting primary sampling frame consists of 310 polygons (Fig.1): 212 occupied (68.4%) and 98 abandoned (31.6%), thus current occupancy is 0.68. Polygons within the sampling frame represent the range of aspects, slopes, elevations and polygon sizes across the park.

Although new colonisations of habitats not previously occupied are thought to be unlikely (Suzanne Griffin, pers. comm.), the proposed occupancy monitoring program will contain an additional component, outside the primary sampling frame, to sample for possible colonisations of new habitats. The colonization sampling will be conducted based on a detailed marmot habitat model (Griffin et al. unpubl. data) to identify potentially suitable empty habitat with no record of previous occupancy. Each year, a different set of polygons (10% of the number sampled from the primary sampling frame) will be sampled.

3.3.2 Sampling plan

Although convenience sampling of sites near trails would minimize monitoring costs, it would greatly decrease the inferential scope of the study. On the other hand random selection of single polygons (simple random sampling) would be an inefficient use of the observer time in the rugged terrain of the mountainous park. Additionally,

before locating a sampled polygon, the observer often may walk through several other polygons without recording observed marmots.

Here I present a sampling design which relies on randomly chosen clusters of polygons (closely located groups of polygons). Cluster sampling represents a trade-off between randomization and cost-efficiency of sampling. Polygons to be sampled are naturally clustered on separated mountaintops. Much more time is needed to travel between clusters (from several-hour to two-day-long hikes) than to visit several nearby polygons within the cluster. Also, cluster sampling decreases the number of time-consuming ascents, increases observer familiarity with an area, and is logistically efficient because several polygons can be sampled from a single backcountry base camp. Collectively, the benefits of cluster sampling should greatly increase the number of surveyed polygons per sampling period per observer, while preserving the intent to obtain a representative sample of the marmot population in the park.

3.3.3 Sampling plan calculations

In this section I consider the necessary sample sizes and efficiencies when sampling the universe of potential polygons in the sampling frame using either simple random sampling or one of three variants of cluster sampling. To conduct cluster sampling I first divided the sampling frame into 66 clusters. Clusters were created exclusively with respect to time efficiency of the survey. To determine the most effort-efficient clusters I used my personal knowledge of the area and a GIS model of topography. The predetermined size of the cluster was 5 polygons and 86% of all clusters consist of 4 to 6 polygons, but because of logistical constraints, some smaller or larger

clusters were included (overall $\bar{x} = 4.7$; minimum = 1; maximum = 7). Five of the clusters are highly isolated and predominantly abandoned (only two polygons constituting these clusters are occupied, Fig. 1). Because of their remote location these clusters would be very costly to survey, thus I evaluated plans both with and without these five clusters. After excluding these clusters, current observed occupancy (proportion of occupied polygons) in the sampling frame changed from 0.68 to 0.72.

As the cluster size was chosen a priori, I also assessed another variant of monitoring by dividing the sampling frame into groups approximately twice as large. In effect I created 33 big clusters (containing 9 polygons on average). Finally, I also considered simple random sampling (SRS) as an alternative to cluster sampling, excluding the 5 remote clusters.

Thus I evaluated four different variants of sampling universes: a) 66 small clusters (full sampling frame of 310 polygons and approximately 5 polygons per cluster); b) 61 small clusters (reduced frame of 292 polygons); c) 33 large clusters (292 polygons and approximately 9 polygons per cluster); d) Simple Random Sampling (292 polygons). To compare all scenarios, I calculated required sample sizes to attain a prescribed level of precision and then estimated the sampling effort necessary to achieve this under each plan.

For each variant of the sampling plan, I computed the minimum sample size necessary to estimate occupancy to within 10% of the true occupancy with 95% confidence.

I determined necessary sample sizes for cluster sampling based on the standard formula (Thompson 2002: 36):

$$n = \frac{1}{\frac{1}{N} + \frac{d^2}{s^2 z^2}} \quad (1)$$

where: n = sample size, N = the total number of clusters in the sampling frame (66, 61 or 33 depending on the variant considered), d = the maximum allowable difference between the true occupancy and its estimate (0.1 in this case), s^2 = variance of the occupancy between clusters (determined from the current marmot occupancy data to be 0.13, 0.11 and 0.09 for variants a , b and c , respectively), z = standard normal quintile corresponding to the chosen alpha level ($\alpha = 0.05$).

Notice that variance in occupancy among clusters that may arise from spatial autocorrelation in occupancy or other heterogeneity is accounted for by the variance term, s^2 .

The sample size required for simple random sampling was determined based on the formula (Thompson 2002: 42):

$$n = \frac{1}{\frac{N-1}{Nn_0} + \frac{1}{N}} \quad \text{where: } n_0 = \frac{z^2 p(1-p)}{d^2}, \quad (2)$$

N = the total number of polygons in the sampling frame (292), p = the current proportion of occupied polygons in the sampling frame (0.72), z and d are defined as in equation (1).

To assess relative effort of each sampling plan I drew 15 random samples of the required size for each of the sampling variants *a* through *d*. For each of the simulated monitoring scenarios I calculated the minimum total distance needed to be traveled by foot to reach all sampled polygons as well as total driving time from the Olympic National Park headquarters (Table 1).

Based on the computations presented above, the most efficient sampling design would be variant *b* with 61 small clusters of approximately 5 polygons per cluster (Table 1). Eliminating the five remote and predominantly abandoned clusters decreased variance of occupancy between clusters, reducing the required sample size and sampling effort compared to the sampling frame that included the remote sites (Fig. 2). Although the larger 9-polygon clusters (variant *c*) required similar effort as variant *b* (Table 1, Fig. 2), the use of 33 instead of 61 clusters has the considerable disadvantage of sampling only about half as many areas in the Park. Simple random sampling allows for the smallest sample size but is very inefficient in terms of sampling effort because the selected polygons are highly scattered (Table 1, Fig. 2).

Using the current known occupancy of approximately 0.72, a small-scale simulation was performed to evaluate the chosen sampling plan from the current sampling frame in ONP. I drew 1000 simulated samples of 25 clusters (the sample size necessary for the preferred sampling design with a sample universe of 61 clusters; Table 1) and for each simulation computed the proportion of occupied polygons in the sample, constituting an estimate of population occupancy. The histogram of sampling distributions of occupancy estimates (Fig. 3) shows that cluster sampling gives accurate

estimates, centered around the true proportion of occupied polygons ($\bar{x} = 0.71$, $sd = 0.05$).

3.3.4 Sampling plan implementation and timing of surveys

The proposed monitoring program will be based on annual repeated sequences of surveys, following the preferred sampling scheme of variant *b*. I will randomly select 25 clusters (containing approximately 120 polygons) from the list of 61 clusters of polygons in the sampling frame (both the full list and the randomly selected polygons grouped into clusters will be provided to the Park). Every next year, the same, initially selected set of clusters will be surveyed (Mackenzie et al. 2006). Collected information about status of surveyed polygons over multiple years will be used for estimation of the trend in occupancy (one of the possible methods is use of regression of the logarithm of occupancy estimates versus time, Thompson et al. 1998). To specifically monitor for new colonizations, each year a new set of 15 polygons will be randomly selected from the list of remaining (not included in the primary sampling frame) polygons constituting suitable marmot habitat (from the model developed by Griffin et al.); up to 5 of these could be deleted each year due to inaccessibility.

Each polygon will be visited at approximately the same time (season, time of day) across years to control for factors such as phenology of vegetation and seasonal changes in activity patterns which could affect observability. All monitoring activities should target the activity peak period, when the probability of observing marmots on the colony area is the highest (Bryant 1998, Cortot et al. 1996, Lenti Boero 1999, Leontieva et al.

1997). Presence should be estimated based on direct sightings, pellets, calls, and active burrows (Appendix C).

Marmot daily activity patterns depend on the season and weather (Barash 1973, personal observation). In May, when marmots emerge from hibernation, activity is unimodal with the peak around noon. In the summer it becomes bimodal with the mid-day being a siesta time when marmots are entirely absent from the surface. During September above ground activity shifts to later hours in the morning and earlier in the evening. Before hibernation it becomes unimodal again. Therefore optimal times for summer surveys are morning and late afternoon hours (before 11:00 and after 16:00; mid-June to mid-September).

Accessibility of polygons (snow conditions on trails, road openings) will be an additional factor influencing seasonal timing of monitoring activities (some remote sites could often be inaccessible in June). Accessibility of polygons will dictate the logical order of monitoring surveys.

3.3.5 Personnel

Critical pieces of information for the monitoring design are the type and number of personnel that will be available (seasonal rangers, biologists, volunteers, park interns). This will require consultations with park managers. For monitoring purposes there are advantages to using both inexperienced amateur observers, as well as park personnel to conduct repeated surveys across years. A presence-absence survey of backcountry alpine meadows throughout the park is feasible for inexperienced observers and constitutes an attractive project for recruiting volunteers. Amateurs do not know previous marmot

distributions, thereby eliminating a potential source of bias. By contrast, observers experienced in sampling marmots in the park can unconsciously put less effort into areas where marmots were absent in previous periods, thereby failing to record colonisations of new sites or identification of previously undetected colonies. On the other hand, inexperienced observers may have higher error rates in determining polygon occupancy status (difficulties in distinguishing between marmot and mountain beaver (*Aplodontia rufa*) burrows, inadequate searching behavior, etc.). Therefore, adequate training is necessary (Appendix C).

3.4 Conclusions

The example of the highly endangered Vancouver Island marmot, where dramatic decline was recognized much too late (Bryant 1998), underscores the importance of long-term studies. A monitoring design for Olympic marmots could also contribute to the knowledge needed for recovery programs of other threatened marmot species, including not only the Vancouver Island marmot but also Asian species: *M. camtchatica*, and *sibirica* (Bibikov 1999, Janz et al. 2000, Karels et al. 2004). Annual sampling of presence-absence of marmots in ONP, based on random sampling of 25 clusters, each containing approximately 5 polygons with current or previous occupancy (and supplemented by sampling 10-15 never-occupied polygons), provides an efficient method for tracking extinction and recolonization dynamics of Olympic marmots.

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Table 1. Results of the sample size computation and effort evaluation based on 15 simulated samples from each of 4 variants of sampling plan for Olympic marmot.

Sampling design	Number of polygons	Mean number of polygons per cluster	Required sample		Minimum Effort Required			
			Number of clusters	Number of polygons	Driving (hours)	SD	Hiking (km)	SD
a) 66 clusters	310	4.7	29	136 ¹	31	4.8	430	49.5
b) 61 clusters	292	4.8	25	120 ¹	26	3.1	322	33.0
c) 33 clusters	292	8.8	17	150 ¹	24	3.7	315	24.2
d) SRS	292	-	-	62	30	2.1	399	20.5

¹calculated as a product of the mean number of polygons per cluster and number of clusters

Figures

Figure 1. Distribution of occupied and abandoned colonies of the Olympic marmot within Olympic National Park recorded during the surveys in 2002-2005; white triangles – occupied colonies, black triangles – abandoned colonies, grey area – potential marmot habitat, solid lines – roads, thin dashed lines – trails, thick dashed line – park boundary. *NA* - clusters of polygons removed (19 polygons total) from the sampling frame because of inaccessibility, *R* - remote and isolated clusters of polygons removed for variants *b*, *c* and *d* (18 polygons total).

Figure 2. Boxplots of the total minimum hiking distances computed for four monitoring scenarios ($n=15$, $F=42.8$, $P < 0.0001$).

Figure 3. Histogram presents the results of occupancy estimation for 1000 simulated samples of the required size (25 clusters) for the monitoring variant with 61 clusters. Histogram shows that cluster sampling gives accurate estimates, centered around the true proportion of occupied polygons (0.7).

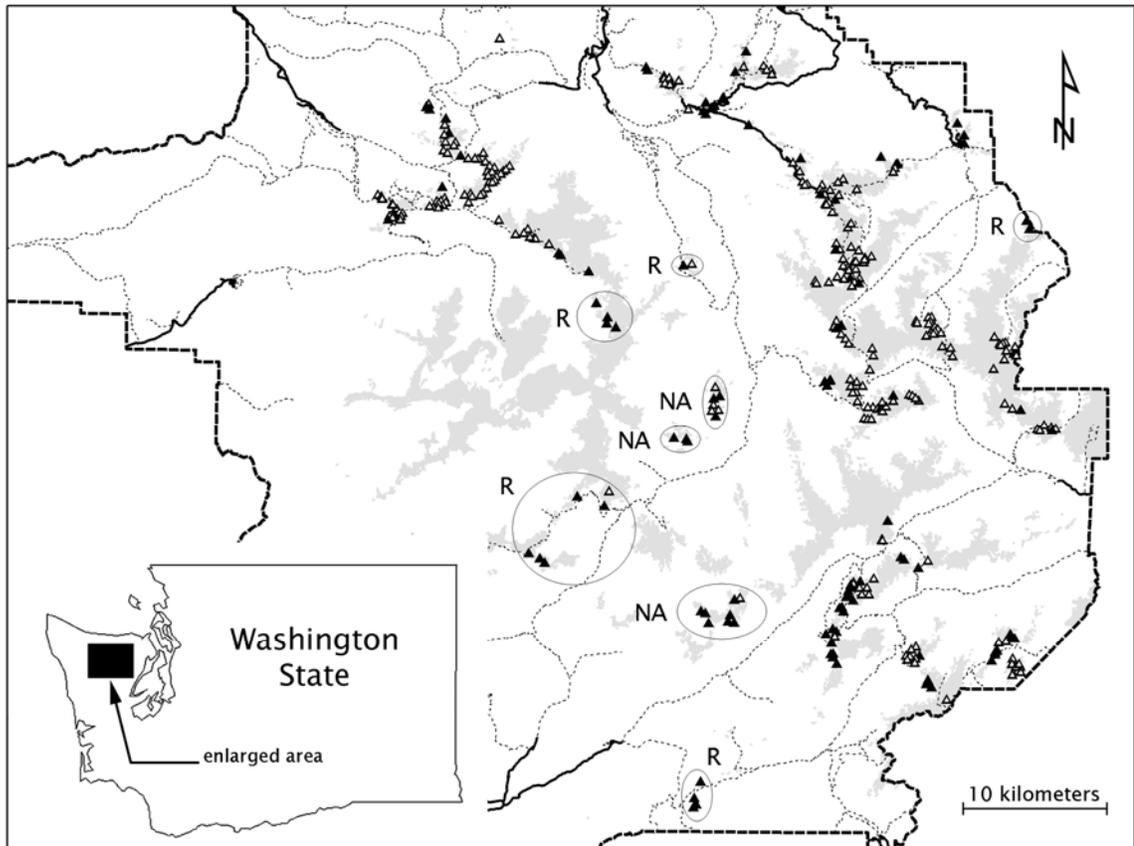


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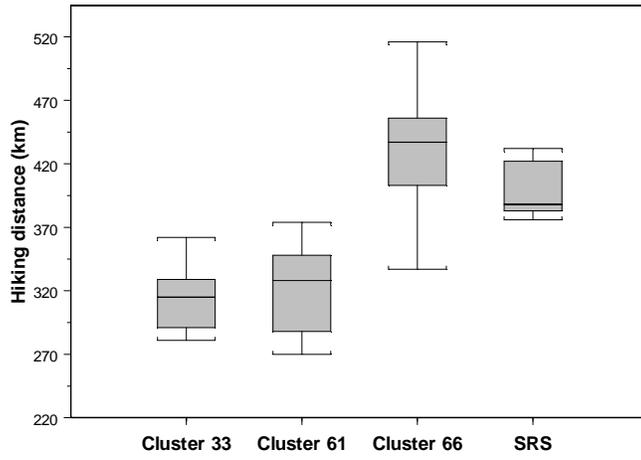


Figure 2. Boxplots of the total minimum hiking distances computed for four monitoring scenarios ($n=15$, $F=42.8$, $P < 0.0001$).

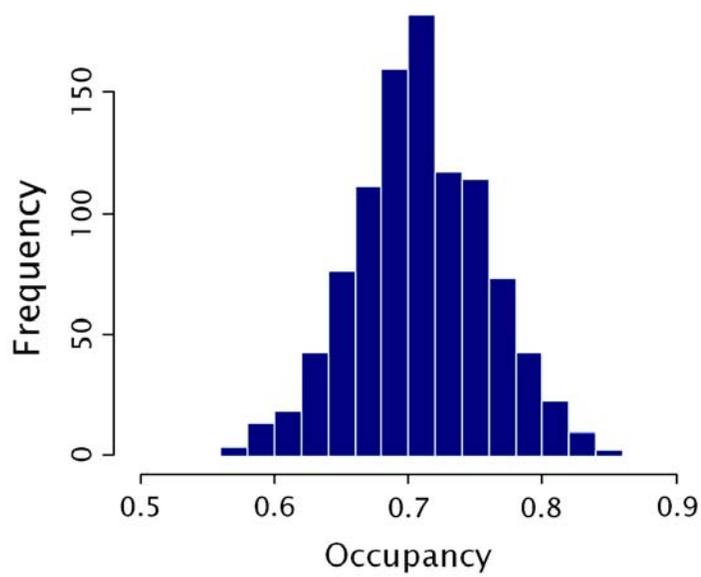


Figure 3. Histogram presents the results of occupancy estimation for 1000 simulated samples of the required size (25 clusters) for the monitoring variant with 61 clusters. Histogram shows that cluster sampling gives accurate estimates, centered around the true proportion of occupied polygons (0.72).

APPENDIX A

Non-invasive fecal genotyping protocol (adapted from Frantz et al. 2003)

- 1) Run all samples twice
- 2) Loci that give rise to the same heterozygote twice are accepted
- 3) Step-wise amplification until each allele is observed twice
 - a. Rerun all samples with homozygote locus, samples where heterozygote was observed only once, or which did not amplify for all loci
 - i. For samples which did not amplify at one or more loci, sample must be rerun at least twice for that marker
 - ii. For samples where a homozygote was observed once and the marker did not amplify, sample must be rerun twice
 - b. Go to step 4 before deciding which samples need additional reruns
 - c. Max of 7 positive PCRs per locus
 - d. If ambiguous, (1 heterozygote, 6 homozygote runs) follow Miller et al.(2002) and count it as a half-locus using the homozygote allele
- 4) Homozygotes are *provisionally* accepted if 3 positive PCRs give rise to the same allele
- 5) Identical provisional profiles are matched and classified as originating from the same individual
- 6) Individuals are accepted if:
 - a. a single sample was heterozygote at all loci and 100% match for two runs

- b. a group of 2 or more samples are 100% match where some markers are homozygote where all homozygotes were confirmed 3 times
- 7) After grouping genetic profiles (regionally or by genetic similarity), pair-wise comparisons are performed between 'individuals' with the fewest mismatches
- a. If 3 different alleles are observed at a specific locus the groups are declared different
 - b. If only two different alleles are present, ADO cannot be ruled out and they could originate from the same individual
 - i. How many loci (and samples) are different from most similar group?
 - 1. 1-2 loci (1 sample):
 - a. Is the locus the one(s) showing the most ADO in the multiplex(es)?
 - i. If so, likely ADO
 - ii. If not, maybe a rare sampling event for a sib/offspring - amplify an additional 4 times
 - 2. 3 or more loci (2 or more samples):
 - a. Is the sample degraded (poor repeatability, weak amplification)?
 - i. Yes, re-extract or drop
 - ii. If not, maybe a rare sampling event for a sib/offspring - amplify an additional 4 times

- 8) Incomplete profiles are grouped with provisional profiles as long as a consensus genotype was obtained at the most informative locus (P_{ID-Sib})
- a. Gives a conservative estimate of pop size
 - b. The M_h -Jackknife estimator is robust when dealing with ‘shadow effect’ error (Frantz et al. 2003, Mills et al. 2000)

Literature cited:

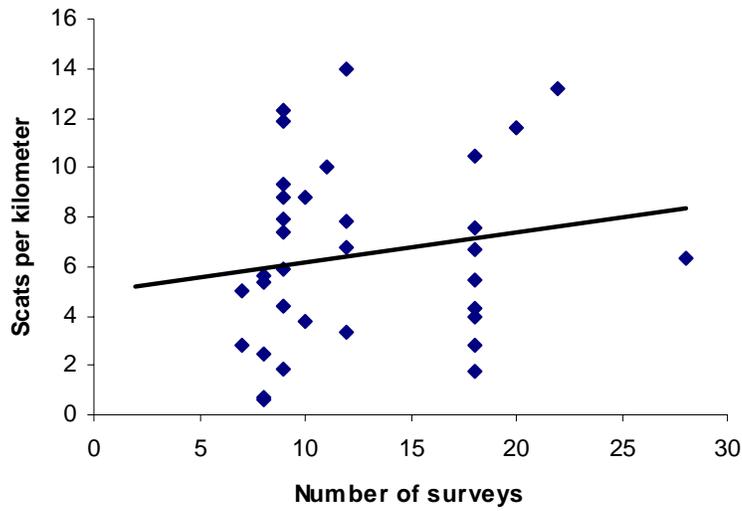
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APPENDIX B

Evidence that the number of scats collected on the subtransects is not affected by differences in sampling effort

Appendix B, Table 1. The total number of surveys and the number of scats per kilometer for all subtransects in the study area. The correlation coefficient between the number of scats per kilometer and the number of surveys is low: $r = 0.18$.

Transect	Subtransect	Number of surveys across two years	Number of scats per km
Badger	Badger	9	8.8
	Elk Mountain	10	8.8
	Maiden	9	5.9
	Shortcut	9	11.9
Cameron	Cameron	9	7.9
	Lost	9	12.3
Dosewallips	Dosewallips	9	4.4
Grand	Grand	12	7.8
	Grand switchback	9	9.3
	Lilian	12	6.8
Gray Wolf	Gray Wolf	9	7.4
Hurricane	Elwha	10	3.8
	Picnic I	18	4.3
	Picnic II	18	4.0
	Picnic trail	18	7.6
	Road after VC	18	5.4
	Trail	28	6.3
	VC loops	18	2.9
Klahhane	Klahhane	11	10.0
	Road before VC	20	11.6
	Sunrise	12	14.0
	Switchback	12	3.3
Lena	Lena trail	9	1.8
	Milk Lake	7	5.0
	Mt Lena	7	2.9
Obstruction	Obstruction	22	13.2
Royal	Above lake	18	1.8
	Below lake	18	6.7
Seven Lakes	Appleton	8	2.5
	Deer Lake	8	0.6
	High Divide	8	0.7
	Sol Duc	8	5.4
	Traverse	8	5.7
Steeple	Steeple	18	10.5



Appendix B, Figure 1. The number of scat per kilometer of a given subtransect versus number of surveys conducted on subtransect. The regression line shows only a weak relationship between the number of discovered scats and the number of surveys ($R^2=0.031$, $P=0.32$).

APPENDIX C

THE OLYMPIC MARMOT SURVEY PROTOCOL

Survey dates (preferable survey dates):	Jun 1 – Sep 15 (Jul 1 – Aug 31)
Time of the day to conduct surveys:	From 1 hour after dawn to 1 hour prior to dark, except 11:00-16:00
Survey duration at each polygon:	Until marmots presence is confirmed or entire polygon is checked (~0.5–2 hours depending on a polygon size)
Number of visit per polygon:	1 if presence of marmots was confirmed during first visit, 2 otherwise
Weather conditions:	All except precipitation and heavy fog

C.1 Training workshop for observers

All observers are required to attend a training workshop. The workshop should consist of two parts: indoor, and outdoor. During the first part volunteers should be briefly familiarized with marmot ecology, monitoring purposes, survey data sheets, park backcountry rules and safety issues. During outdoor workshop observers will be familiarized with marmots, their burrows (occupied and abandoned), pellets and calls. Observers should see a variety of burrows e.g. under rocks, in sedge (*Carex* spp.) clumps etc. It is also important to show mountain beaver burrows as they can be confused with marmot burrows. Observers should also have an opportunity to learn how to use GPS units and locate their position on the map. The best places for an outdoor workshop

would be Hurricane Hill or the marmot colonies along the Obstruction road. Abandoned marmot burrows can be found at Sunrise site near Hurricane Hill VC.

C.2 Surveys

Preferably, surveys should be conducted by two-person teams. Before beginning the survey at each polygon, the observer should first find his position on the map with GPS to confirm his location within the polygon to be surveyed. The observer should traverse the polygon looking for marmots, signs of marmot presence and listening for marmot calls. Clues for likely marmot burrows include distinctive clumps of tall sedge and spots with bare soil (possibly an effect of marmot digging activity). It is also useful to check for burrows under big boulders especially if surrounded by sedge. If burrows are located, observer should next carefully look for marmot pellets around the entrances. The survey should be continued until marmot presence is confirmed or entire polygon is checked (but not longer than 2 hours). If survey is conducted by a two-person team, observers should work independently (surveys on separate routes). During rain, snow and heavy fog (visibility less than 100 meters) surveys should be postponed until weather conditions will improve. On polygons where marmot presence was not detected, survey should be repeated during the same or on the other trip. It is recommended to repeat the survey in different time of the day than the first survey. The second survey should be conducted following the same protocol.

During each polygon survey the observer should complete the data sheet with all required information: polygon number, date, start and end time, weather category, polygon status, number of marmots seen, marmot presence signs found (burrows, pellets,

calls). If marmot or burrows are found, the observer should record UTM coordinates for each marmot or burrow up to a total of 7 (if more than 1 marmot or burrow found, UTM coordinates should be spread around the polygon). Also it is recommended to record all predator sightings.

C.2.1 Polygon status categories:

- **Occupied:** Preferably confirmed by marmot sightings and detection of fresh marmot pellets. A polygon may also be designated occupied if marmot calls are heard within the polygon or active burrows are detected with numerous confirmations of use (see below for active burrows description).
- **Abandoned:** Abandoned burrows detected (see below).
- **Null:** No signs of marmot activity detected.

C.2.2 Burrow status categories:

- **Occupied burrow:** one or more of the following conditions: fresh digging, marmot pellets, trampled vegetation by the entrance, paths in vegetation between burrows, marmot smell, flies.
- **Abandoned burrow:** none of the above, could be collapsed and with vegetation in the entrance tunnel.

C.2.3. Differentiation between marmot and mountain beaver burrows

- **Marmot burrows** usually have entrances over 20 cm wide. Main burrows have flat porches made of dirt and located on the downhill side, used by marmots for resting and watching.
- **Mountain beaver burrow** systems are often located in wet places, near running water. They have many closely located entrances, usually 10-20 cm in diameter. Cone-shaped piles of loose dirt are sometimes present by entrances, and especially in late summer, bundles of clipped vegetation.

C.2.4 Sky conditions categories:

- Clear or a few clouds
- Partly cloudy or variable sky
- Cloudy or overcast
- Slight fog
- Drizzle

C.2.5 Wind conditions categories:

- No wind
- Slight wind
- Moderate wind
- Strong wind

C.3 Camping

When choosing between designated campsites observer should camp on the closest available spot to the polygons to be surveyed. In cross-country it is recommended to camp in close vicinity or within sampled polygons. Observers should always respect the park rules while camping and report their presence to rangers.

C.4 Equipment

Each observer should have the following equipment:

1. binoculars with power range from 7 to 10 and light from 40 to 50;
2. handheld GPS units;
3. survey sheets and 3 pencils;
4. GIS maps with polygons delineated;
5. hiking map;
6. walkie-talkie radio;
7. first aid kit;
8. park radio (optional).

C.5 Proposed survey data sheet

THE OLYMPIC MARMOT SURVEY DATA SHEET

Cluster name:	Polygon #:	Date:	Polygon status: <input type="checkbox"/> Occupied <input type="checkbox"/> Abandoned <input type="checkbox"/> Null		
Observer's name:		Start time:		End time:	
Sky condition: <input type="checkbox"/> Clear <input type="checkbox"/> Partly cloudy <input type="checkbox"/> Cloudy <input type="checkbox"/> Slight fog <input type="checkbox"/> Drizzle					
Wind conditions: <input type="checkbox"/> No wind <input type="checkbox"/> Slight <input type="checkbox"/> Moderate <input type="checkbox"/> Strong					
MARMOTS SEEN: <input type="checkbox"/> Yes <input type="checkbox"/> No			Total # of marmots seen:		
Marmot calls: <input type="checkbox"/> Yes <input type="checkbox"/> No			Marmot and burrow locations		
Burrows status: <input type="checkbox"/> Active <input type="checkbox"/> Abandoned			Marmot / Burrow	UTM E	UTM N
Active burrows confirmation: <input type="checkbox"/> pellets <input type="checkbox"/> fresh digging <input type="checkbox"/> trampled vegetation by the entrance <input type="checkbox"/> paths between burrows <input type="checkbox"/> marmot smell					
Predator sightings:					
Comments:					

C.6 Checklist

a) technical equipment:

1. binocular;
2. GPS unit;
3. survey sheets, 3 pencils;
4. GIS maps;
5. hiking map;
6. walkie-talkie radio;

7. first aid kit;
8. park radio (optional).

b) Camping equipment:

1. tent;
2. sleeping bag;
3. sleeping pad;
4. camping stove, fuel, lighter;
5. pot;
6. water filter;
7. rain coat;
8. warm clothes.

APPENDIX D

Pilot data for detectability of marmots during presence-absence surveys

Surveys were conducted in June, July and August 2006, by a naïve observer with no previous experience with any species of marmots or in the Park; the observer was accompanied by myself (JW) to provide guidance of where to sample, although I was careful to give no clues as to occupancy status. To keep the naïve observer from having an expectation of finding marmots, we sampled both the 94 polygons determined to have been occupied by marmots in at least one of the previous seasons 2002-2005, as well as an additional 30 polygons known (by JW) to be unoccupied.

The detectability of the naïve observer was very high. On 92% (87 of 94) of polygons previously determined as occupied, presence of marmots or signs of current marmot occupancy were found (Table 1). Importantly, this raw detectability rate may have been biased low for two reasons. First, previously occupied polygons may have been abandoned since the last survey. Second, all 7 of the previously occupied sites where the naïve observer did not find marmots were atypical in that they were not on meadows but rather on rocky sites that may have been peripheral habitats that were inconsistently occupied or perhaps infrequently visited without permanently used burrows. Thus, the true detectability for a naïve observer can be considered to range between 92% and 100%. Figure 1 shows the proportion of different cues used by the naïve observer for occupancy determination. In the majority (85%) of polygons the preferred clues were found - marmot sightings or scats found on the burrow porches.

Appendix D, Table 1. Results of the pilot polygon surveys for the detectability assessment.

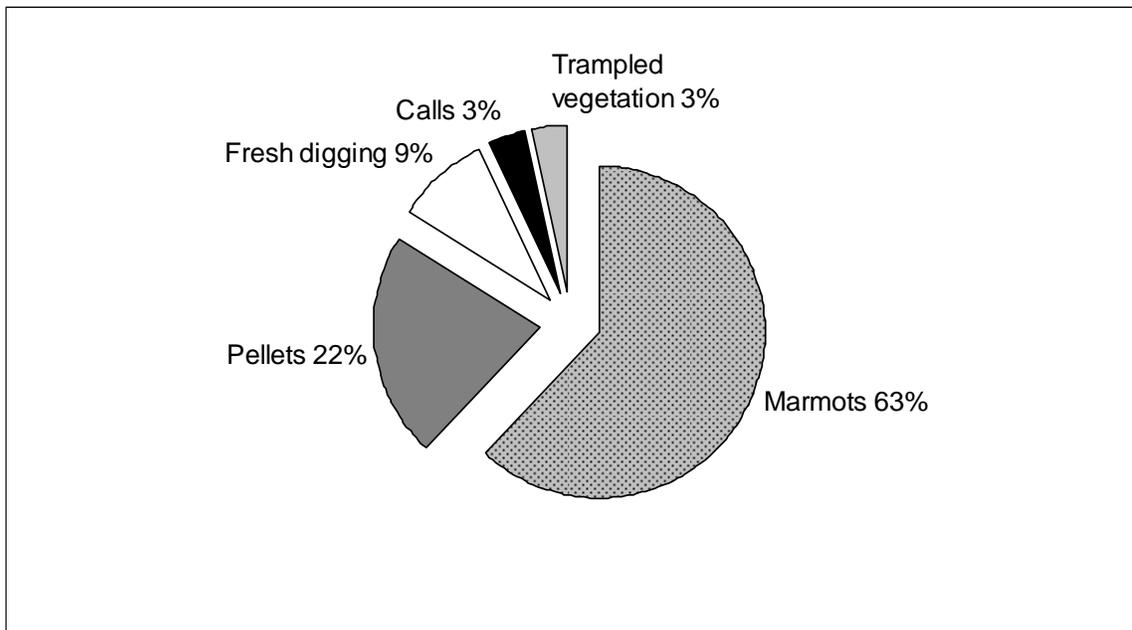
Survey number	Polygon ID	Status Determined	Number of marmots seen	Calls	<u>Active burrows confirmation</u>				
					Pellets	Fresh digging	Trampled vegetation	Paths between burrows	Marmot smell
1	56	1				+			+
2	128	1			+				
3	172	1	2						
4	183	1	2						
5	189	1	2						
6	199	1	2						
7	518	1		+	+		+		
8	523	1				+			
9	524	1			+				
10	559	1			+				
11	575	2							
12	594	1				+			
13	598	0							
14	600	1	1	+					
15	650	1			+		+	+	
16	657	1					+		
17	674	1			+				
18	731	1	1		+				
19	790	1			+				
20	791	1			+				
21	803	1			+				+
22	830	1	1						
23	831	1	2						
24	859 W	1	4						
25	859 E	1				+		+	
26	876	1	1	+					
27	980	1			+			+	
28	983	1	1						
29	1009	1	1						
30	1031	1	1						
31	1040	1	2						
32	1043	1			+				
33	1086	1	1						
34	1106	0							
35	1116	1	1						
36	1132	1		+					
37	1133	1	2						
38	1154	1					+	+	+
39	1164	1			+				
40	1170	1		+				+	+
41	1173	1	1	+					
42	1177	1			+				

Appendix D, Table 1 continued:

Survey number	Polygon ID	Status Determined	Number of marmots seen	Calls	Active burrows confirmation				
					Pellets	Fresh digging	Trampled vegetation	Paths between burrows	Marmot smell
43	1178	1	5	+					
44	1210	1				+			
45	1250	1	1						
46	1264	1				+			
47	1273	1	1						
48	1322	1			+				
49	1331	1	1		+				
50	1370	1	1						
51	1404	1	1						
52	1434	1	1	+					
53	1544	1	1		+				
54	1545	1	1		+				
55	1823	1	5						
56	1882	1			+				
57	2045	1	2						
58	2147	1			+				
59	2232	0							
60	2259	0							
61	2318	1	1						
62	2442	1	1						
63	2531	1	1						
64	2566	1	1						
65	3587	1	1						
66	3615	1	3						
67	3643	1	6						
68	3688	1	3						
69	3785	1			+				
70	3815	1			+				
71	3913	1	1						
72	3996	1	3						
73	4066	1	1						
74	4202	1	6						
75	4290	1	5						
76	4318 W	1	2						
77	4318 E	1	1						
78	4600	1	2	+					
79	5038	1	1						
80	5521	1	1						
81	5607 E	1	1						
82	5607 W	1	1						
83	5620	1					+		
84	6005	1	1						
85	6051	1			+				

Appendix D, Table 1 continued:

Survey number	Polygon ID	Status Determined	Number of marmots seen	Calls	Active burrows confirmation				
					Pellets	Fresh digging	Trampled vegetation	Paths between burrows	Marmot smell
86	6287	1				+			
87	11285	0							
88	11313	1	1						
89	11341	1	1						
90	11342	1				+		+	
91	11357	1	1	+					
92	11394	1	2	+					
93	11401	2							
94	Lena lake	1				+			



Appendix D, Figure 1. Main cues used for polygon status determination in pilot surveys for detectability assessment (n=87).